Atypical structure and subunit composition of respiratory complexes in *Euglena gracilis*

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INTRODUCTION

Euglena gracilis, a non-parasitic secondary green alga related to trypanosomes, has a complex mitochondrial oxidative phosphorylation system constituted by atypical respiratory enzymes (complexes I - V). Recently, the analysis of the subunit composition of respiratory complexes by 2D BN/SDS PAGE has shown that at least 41 of the non-canonical subunits reported in trypanosomes are also present in this alga along with 48 classical subunits described in other eukaryotes including green plants[1]. In the present study the complexes I, III, IV and V were further purified and the subunit composition of each were resolved using a 3D BN/SDS/SDS PAGE analysis. Also the quaternary structure from Complexes I and V was explored by EM and the supramolecular association between the complexes using mild detergents was resolved.

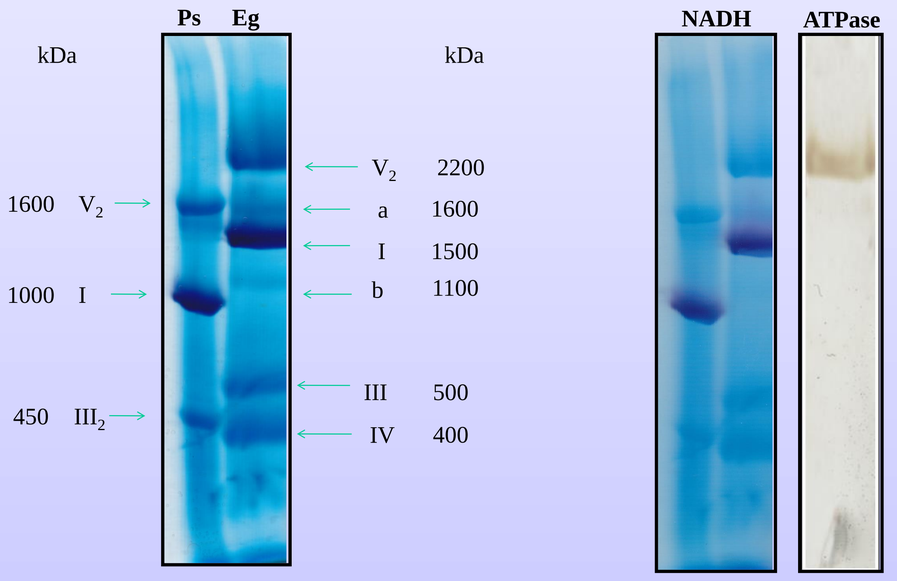

Figure 1. Electrophoretic patterns of mitochondrial complexes from *Euglena gracilis* and *Polytomella* sp. Total mitochondrial protein (1 mg) from *Euglena gracilis* (Eg) and *Polytomella* sp. (Ps) were solubilized in presence of β-dodecyl-n-maltoside and subjected to a BN-PAGE. Estimated molecular masses and identities of protein complexes are indicated. *In-gel* activity stain for Complex I (NADH) and ATP hydrolysis (ATP).

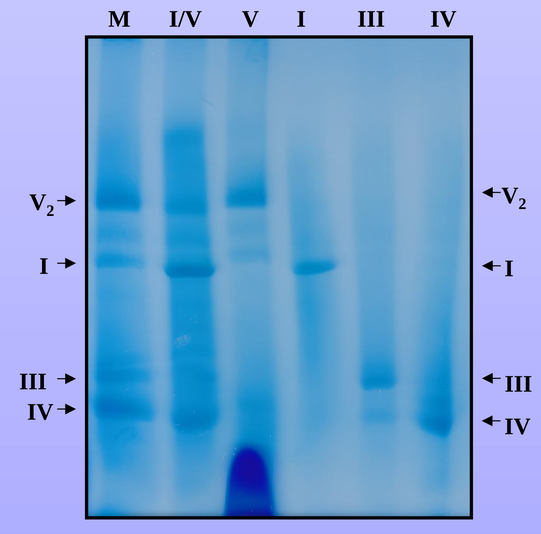

Figure 2. Purified respiratory complexes from *Euglena gracilis*. The mitochondial complexes were solubilized in presence of β-dodecyl-n-maltoside (DDM) and purified by ion exchange/size exclusion chromatography, enriched fractions of each complex were obtained. M: solubilized mitochondria; I/V: Mix of complexes I and V; V: dimeric ATP synthase; I: monomeric NADH:ubiquinone oxidoreductase; III: coenzyme Q:cytochrome c — oxidoreductase; IV: cytocrome c oxidase.

