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Evidence for Cooperative Effects in the Exchange Reaction Catalysed by the Oxoglutarate Translocator of Rat-Heart Mitochondria

Francis E. SLUSE, Claudine M. SLUSE-GOFFART, Claire DUYCKAERTS, and Claude LIÉBECQ

with the technical assistance of Laurette Bertrand and Eli Dethier

Laboratoire de Biochimie et de Physiologie Générale, Institut Supérieur d'Education Physique de l'Université de Liège

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The initial rates of the exchange external oxoglutarate/internal malate through the inner membrane of rat-heart mitochondria, for various concentrations of the two substrates, have been reinvestigated for an extended range of concentrations of the external oxoglutarate. This has been made possible by use of the inhibitor-stop technique that allows 100 times smaller incubation times than the centrifugation-stop technique used previously.

Under the experimental conditions the uptake of the external-labelled oxoglutarate into the mitochondrial-matrix space is mediated by the oxoglutarate translocator performing a ono-to-one exchange of the anions oxoglutarate (external) and malate (internal).

Two intermediary-plateau regions are observed in the kinetic saturation curve of the translocator by the external oxoglutarate, revealing a complex rate equation which is found to be the product of two one-substrate functions. Analysing these features it is shown that the model, proposed earlier, of a "double carrier" as catalyst in a rapid-equilibrium random bi-bi mechanism, is still applicable but that several external binding sites have to be considered. As already noticed the external and the internal substrates bind to their respective sites independently of each other.

Furthermore, some additional requirements imposed by the observed kinetics suggest that the exchange reaction is performed by only one translocator species made of identical interacting subunits.

The anion exchange is tentatively viewed as a rotation of a subunit around an axis situated in the plane of the membrane after two independent local configuration changes induced by the binding of the two substrates on this subunit.

The mitochondrial inner membrane contains a battery of translocators catalysing exchange-diffusion reactions between intramitochondrial and extramitochondrial anions. The translocations follow a one-to-one stoechiometry [1] and the translocators show a definite selectivity for the substrates exchanged and a specific sensitivity to inhibitors (for a review, see [2]). The physiological rôle of these translocators is to couple metabolic processes occurring in the cytosol and in the mitochondrial matrix through the interchange of common intermediates. This coupling is determined by the properties of the translocators and the kinetic characteristics of the translocations they take part in. Extensive quantitative data are therefore desirable not only to elucidate their catalytic mechanism but also to understand their possible regulatory rôle.

Note. In case of statistical analyses, the values reported are mean ± standard deviation.

The kinetic study of the exchange reactions catalysed by the oxoglutarate translocator had been undertaken by measuring the initial rate of 12 exchanges at different internal-anion and external-anion concentrations [3]. The favourable experimental conditions allowed a rigorous kinetic analysis because only one translocator took part in the exchange studied.

This study led to the determination of an equation that related the rate of the reaction to the concentration of the substrates and of the translocator. This equation included the kinetic constants that were functions of the rate constants of the elementary steps of the reaction. A careful examination of the kinetic constants allowed one to choose the simplest mechanism that best applied to the exchange reaction at 4 °C. It was the so-called mechanism of rapid equilibrium random bi-bi. This mechanism was confirmed by the study of external-product inhibition which revealed a mixed dead-end and product inhibition [4]. It was concluded

that the translocator should possess two independent binding sites for substrates, one internal and one external, and that the transport of anions should be achieved by a simultaneous displacement of the two anions, which would be the limiting step of the exchange reaction [3–5].

The rather limited concentration range of external substrates studied was imposed by the slowness of the centrifugation-stop technique used to block the exchange reaction. Accordingly the conclusions reached may be valid only within this limited concentration range. To investigate the exchange at lower or higher external concentrations, an inhibitor-stop technique was developed to reduce the incubation time; phenylsuccinate was used as an inhibitor [6].

This paper describes the kinetics of the exchange of external oxoglutarate for internal malate for a very large range of oxoglutarate concentrations (0.1 μM to 100 μM) and also for a somewhat larger range of internal-malate concentration. These results have been briefly presented elsewhere [7–9].

MATERIALS AND METHODS

Materials

Special reagents were obtained from the following sources: 2-oxo[5-^{14}C]glutarate, [U-^{14}C]sucrose and tritiated water (The Radiochemical Center, Amersham, England); rotenone (Sigma Chemical Company, Saint-Louis, Missouri, U.S.A.); mersalyl, acid form (Mann Research Laboratories, New York, U.S.A.); phenylsuccinate (Aldrich, Milwaukee, Wisconsin, U.S.A.). Because aqueous solutions of 2-oxo[5-^{14}C]-glutarate are unstable if frozen and thawed, due to intramolecular and intermolecular aldol-type condensations, a fresh dry sample was used for each experiment; this precaution was taken before but not mentioned [3,4].

Preloading of the Mitochondria

Mitochondria from rat-heart ventricle were prepared according to Tyler and Gonze [10] and loaded with malate during a 30-min period at 0 °C in the following medium: 157.5-mM mannitol, 52.5-mM sucrose, 20-mM Tris · Cl pH 7.4, 10 or 5-mM malate and 0.035-mM EDTA.

The varied concentrations of internal malate were obtained by washings in large volumes of isolation medium [10] as indicated before [3].

Incubation Medium

The incubation medium contained 15-mM KCl, 5-mM $MgCl_2$, 2-mM EDTA, 50-mM Tris · Cl pH 7.4, 22.5-mM mannitol and 7.5-mM sucrose (the latter two derived from the stock mitochondrial suspension); 0.5 ml of the incubation medium contained 2 μg of rotenone, 0.5 μmol of sodium arsenite, 3.3 μCi of tritiated water and 0.1 μmol of mersalyl.

The amount of mersalyl used (0.2 μmol/mg protein) does not affect the initial rate of oxoglutarate/malate exchange at 4 °C, as verified in our laboratory, and is sufficient to block the malate/phosphate exchange, even at 20 °C [11].

The mitochondrial concentration was the same in each experiment: around 0.47 mg of protein per 0.5-ml incubation assay. The concentration of oxoglutarate was varied from 0.094 to 105 μM. The final concentration of the inhibitor (phenylsuccinate) was 22.7 mM.

Timing

The mitochondria were preincubated 1 min at 2 °C in 0.35 ml of incubation medium, then supplemented by labelled oxoglutarate dissolved in 0.15 ml of the same medium to avoid osmotic shock. After 0.2–1-s incubation, 0.2 ml of 80-mM phenylsuccinate was added to block the exchange reaction.

This high concentration of phenylsuccinate is necessary to produce a satisfactory inhibition (K_i = 250 μM, 98% inhibition at the final 22.7-mM concentration) [6], but increases the osmolarity of the final suspension.

As a result, the mitochondrial pellet shrinks at the expense of the matrix space (the sucrose space remains constant). This must be carefully measured to calculate the exact amount of labelled substrate taken up.

Exactly 7 s after the addition of phenylsuccinate, mitochondria were separated by rapid centrifugation in an Eppendorf microcentrifuge (model 3200) for 50 s. The supernatant was acidified, the walls of the tube and the surface of the pellet containing the trapped labelled oxoglutarate were rinsed with the isolation medium [3], and the tube was blotted rapidly and the pellet acidified.

The radioactivities were determined in a Packard liquid-scintillation counter with Insta-Gel (Packard) as scintillation medium.

Internal-malate concentration was determined enzymically with malate dehydrogenase [12] and mitochondrial proteins were determined by the biuret method [13] using bovine serum albumin (fraction V) as standard.

Equipment

The inhibitor-stop technique made it possible to perform very short incubations, corresponding to

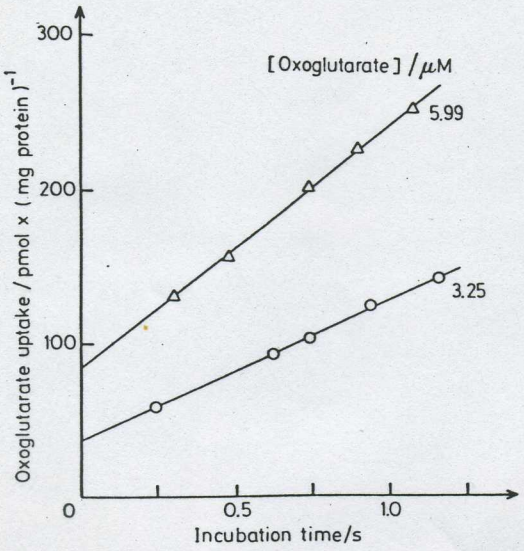

Fig. 1. *Initial-rate determination of oxoglutarate translocation in malate-loaded mitochondria.* Mitochondria (0.48 mg of protein) were prepared and incubated as described in Materials and Methods

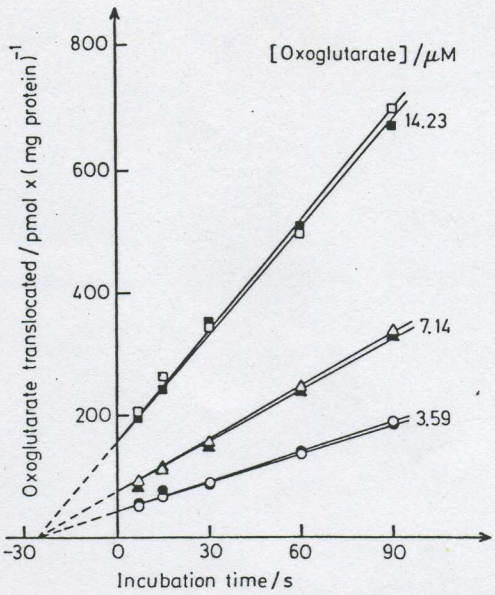

Fig. 2. *Residual rate of oxoglutarate uptake in the presence of 22.7-mM phenylsuccinate.* Uptake was initiated by simultaneous addition of phenylsuccinate (22.7 mM) and labelled oxoglutarate and for two mitochondrial preparations (0.46 and 0.45 mg of protein). The incubation time represented the time interval between the addition of substrate plus inhibitor and the beginning of the centrifugation. Translocated oxoglutarate was the total oxoglutarate uptake minus the oxoglutarate contained in the sucrose space. The value of the intercept with the axis of abscissa (25.7 ± 0.9 s) was calculated by imposing a common intersection, on the abscissa, to the straight lines calculated by the method of least squares

the time interval between the additions of the substrate and that of the inhibitor. This required an extremely quick addition of the two compounds, instantaneous mixing, and a very precise determination of the duration of the incubation period. These requirements were fulfilled by using Hamilton syringes (CR700 Series), large-volume additions (40% of the preceding incubation volume) and electric coupling of the injecting syringes to a Jaquet chronoscope (model 320) of 10-ms precision.

The time resolution of the assay is illustrated in Fig. 1. The five incubation times, required to determine the slope satisfactorily, are distributed between 0.24 and 1.16 s.

Requirements

The time interval between the addition of the inhibitor and the beginning of the centrifugation must be kept constant (a 7-s period was selected for the facility of the operator). This is essential because there remains a residual exchange activity in the presence of the inhibitor that cannot be neglected, since it may last as much as 100 times the incubation period itself.

This residual activity may be determined by measuring the uptake of labelled oxoglutarate after simultaneous injection of both substrate and inhibitor; this is illustrated in Fig. 2 where the amount of oxoglutarate transported, after subtraction of the sucrose-space contamination, is expressed as a function of the time preceding the onset of centrifugation. The slopes of the straight lines are the residual initial rates of the phenylsuccinate-inhibited reactions. The six straight lines intersect with the abscissa on a common point at −26 s representing the time required to stop the residual exchange by centrifugation if bound oxoglutarate may be neglected.

The period for which the residual activity persists in ordinary experiments, is therefore made up of two parts, the 7 s that separate the injection of the inhibitor from the onset of the centrifugation and the 26 s required for interruption by centrifugation, thus a total of 33 s or at least 30 times the longest incubation period. The amount of oxoglutarate transported during this period may not be neglected, even though the activity represents only 2% of the initial non-inhibited rate. A non-negligible part of this activity is still due to the translocator as indicated by its saturation behaviour (not shown).

The extrapolated ordinates ($t = 0$), in ordinary experiments (Fig. 1) represent the oxoglutarate uptake during 33 s in the presence of the inhibitor, plus the sucrose-space contamination, plus binding contamination.

The tritiated-water space after phenylsuccinate is used to calculate the total oxoglutarate uptake in

the mitochondrial pellet; this is corrected by subtraction of the sucrose-space contamination to give the true translocated oxoglutarate. The internal-malate concentration must be determined on the matrix volume before shrinking, and requires sucrose-space determination in the absence of the inhibitor.

Typical Experiment

In a typical experiment we measure the uptake of radioactivity at three concentrations of external oxoglutarate for three concentrations of internal malate; each rate of uptake is calculated from five incubation times. The 15 incubations corresponding to one of the three concentrations of internal malate are realized within 7 min; during this time the internal-malate concentration is essentially stable, as verified by measuring malate concentration before and after the experimental series.

RESULTS

Time-Course of Oxoglutarate Uptake

The time-course of 2-oxo[5-^{14}C]glutarate uptake in malate-loaded mitochondria at 2 °C is illustrated in Fig. 3. Oxoglutarate uptake increased linearly with time for 3 s in the conditions specified in the legend.

Equilibrium was reached after 100 s with a half-equilibration time of 18 s. Earlier studies [4] had been carried out with 3 times less mitochondria.

Variation of Internal-Malate Concentration

The dependence of the rate of the external-oxoglutarate/internal-malate exchange on the internal-malate concentration was investigated within the 1.5–9-mM range for different fixed concentrations of the external oxoglutarate between 0.54 and 53.8 μM. The saturation curves were hyperbolic.

Fig. 4 shows the double-reciprocal plot made of straight lines converging to a common point situated on the abscissa. This common point corresponds to a K_m of 2.43 ± 0.32 mM independent of the external-substrate concentration.

The same was observed previously using a centrifugation-stop technique, with only three concentrations of each substrate: from 2.5 to 3.2-mM internal malate, and from 0.2 to 1-μM external oxoglutarate in one experiment [3], from 8.9 to 12.2-mM internal malate and from 1 to 3-μM external oxoglutarate in another experiment [4]. The K_m-value for internal malate was 3.60 ± 0.44 mM [4], somewhat greater than the present value.

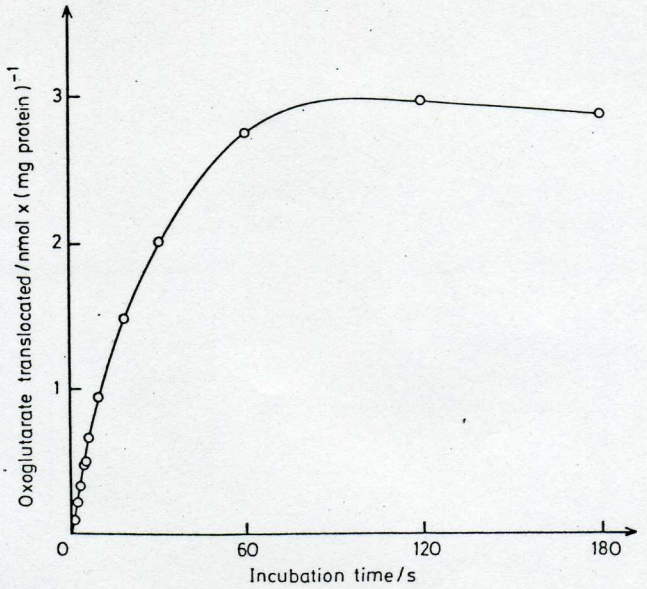

Fig. 3. *Time-course of oxoglutarate translocation.* Mitochondria (0.64 mg of protein) were prepared and incubated as described in Materials and Methods, except that the volume of the incubation medium was 0.85 ml. Oxoglutarate concentration was 3.12 μM