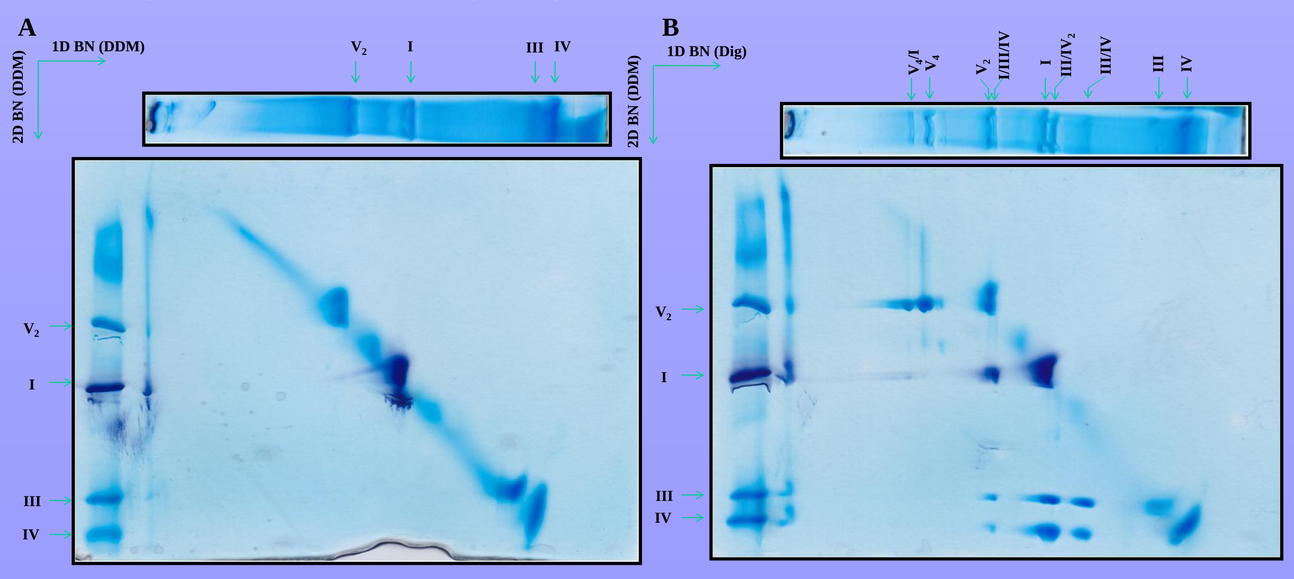

Figure 5. Resolution of respiratory supracomplexes in *Euglena gracilis*. Mitochondria were solubilized in presence of β-dodecyl-n-maltoside (DDM) (A) and Digitonin (Dig) (B), then subjected to a BN-PAGE. The 1D gel was then subjected to 2D BN–PAGE with DDM in the cathode buffer. The 2D gel was subjected to a *in-gel* activity stain for Complex I and stained with Coomassie brilliant blue. The identified supra-complex associations are indicated.