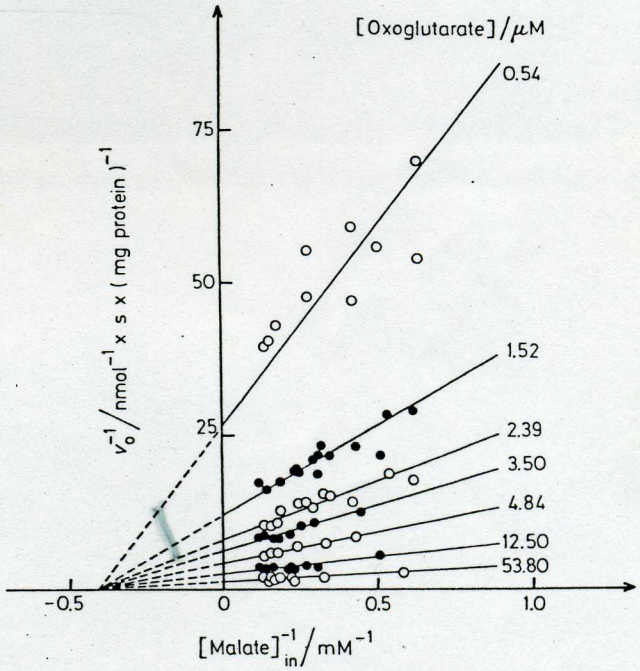

Fig. 4. *Dependence of the rate of oxoglutarate/malate exchange on the internal-malate concentration.* Mitochondria (0.47 mg of protein) were prepared and incubated as described in Materials and Methods. The straight lines were calculated by the method of least squares, by imposing a common intersection on the abscissa, unknown *a priori* [4]

The agreement between the two sets of experiments shows that the centrifugation-stop technique, despite its relative crudeness, provides valuable initial-rate measurements when the substrate concentrations and amount of mitochondria are properly chosen.

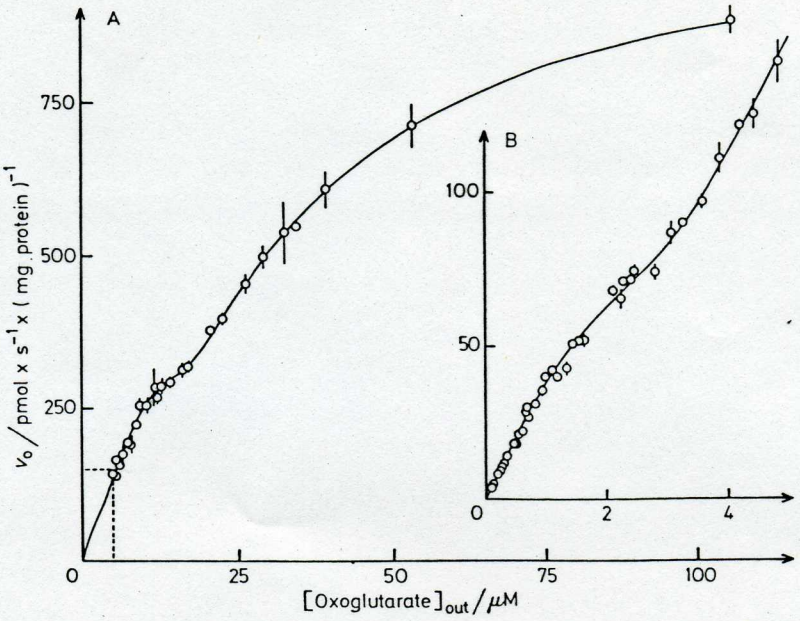

Fig. 5. *Initial rate as a function of the external oxoglutarate concentration at 4-mM internal malate.* Mitochondria (0.47 mg of protein) were prepared and incubated as described in Materials and Methods. Each of the 61 points was obtained by measuring the rate (slope calculated from 5 incubation times) at three internal-malate concentrations around 4 mM. Vertical bars represent the standard error

Variation of External-Oxoglutarate Concentration

The dependence of the rate of the external-oxoglutarate/internal-malate exchange on the external-oxoglutarate concentration was studied between 0.1 and 105 μM for a 4-mM internal-malate concentration. If necessary, the initial rate for 4-mM internal malate was obtained by linear interpolation of the double-reciprocal plots obtained with three internal-malate concentrations around 4 mM. This is justified by the linear relations obtained between the reciprocal of the initial rate and the reciprocal of the internal-malate concentration (Fig. 4).

The variation of the initial rate as a function of external-oxoglutarate concentration is illustrated in Fig. 5A and 5B. The curve obtained deviates from simple hyperbolic saturation: there is a hyperbola-like part for oxoglutarate concentrations lower than 2.5 μM (Fig. 5B) followed by two sigmoïdal curves when the oxoglutarate concentration increases (Fig. 5A). The curve presents two intermediary regions (near 3 and 15-μM oxoglutarate) like intermediary plateaus. In our early experiments [3,4], the external-oxoglutarate concentration was $\leqslant$ 3 μM.

Fig. 6 shows the Lineweaver-Burk plot of the data in which the non-Michaelian behaviour of the kinetics appears clearly. The graph can be divided into different regions.

For very low oxoglutarate concentrations (0.1–0.7 μM) a straight line is observed that may be extended to the origin (Fig. 6A); this part of the curve will be designated region 1.

Between 0.7 and 3 μM another straight line is observed; that part will be named region 2 (Fig. 6B). To this correspond a pseudo Michaelis constant (K_{ps}) of 3.96 $\pm$ 0.69 μM and a pseudo maximal rate (V_{ps}) of 187 $\pm$ 25 pmol $\cdot$ s^{-1} $\cdot$ mg^{-1}.

Between 3 and 105 μM the double-reciprocal plot presents two jumps (Fig. 6B and 6C). The apparent maximal rate, obtained by computer simulation, is 963 pmol $\cdot$ s^{-1} $\cdot$ mg^{-1}, that is five times the pseudo maximal rate obtained by linear extrapolation of region 2.

In previous experiments in which the centrifugation-stop technique had been used [3–5], the limits of external-oxoglutarate concentrations for the whole set of experiments had been 0.2 and 3 μM. This concentration range covers regions 1 and 2. Since in each experiment only three points had been determined for a given internal concentration and since the determination of the initial rates had been less accurate, the deviation from linearity could not be detected. Furthermore, in the interpretation that will be proposed this deviation is dependent on the mitochondrial concentration and is more important when the mitochondrial concentration is higher.

Fig. 7 shows the influence of the internal-malate concentration on the double-reciprocal plots relative to the external oxoglutarate. Extrapolation of the linear parts (region 2) and of the following pseudo-

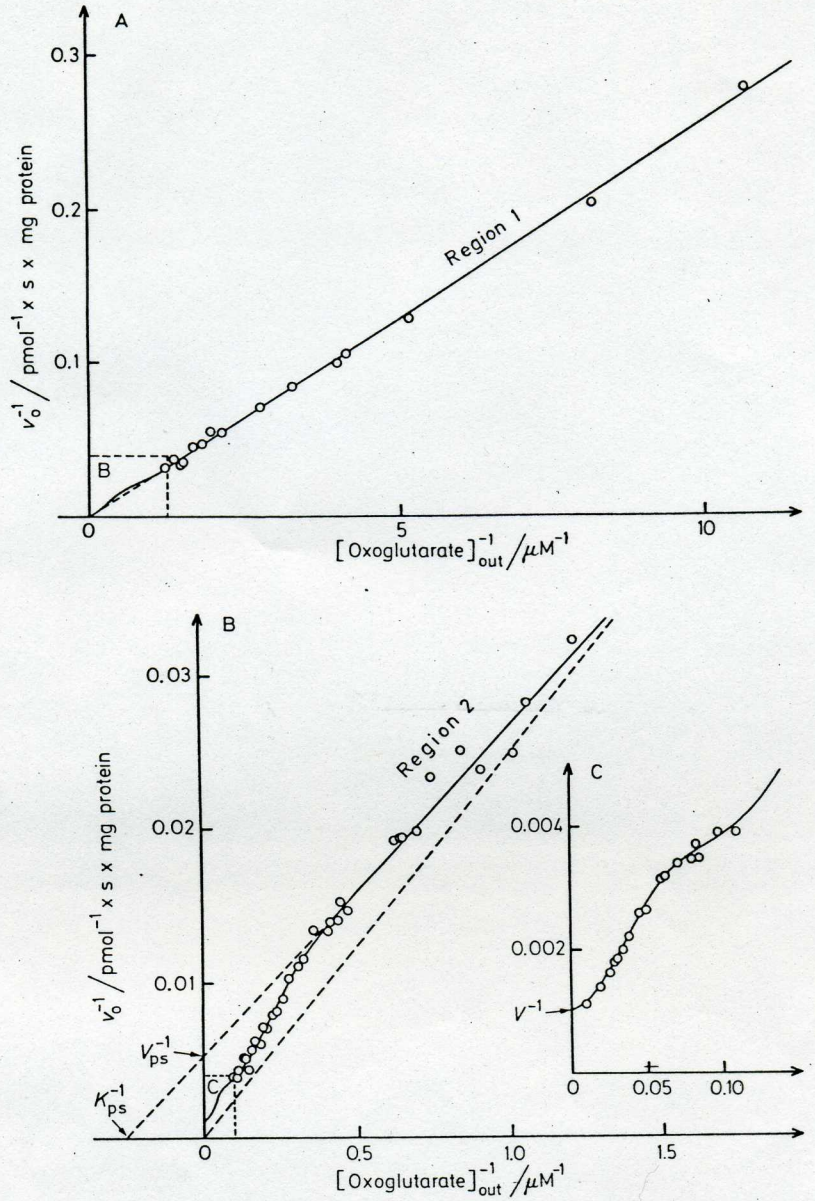

Fig. 6. *Reciprocal of the initial rate as a function of the reciprocal of the external-oxoglutarate concentration at 4-mM internal malate.* Mitochondria (0.47 mg of protein) were prepared and incubated as described in Materials and Methods. (A) Region 1 (0.1 – 0.7-μM oxoglutarate); (B) region 2 (0.7 – 3.0-μM oxoglutarate) and the first jump; (C) the last jump

linear parts to the abscissa provides intersection points independent of the internal-malate concentration.

The unusual kinetic behaviour of the exchange may also be visualized in the Hofstee plot (Fig. 8) and in the Hanes plot (Fig. 9). In these plots, the points corresponding to region 1 are omitted for the sake of clarity, and the linear region corresponds to region 2 of the double-reciprocal plot.

The pseudo Michaelis constant of region 2 determined from the Hofstee plot is 3.65 ± 0.50 μM and the pseudo maximal rate determined from the Hanes plot is 172 ± 17 pmol · s^{-1} · mg^{-1}.

The Hill plot (Fig. 10) may be schematically decomposed into five straight-line segments of which the slopes (h) are alternatively higher and smaller than unity:

h = 1.04 at 0.1 – 0.7 μM oxoglutarate
h = 0.74 at 1.0 – 2.8 μM oxoglutarate
h = 1.18 at 3.2 – 8.0 μM oxoglutarate
h = 0.57 at 9 – 17 μM oxoglutarate
h = 1.80 at 22 – 105 μM oxoglutarate.

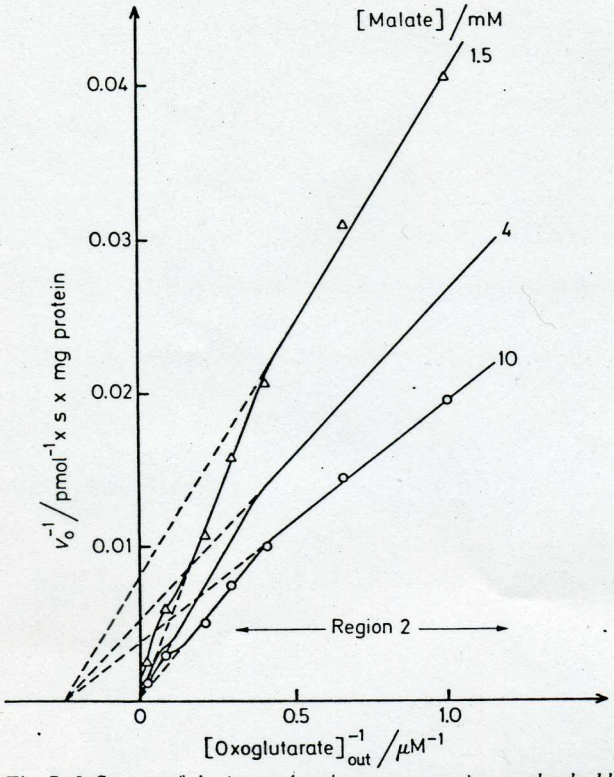

Fig. 7. *Influence of the internal-malate concentration on the double-reciprocal plots relative to external oxoglutarate.* Mitochondria (0.47 mg of protein) were prepared and incubated as described in Materials and Methods. The curve for 4-mM internal malate was taken from Fig. 6B. The points on the two other curves were obtained from Fig. 4

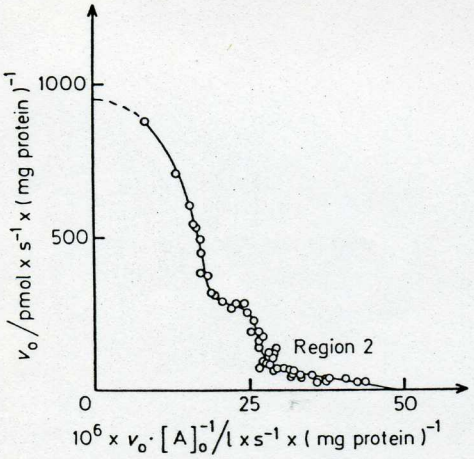

Fig. 8. *Initial rate (v_0) as a function of $v_0/[S]_0^{-1}$ (Hofstee).* Curve obtained from the data of Fig. 5. The linear region 2 was calculated by the method of least squares

The values of the Hill coefficients (h) are obviously dependent on the exact value of the maximal rate but the observed fluctuation is a reliable feature. This will be described as a fluctuating "apparent cooperativity".

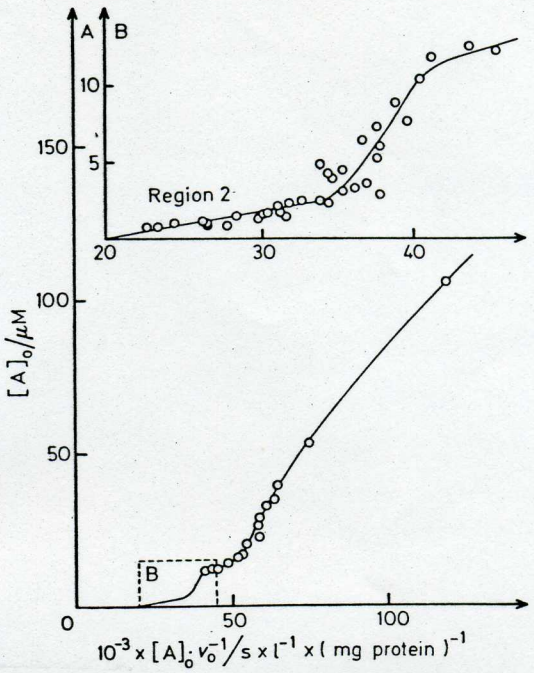

Fig. 9. *Substrate concentration ($[S]_0$) as a function of $[S]_0/v_0$ (Hanes).* Curves obtained from the data of Fig. 5. The linear region 2 was calculated by the method of least squares

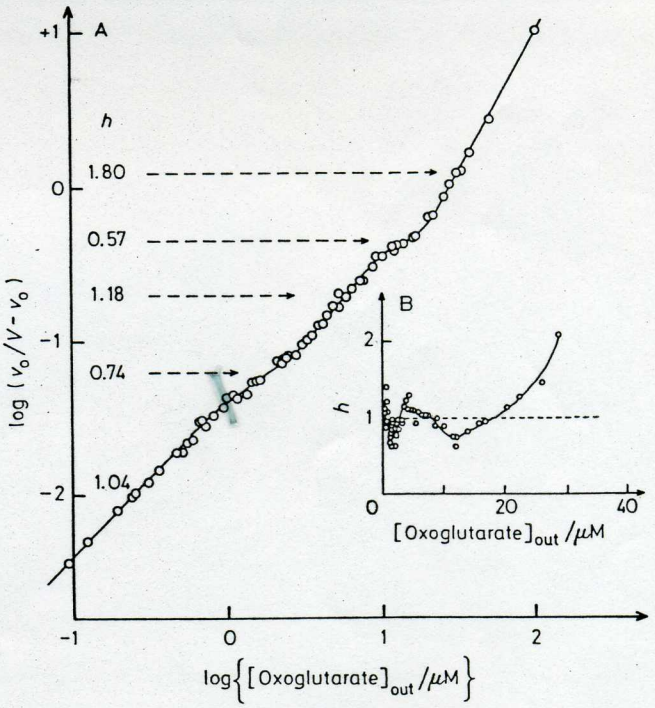

Fig. 10. *(A) Log $v \cdot (V-v)^{-1}$ versus log $[S]_0$ (Hill) and (B) h as a function of $[S]_0$.* Curves obtained from the data of Fig. 5 using $V = 963$ pmol · s^{-1} · (mg protein)$^{-1}$ obtained from Fig. 6C. (A) The h-values are the Hill coefficients corresponding to each straight-line segment. (B) The h-values are calculated from the points of curve (A) by a method of derivation described in [26]

DISCUSSION

As previously [3–5], the exchange of anions through the mitochondrial inner membrane will be viewed as a two-substrate two-product enzymic reaction. The kinetic data will be tentatively interpreted using a general equation of the Wong-Hanes type [14,15].