RESULTS

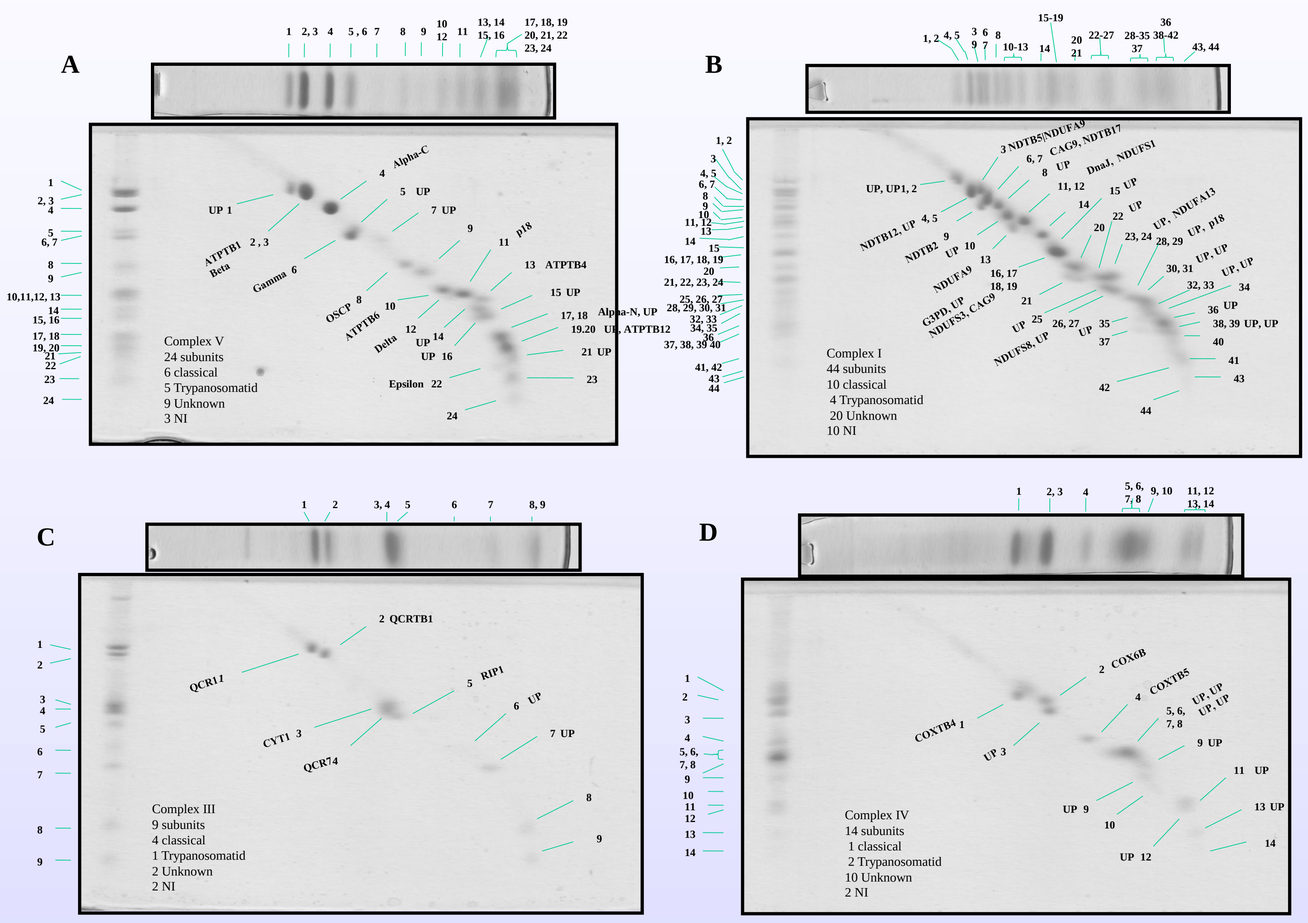

Figure 3. 3D resolution of the polypeptides that constitute the mitochondrial respiratory complexes in *Euglena gracilis*. Each complex was resolved in 1D Blue Native PAGE. The 1D gel was then subjected to a glycine–SDS–PAGE system (12% acrylamide). After, the 2D gel was then subjected to 3D tricine–SDS–PAGE (14% acrylamide) and stained with Coomassie brilliant blue. A) dimeric ATP synthase; B) monomeric NADH:ubiquinone oxidoreductase; C) coenzyme Q:cytochrome c — oxidoreductase; D) cytocrome c oxidase. The identified subunits are indicated by the corresponding labels.