The discussion will first deal with the influence of the concentration of one (external) substrate on the initial rate, the concentration of the other (internal) substrate being fixed. Then the effect of the fixed (external) substrate concentration on the initial rate as a function of the variable (internal) substrate concentration will be explored.

From this the general mechanism of the exchange reaction will be deduced. In the light of the conclusions reached, the kinetic behaviour at low concentrations of external oxoglutarate will be discussed.

Finally, a possible effect of the diffusion of the substrates to the active sites of the translocator will be considered.

DEPENDENCE OF THE INITIAL RATE ON THE EXTERNAL-SUBSTRATE CONCENTRATION

The different graphical representations of the exchange kinetics as a function of the external-oxoglutarate concentration clearly reveal a complex behaviour. To describe such a behaviour it is necessary to use a general equation of the type

$$v_0 = \frac{\sum_1^n a_i [A]_0^i}{\sum_0^n b_i [A]_0^i} \qquad (1)$$

where v_0 is the initial rate and $[A]_0$ the initial concentration of the substrate, external oxoglutarate in the present case. The coefficients a_i and b_i are related to the enzyme concentration, to the second substrate concentration, and to the rate constants for the individual steps of the reaction mechanism. The classical Michaelis-Menten equation is a particular case of Eqn (1) in which $n = 1$.

The degree of the rate equation n may be greater than unity for one or several of the following reasons.

a) The enzyme possesses more than one binding site for the substrates and these sites interact. In the Monod-Wyman-Changeux model [16], the binding sites do not interact but indirect cooperativity is mediated by displacement of the equilibrium between two forms of the enzyme.

b) The reaction follows steady-state kinetics and several pathways are possible. For instance, if there is one binding site for each of the two substrates the more general mechanism gives rise to a rate equation in which $n = 3$ [17].

c) Several enzymes are involved in the reaction.

Saturation curves generated by Eqn (1) when $n > 1$ are differently shaped (pseudo-hyperbolic, sigmoïdal, or composite) according to the values of the coefficients a_i and b_i.

Teipel and Koshland [18] have described the characteristics of the equation when the saturation curve presents one intermediary plateau. They have shown that: (a) n must be $\geqslant 3$; (b) in the case of a multi-site enzyme following rapid-equilibrium kinetics, the occurrence of a cooperativity (affecting the binding constant or the catalytic rate constant) first negative, then positive in the course of the enzyme saturation can generate a plateau.

A four-site model with cooperative interactions occurring only at the level of the affinities accounts fairly well for the saturation curve of glutamate dehydrogenase from *Blastocladiella emersonii* [18].

When the double-reciprocal plot presents abrupt transitions between pseudo-linear sections, negative cooperativity followed by positive cooperativity must affect the catalytic rate constant [19].

Zeylemaker *et al.* [20] observed an intermediary-plateau in the saturation curve of succinate dehydrogenase with succinate and found that steady-state kinetics with several pathways applies better to this particular case than a multi-site-enzyme model.

As shown in the present work, there are two intermediary-plateau regions (four inflection points) in the saturation curve of the oxoglutarate of rat-heart mitochondria. As the degree of the second derivative of the rate equation is $3n - 3$, the degree of the rate equation n must in any case be at least three. However, in our opinion, the existence of two detectable plateaus necessitates a much higher degree, probably five at least as also claimed by Kagan and Doroshko [21]. It cannot be excluded that such a high degree results from the contribution of several translocator species. For example, the saturation curve could be the sum of the contributions from three translocators with a quite different half-saturation concentration, one possessing a hyperbolic curve and two others a sigmoïdal curve.

DEPENDENCE OF THE INITIAL RATE ON THE INTERNAL-SUBSTRATE CONCENTRATION

Two substrates, one external (A) and one internal (B), are in fact involved in the transport reaction which is a one-to-one exchange of anions. Thus the initial

rate equation must be written:

$$v_0 = \frac{\sum_{i=1}^{n_A} \sum_{j=1}^{n_B} a_{ij} [A]_0^i [B]_0^j}{\sum_{i=0}^{n_A} \sum_{j=0}^{n_B} b_{ij} [A]_0^i [B]_0^j} . \quad (2)$$

When the external oxoglutarate concentration ($[A]_0$) is fixed, the dependence of the initial rate on the internal-malate concentration ($[B]_0$) seems to be Michaelian (Fig. 4). This means either that $n_B = 1$ or that the investigated range of internal concentrations is too narrow. That $n_B = 1$ does not necessarily imply that there is only one internal site. Indeed, n_B may be unity if the internal binding sites do not interact either between themselves or with the external sites.

A PARTICULAR PROPERTY OF THE EXCHANGE REACTIONS AND ITS IMPLICATIONS

The kinetics of the exchange reactions catalysed by the oxoglutarate translocator present an important feature: the double-reciprocal straight lines (or pseudo straight lines) relative to the variation of the concentration of one substrate, each of them corresponding to different fixed concentrations of the other substrate, converge to a common point situated on the abscissa (Fig. 4, Fig. 7 and [3–5]). This behaviour demonstrates that the initial rate is in fact the product of two one-substrate functions. So, Eqn (2) takes the particular form:

$$v_0 = \frac{\sum_{1}^{n_A} \alpha_i [A]_0^i}{\sum_{0}^{n_A} \beta_i [A]_0^i} \cdot \frac{\sum_{1}^{n_B} \gamma_j [B]_0^j}{\sum_{0}^{n_B} \delta_j [B]_0^j} . \quad (3)$$

In order to transform Eqn (2) into Eqn (3) the following relations must be verified:

$$a_{ij} = \alpha_i \gamma_j \quad (4)$$

and

$$b_{ij} = \beta_i \delta_j . \quad (5)$$

If the exchange kinetics are of the steady-state type, an equation like Eqn (3) would be possible only if there is a number of quite-fortuitous relations between the kinetic coefficients. This situation may thus be considered improbable and may even be excluded as has been observed for 12 exchanges [3–5] among which are corresponding forward and backward exchanges [22].

In the case of rapid-equilibrium kinetics, the kinetic coefficients are related to the dissociation constants of the enzyme-substrate complexes and to the rate constant of the limiting step of the reaction. Therefore the conditions resulting from Eqn (3) are simpler and possess fundamental significance. On the other hand, rapid-equilibrium kinetics are plausible since the transport step of the exchange reaction may be limiting. The remaining part of the discussion will therefore assume kinetics of the rapid-equilibrium type.

Implications of Eqn (3) (if Several Translocators)

As the exchange kinetics are complex, the possible presence of several oxoglutarate translocator species is to be considered.

If more than one translocator is involved in the exchange reaction, Eqn (3) must apply to the overall rate of exchange. This is possible if each individual rate conforms to an equation like Eqn (3) and if the individual equations have a common part, either the part depending on the external-substrate concentration or the part depending on the internal-substrate concentration. As the overall rate seems to be Michaelian when the internal malate is varied, it is the external part only which would differ from one translocator to another.

This is not very attractive although cannot be excluded.

Implications of Eqn (5)

Eqn (5) applies only to a sequential mechanism and means that the binding of the external substrate to the translocator (a single one, or each of several ones) is independent of the binding of the internal substrate and *vice-versa*. The mechanism is thus a particular case of a rapid-equilibrium random mechanism.

This conclusion is supported by the previous observation that the pseudo Michaelis constant (corresponding to a limited range of concentrations) for a given substrate, either external or internal, is independent of the nature of the counter-ion [3].

The translocator is thus a molecule or a group of molecules that spans the mitochondrial inner membrane and possesses binding sites on the two sides of the membrane. The lack of interactions between internal and external sites is plausible as these sites are probably separated by a distance equal to the thickness of the membrane.

Implications of Eqn (4)

This part of the discussion applies also to a single translocator or to each of several ones.

The rate of reaction corresponding to a translocator E is the sum of the contributions of each active species this translocator may form when combined with the substrates. The a_{ij} coefficients, now under discussion, are related to the activity of the complexes EA_iB_j. These relations will first be expressed in terms of dissociation constants and catalytic rate constants [see Eqn (9) below] in order to see in what conditions they conform to Eqn (4).

Complexes corresponding to a same formula EA_iB_j may differ by the arrangement of the i molecules A and of the j molecules B on the binding sites.

Each external binding site will be numbered by an integer p from 1 to n_A and each internal site by an integer q from 1 to n_B; n_A and n_B are the number of external and internal sites respectively.

The occupancy of a site will be characterized by $l_p = 0$ or 1, if external, and by $m_q = 0$ or 1, if internal. The whole set of l_p and m_q for one enzyme-substrate complex will be noted P and Q. Thus the formula $(EA_iB_j)_{P,Q}$ completely characterises a complex. In this complex, the activity of a particular couple of loaded sites, one external and one internal, is dependent upon a catalytic rate constant $k_{P,Q,p,q}$ which is zero if the exchange is forbidden between the external site number p and the internal site number q.

The contribution of $(EA_iB_j)_{P,Q}$ to the reaction rate is:

$$\sum_{p,q} l_p\, m_q\, k_{P,Q,p,q}\, [(EA_iB_j)_{P,Q}]$$

and the total initial rate is given by:

$$v_0 = \sum_{i,j} \sum_{P,Q} \sum_{p,q} l_p\, m_q\, k_{P,Q,p,q}\, [(EA_iB_j)_{P,Q}]. \qquad (6)$$

If the binding of the external substrate and of the internal substrate are independent, as shown above, the following equations may be written:

$$[(EA_iB_j)_{P,Q}] = [E]\psi_P\, \theta_Q\, [A]_0^i\, [B]_0^j \qquad (7)$$

and

$$[E] = \frac{[E]_0}{\sum_0^{n_A} \beta_i\, [A]_0^i \sum_0^{n_B} \delta_j\, [B]_0^j} \qquad (8)$$

where

$$\beta_i = \sum_P \psi_P \text{ and } \delta_j = \sum_Q \theta_Q.$$

ψ_P and θ_Q are the association constants corresponding to

$$(EA_iB_j)_{P,Q} \rightleftharpoons (EB_j)_Q + iA$$

and to

$$(EA_iB_j)_{P,Q} \rightleftharpoons (EA_i)_P + jB.$$

Comparing Eqn (6), after inclusion of Eqn (7) and Eqn (8) to Eqn (3) one obtains:

$$a_{ij} = [E]_0 \sum_{P,Q} \sum_{p,q} l_p\, m_q\, k_{P,Q,p,q}\, \psi_P\, \theta_Q. \qquad (9)$$

In order to have a_{ij} equal to a product of two functions, the first dependent only on the external characteristics (labelled P and p) and the second dependent only on the internal characteristics (labelled Q and q), $k_{P,Q,p,q}$ must be a product of two such functions. The constant $k_{P,Q,p,q}$ is related to the Gibbs energy of activation of the reaction $\Delta G^{\neq}_{P,Q,p,q}$, by:

$$k_{P,Q,p,q} = \frac{k_B\, T}{h} \exp\left[-\frac{\Delta G^{\neq}_{P,Q,p,q}}{RT}\right] \qquad (10)$$

where k_B, h, R and T are the Bolzman constant, the Planck constant, the gas constant and the absolute temperature respectively. So, the problem is now to express $\Delta G^{\neq}_{P,Q,p,q}$ as a sum of simpler terms.

The activation energy can be decomposed into three components (*vide infra*) if those interactions that depend upon P, modified during the p−q exchange, are localised in the near neighbourhood of the external surface of the membrane while those that depend upon Q are localised in the near neighbourhood of the internal surface. The three components are:

a) an intrinsic component $\Delta G^{\neq *}_{p,q}$ which is independent of the binding sites occupancy;

b) a component $\Delta G^{\neq}_{P,p}$ which depends on the occupancy of the external binding sites only;

c) a component $\Delta G^{\neq}_{Q,q}$ which depends on the occupancy of the internal binding sites only.

Thus,

$$\Delta G^{\neq}_{P,Q,p,q} = \Delta G^{\neq *}_{p,q} + \Delta G^{\neq}_{P,p} + \Delta G^{\neq}_{Q,q} \qquad (11)$$

and

$$k_{P,Q,p,q} = k^*_{p,q}\, \chi_{P,p}\, \chi_{Q,q} \qquad (12)$$

where $k^*_{p,q}$ is the intrinsic catalytic rate constant for the couple of sites p−q. $\chi_{P,p}$ and $\chi_{Q,q}$ are factors dependent upon the occupancy of the external sites and of the internal sites respectively. Eqn (9) transforms into:

$$a_{ij} = [E]_0 \sum_{P,Q} \sum_{p,q} k^*_{p,q}\, (l_P\, \chi_{P,p}\, \psi_P)\, (m_q\, \chi_{Q,q}\, \theta_Q) \qquad (13)$$

If the external sites are different from each other in the absence of substrate and if this is also the case for the internal sites, Eqn (4) cannot be verified

The necessary and sufficient condition to derive Eqn (4) from Eqn (13) is that the internal substrate does not distinguish between internal active sites when none of them is loaded, or that the external substrate does not distinguish between external active sites under the same condition. The following demonstration will concern the case of identical external

sites but also applies *mutatis mutandis*, to the case of identical internal sites.

If the n_A external sites are identical when unloaded, ψ_P and $\chi_{P,p}$ depend on the relative position (X) of the i loaded external sites only. Among the i loaded external sites of a configuration X, some may be identical and some not. Each category of identical loaded sites will be designated by a letter, s for instance, and n_s will be the number of loaded sites in this category.

ψ_X will be the association constant corresponding to

$$(EA_iB_j)_X \rightleftharpoons EB_j + iA\,.$$

With these notations, Eqn (13) becomes:

$$a_{ij} = [E]_0 \left(\sum_X \sum_s \frac{n_s}{n_A} \chi_{X,s}\, \psi_X\right)\left(\sum_Q \sum_{p,q} k^*_{p,q}\, m_q\, \chi_{Q,q}\, \theta_Q\right). \quad (14)$$

This equation is of the same form as Eqn (4). Some particular cases may be of interest.

It can first be supposed that the internal active sites are also identical in the absence of substrate. The corresponding equation is:

$$a_{ij} = [E]_0 \left(\sum_{p,q} k^*_{p,q}\right)\left(\sum_X \sum_s \frac{n_s}{n_A} \chi_{X,s}\, \psi_X\right)\left(\sum_Y \sum_t \frac{n_t}{n_B} \chi_{Y,t}\, \theta_Y\right) \quad (15)$$

in which Y, t, n_t, $\chi_{Y,t}$ and θ_Y have the same significance for the internal sites as X, s, n_s, $\chi_{X,s}$ and ψ_X for the external sites.

This equation also applies to the case of n identical subunits disposed in a symmetrical way, each subunit possessing a couple of sites (one external, one internal) able to perform an exchange. Eqn (15) then takes the form

$$a_{ij} = n\,[E]_0\, k^*\left(\sum_X \sum_s \frac{n_s}{n} \chi_{X,s}\, \psi_X\right)\left(\sum_Y \sum_t \frac{n_t}{n} \chi_{Y,t}\, \theta_Y\right) \quad (16)$$

where k^* is the intrinsic catalytic rate constant per loaded subunit; it does not take into account the subunit interactions.