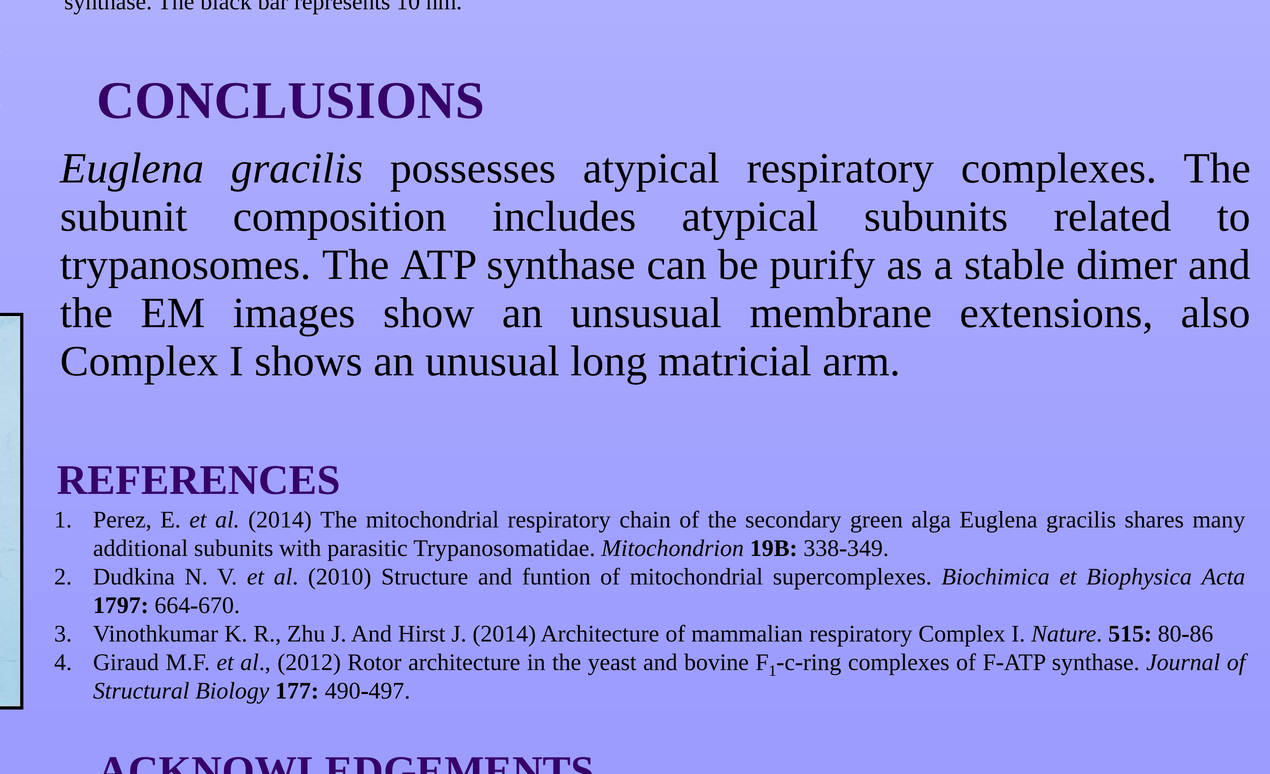

Figure 4. Atypical structure of mitochondrial complexes I and V in *Euglena gracilis* determined by EM. Averaged side views of Complex I from Bovine[2] (A) and *E. gracilis* (B). Overlap of the electronic density map from Bovine complex I (EMD: 2676)[3] over side image from *E. gracilis* (C), the green arrow shows an extra domain in the algal complex. Averaged side views of dimeric ATP synthase from Yeast (D) and *E. gracilis* (E, F). Overlap of the electronic density from the yeast F_1-C_{10} crystal[4] over the Yeast (D) and *E. gracilis* (F) dimer, the green arrows show atypical domains to a mitochondrial ATP synthase. The black bar represents 10 nm.

CONCLUSIONS

Euglena gracilis possesses atypical respiratory complexes. The subunit composition includes atypical subunits related to trypanosomes. The ATP synthase can be purify as a stable dimer and the EM images show an unsusual membrane extensions, also Complex I shows an unusual long matricial arm.

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