The existence of one translocator made of identical subunits (probably five at least) is undoubtedly the most attractive interpretation of the observed kinetics. The simplest model that may be proposed for the exchange reaction is the rotation of a subunit around an axis situated in the plane of the membrane made possible by two local configuration changes: one induced on the external side of the subunit by binding of the external substrate and one independently induced on the internal side by the internal-substrate binding.

KINETIC BEHAVIOUR AT LOW OXOGLUTARATE CONCENTRATIONS

At low concentrations of a substrate, the reciprocal of the initial rate of a reaction tends asymptotically to a linear function of the reciprocal of the substrate concentration when the substrate concentration tends to zero [13].

At low external-oxoglutarate concentrations ($[A]_0$), the initial rate may be approximated by:

$$v_0 = \frac{\alpha_1^2\,[A]_0}{\alpha_1 + (\alpha_1\beta_1 - \alpha_2)\,[A]_0} \cdot \frac{\sum_1^{n_B} \gamma_j\,[B]_0^j}{\sum_0^{n_B} \delta_j\,[B]_0^j}\,. \quad (17)$$

Deviation from Eqn (17) may be observed if, at very low concentrations, $[A]_0$ is no longer a good approximation of the free-substrate concentration [23]. At low external-oxoglutarate concentrations, the observed kinetics conform well to these predictions (Fig. 6). Indeed, region 2 may correspond to Eqn (17) and region 1 to the expected deviation.

If some additional assumptions are made, as will be seen in the next paragraph, this deviation can be used to determine the translocator concentration.

Before that, it is of interest to discuss the significance of the kinetic constants (V_{ps} and K_{ps}) calculated from region 2. These constants are:

$$V_{ps} = \frac{\alpha_1^2}{\alpha_1\beta_1 - \alpha_2} \cdot \frac{\sum_1^{n_B} \gamma_j\,[B]_0^j}{\sum_0^{n_B} \delta_j\,[B]_0^j}$$

$$= 172 \pm 17\ \text{pmol} \cdot \text{s}^{-1} \cdot (\text{mg protein})^{-1} \quad (18)$$

and

$$K_{ps} = \frac{\alpha_1}{\alpha_1\beta_1 - \alpha_2} = 3.65 \pm 0.50\ \mu\text{M}\,. \quad (19)$$

As V_{ps} and K_{ps} are positive, one has:

$$\alpha_1\beta_1 > \alpha_2 \quad (20)$$

$$V_{ps} \geqslant \frac{\alpha_1}{\beta_1} \cdot \frac{\sum_1^{n_B} \gamma_j\,[B]_0^j}{\sum_0^{n_B} \delta_j\,[B]_0^j} \quad (21)$$

$$K_{ps} \geqslant \frac{1}{\beta_1}\,. \quad (22)$$

The inequality (20) means that the "cooperativity" may be either nul (if $\alpha_2 = [(n_A - 1)/n_A]\alpha_1\beta_1$) or

positive (if $[(n_A - 1)/n_A]\alpha_1\beta_1 < \alpha_2 < \alpha_1\beta_1$) or negative (if $\alpha_2 < [(n_A - 1)/n_A]\alpha_1\beta_1$.

In the double-reciprocal plot (Fig. 6B), the part of the curve adjacent to region 2 on the high oxoglutarate concentrations side possesses a negative concavity and this implies that α_2 cannot be $> [(n_A - 1)/n_A]\alpha_1\beta_1$. The case of "positive cooperativity" is thus excluded. The case of "nul cooperativity" is improbable. More plausible is the case of "negative cooperativity". Moreover, the product-inhibition pattern [4,5] seems to indicate that α_2 is negligeable compared to $\alpha_1\beta_1$.

Relation (22) provides the information that $\beta_1 \geqslant 2.5 \times 10^5\ M^{-1}$ at 2 °C. The constant β_1 characterises the affinity of oxoglutarate for the free translocator or the sum of them if there are several translocators.

Relation (21) is not of direct interest but may be used in the comparison of V_{ps} with the total apparent maximal rate V:

$$\frac{V}{V_{ps}} = 5 \pm 1 \leqslant \frac{\alpha_{n_A}\,\beta_1}{\alpha_1\,\beta_{n_A}}. \qquad (23)$$

In the plausible case of external sites all identical when unloaded, relation (23) becomes:

$$\frac{\chi_{1A}}{\chi_{n_A A}} \leqslant \frac{n_A}{5}. \qquad (24)$$

One can imagine that the catalytic rate constant is not influenced by binding site interactions assuming that the sites once loaded escape to the interactions with neighbouring sites by a local conformational change analogous to a subunit dissociation. In that case $\chi_{1A} = \chi_{n_A A} = 1$ and n_A must be $\geqslant 5$. This later condition is consistent with the evaluation of the number of external sites performed at the beginning of the discussion.

CONCENTRATION OF THE TRANSLOCATOR

As stated in the preceding section, the initial rate for low external-oxoglutarate concentrations (regions 1 and 2) obeys the following equation

$$v_0 = \frac{V_{ps}\,[A]}{K_{ps} + [A]} \qquad (25)$$

in which [A] is the free-substrate concentration.

If the contribution of one translocator E is predominant at low external-oxoglutarate concentration, and if the substrate bound to the membrane is exclusively bound to the translocator, one also has:

$$[A]_0 = [A] + \sum_{i=1}^{n_A} \sum_{j=0}^{n_B} i\,[EA_iB_j] \approx [A] + \frac{[A]\,[E]_0\,\beta_1^2}{\beta_1 + (\beta_1^2 - 2\,\beta_2)\,[A]}. \qquad (26)$$

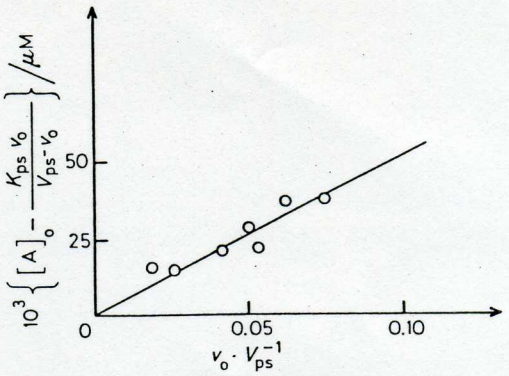

Fig. 11. *Determination of the total concentration of the translocator ($[E]_0$).* Calculated from the data of Fig. 6 by imposing an intersection with the origin to the straight line calculated by the method of least squares

If the fixation of a first substrate molecule to an external site of E exhibits an important negative effect on the remaining external sites *i.e.* if $\beta_1^2 - 2\,\beta_2 \approx \beta_1^2$ and if $\alpha_1\beta_1 - \alpha_2 \approx \alpha_1\beta_1$, the EAB_j complexes are predominant and

$$[A]_0 \approx [A] + \frac{[A]\,[E]_0}{K_{ps} + [A]}. \qquad (27)$$

Eqn (25) gives:

$$[A] = K_{ps}\,\frac{v_0}{V_{ps} - v_0} \qquad (28)$$

and

$$\frac{[A]}{K_{ps} + [A]} = \frac{v_0}{V_{ps}}. \qquad (29)$$

Thus Eqn (27) becomes:

$$[A]_0 - K_{ps}\,\frac{v_0}{V_{ps} - v_0} \approx [E]_0\,\frac{v_0}{V_{ps}}. \qquad (30)$$

This relation may be used to determine $[E]_0$, the total concentration of the translocator. The value obtained for $[E]_0$ is 0.5 μM (Fig. 11) that is 0.5 μmol/g of mitochondrial protein.

From binding experiments, Klingenberg has determined the concentration of the adenine-nucleotide translocator [24]. In rat-heart mitochondria the content of "high-affinity sites" for ADP is 0.3 μmol/g of protein and the content of "low-affinity sites" for ADP is 0.9 μmol/g of protein.

DIFFUSIONAL RESISTANCE

The interpretation proposed so far supposes that the diffusion of the substrates to the appropriate face of the mitochondrial inner membrane is sufficiently rapid to avoid substrate depletion near the active sites. The rate of uptake measured was assumed to depend

only on the substrate concentrations in the bulk solutions and on the characteristics of the active complexes. However, deviations from the Michaelis equation due to diffusional resistance must be considered in the case of immobilized enzymes.

The system studied here seems a perfect candidate for external diffusion effects because (a) the internal and external solutions are unstirred, (b) the external substrate has to diffuse through the mitochondrial outer membrane to reach the surface of the inner membrane. Furthermore, electrostatic effects may be expected as the substrates carry electric charges and as the membrane may be electrically charged as well [25].

In a stationary state, the exchange rate (v) must be equal to the diffusional rate of each substrate:

$$v = h_A [A]_0 - h'_A [A] = h_B [B]_0 - h'_B [B]. \quad (31)$$

$[A]_0$ and $[A]$ are the external substrate concentrations in the bulk external solution and in the environment of the active sites. The concentrations of the internal substrate, $[B]_0$ and $[B]$ are defined in the same way. h_A, h'_A, h_B and h'_B are the transport coefficients implied in the expression of the diffusion rates.

As the exchange rate v is a function of the substrate concentrations near the sites, $[A]$ and $[B]$, Eqn (31) implies that $[A]$ will depend not only on $[A]_0$ but also on $[B]_0$. Similarly $[B]$ will depend on $[B]_0$ and on $[A]_0$. Under such conditions, the rate of exchange as a function of the bulk concentrations $[A]_0$ and $[B]_0$ cannot be a product of two one-substrate functions. As this behaviour is observed, it is concluded that the diffusion of the substrates is not rate-limiting.

CONCLUSIONS

The kinetic study of the oxoglutarate translocator of rat-heart mitochondria carried out at 2 °C leads to the following observations. (a) The oxoglutarate uptake does not conform to a simple Michaelis-Menten kinetics: the saturation curve by external oxoglutarate presents two intermediary-plateau regions in the extended range of concentrations investigated. (b) The initial rate equation is the product of two one-substrate functions.

Important conclusions may be reached from the analysis of this kinetics.

a) If several translocator species participate in the exchange reaction, their individual rate equations have a common part, the external or the internal substrate function. This is not quite plausible and the assumption of only one translocator species is prefered.

b) The content of the oxoglutarate translocator is of the order of 0.5 μmol/g of mitochondrial protein.

c) The translocator is a "double carrier" (*i.e.* with an external and an internal face) [1] which possesses several external binding sites (probably five at least) and one internal site at least. On one side of the membrane at least, the binding sites are identical when none of them is loaded. This is quite compatible with the attractive model of a translocator made of a symmetrical association of identical subunits each of them bearing a couple of binding sites (one internal and one external).

d) The external and the internal substrates bind to the carrier independently of each other.

e) The apparent association constant of the free translocator with one external oxoglutarate molecule is $2.5 \times 10^5\ M^{-1}$.

f) The saturation of the external sites by oxoglutarate has on the exchange rate an alternating cooperative effect which is first negative.

g) Substrate diffusion to the active sites and substrate binding to the translocator are not rate-determining while the exchange step is rate-limiting (rapid-equilibrium kinetics).

The reaction mechanism stated previously from measurements performed in narrower concentration ranges [3–5] is thus extended now to a multi-site translocator. The estimation of the minimum number of binding sites is susceptible to revision since it depends on the still limited concentration ranges investigated and on the possible presence of an unidentified effector. More critical is perhaps the fact that the experiments were carried out at low temperature. Indeed, we do not know if all the conclusions are tenable at physiological temperatures. For instance, subunit associations could be stable at low temperatures only and the complex kinetics described might be without physiological significance. However, subunit associations may be a key feature of anion transport through the mitochondrial inner membranes: a small number of subunit species could perhaps account for the various anion translocators according to the way they are associated between them and with other components of the membrane.

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REFERENCES

1. Klingenberg, M. (1970) *Essays Biochem. 6*, 119–159.
2. Papa, S., Lofrumento, N. E., Quagliariello, E., Meijer, A. J. & Tager, J. M. (1970) *J. Bioenerg. 1*, 287–307.
3. Sluse, F. E., Ranson, M. & Liébecq, C. (1972) *Eur. J. Biochem. 25*, 207–217.
4. Sluse, F. E., Goffart, G. & Liébecq, C. (1973) *Eur. J. Biochem. 32*, 283–291.

5. Sluse, F. E. & Liébecq, C. (1973) *Biochimie (Paris) 55*, 747–754.
6. Palmieri, F., Prezioso, G., Quagliariello, E. & Klingenberg, M. (1971) *Eur. J. Biochem. 22*, 66–74.
7. Sluse, F. E., Sluse-Goffart, C. M., Duyckaerts, C. & Liébecq, C. (1974) *Abstr. Commun. 32nd FEBS Adv. Course.*
8. Sluse, F. E. & Sluse-Goffart, C. M. (1974) *Abstr. Lect. 27th FEBS Adv. Course.*
9. Sluse-Goffart, C. M. & Sluse, F. E. (1974) *Abstr. Lect. 27th FEBS Adv. Course.*
10. Tyler, D. D. & Gonze, J. (1967) *Methods Enzymol. 10*, 75–77.
11. Sluse, F. E., Meijer, A. J. & Tager, J. M. (1971) *FEBS Lett. 18*, 149–153.
12. Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.) pp. 328–332, Academic Press, New York.
13. Beisenherz, G., Boltze, H. J., Bücher, T., Czok, R., Garbade, K. H., Meyer-Arendt, E. & Pfleiderer, G. (1953) *Z. Naturforsch. Teil B, 8*, 555–577.
14. Wong, J. T.-F. & Hanes, C. S. (1962) *Can. J. Biochem. Physiol. 40*, 763–804.
15. Petterson, G. (1970) *Acta Chem. Scand. 24*, 1271–1274.
16. Monod, J., Wyman, J. & Changeux, J. P. (1965) *J. Mol. Biol. 12*, 88–118.
17. Sweeny, J. R. & Fisher, J. R. (1968) *Biochemistry, 7*, 561–565.
18. Teipel, J. & Koshland, D. E., Jr (1969) *Biochemistry, 8*, 4656–4663.
19. Engel, P. C. & Ferdinand, W. (1973) *Biochem. J. 131*, 97–105.
20. Zeylemaker, W. P., Jansen, H., Veeger, C & Slater, E. C. (1971) *Biochim. Biophys. Acta, 242*, 14–22.
21. Kagan, Z. S. & Doroshko, A. I. (1973) *Biochim. Biophys. Acta, 302*, 110–128.
22. Lueck, J. D., Ellison, W. R. & Fromm, H. J. (1973) *FEBS Lett. 30*, 321–324.
23. Reiner, J. M. (1959) *Behavior of Enzyme Systems*, Burgess Publ. Co., Minneapolis.
24. Weidemann, M. J., Erdelt, H. & Klingenberg, M. (1970) *Eur. J. Biochem. 16*, 313–335.
25. Kobayashi, T. & Laidler, K. J. (1974) *Biotechnol. Bioeng. 16*, 77–97.
26. Cantone, B., Emma, V. & Grasso, F. (1968) *Adv. Mass Spectrom. 4*, 599–606.

F. E. Sluse, C. Duyckaerts, and C. Liébecq, Laboratoire de Biochimie et de Physiologie Générale, Institut Supérieur d'Education Physique de l'Université de Liège, 1 Rue des Bonnes-Villes, B-4020 Liège, Belgium

C. M. Sluse-Goffart, Département de Chimie Générale et de Chimie Physique, Institut de Chimie, Université de Liège au Sart-Tilman, B-4000 par Liège 1, Belgium

ERRATUM

Evidence for cooperative effects in the exchange reaction catalysed by the oxoglutarate translocator of rat-heart mitochondria, by F.E. Sluse, C.M. Sluse-Goffart, C. Duyckaerts and C. Liébecq

Page 12, column 1, lines 3-12 : *should be replaced by* In the double-reciprocal plot (Fig.6B), the part of the curve adjacent to region 2 on the high-oxoglutarate-concentration side possesses a negative concavity. This does not imply that α_2 cannot be $> [(n_A - 1)/n_A]\alpha_1\beta_1$. The case of " positive cooperativity " cannot be excluded in this way.