Biochimica et Biophysica Acta 1777 (2008) 740–746

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Minireview

Is supercomplex organization of the respiratory chain required for optimal electron transfer activity?

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ARTICLE INFO

Article history:
Received 5 December 2007
Received in revised form 31 March 2008
Accepted 5 April 2008
Available online 11 April 2008

Keywords:
Respiratory chain
Supercomplex
Coenzyme Q
Cytochrome c

ABSTRACT

The supra-molecular assembly of the main respiratory chain enzymatic complexes in the form of "supercomplexes" has been proved by structural and functional experimental evidence. This evidence strongly contrasts the previously accepted Random Diffusion Model stating that the complexes are functionally connected by lateral diffusion of small redox molecules (i.e. Coenzyme Q and cytochrome *c*). This review critically examines the available evidence and provides an analysis of the functional consequences of the intermolecular association of the respiratory complexes pointing out the role of Coenzyme Q and of cytochrome *c* as channeled or as freely diffusing intermediates in the electron transfer activity of their partner enzymes.

1. Introduction

The electron transfer chain consists of four major multi-subunit complexes designated as NADH:CoQ reductase (Complex I), succinate:CoQ reductase (Complex II), ubiquinol:cytochrome *c* reductase (Complex III) and cytochrome *c* oxidase (Complex IV). The best fit unit stoichiometry between complexes in beef heart mitochondria is 1.1 Complex I: 1.3 Complex II: 3 Complex III: 6.7 Complex IV [1]. In addition there are 0.5 ATP synthase (also called Complex V) and 3–5 units of the ADP/ATP translocase (catalyzing the equimolar exchange of ADP and ATP across the inner membrane) for each cytochrome oxidase, and there is one $NADH/NADP^+$ transhydrogenase per Complex I [2]. Indeed, wide differences in cytochromes, Coenzyme Q and pyridine nucleotide contents of mitochondria from different species, as well as from different organs of the same species, have been reported [3–6]; data in the literature indicate that even the molar ratios of the respiratory components vary significantly.

2. Supra-molecular organization of the mitochondrial respiratory chain

2.1. Electrophoretic evidence

The model of a random distribution of mitochondrial complexes (Random Diffusion Model), with electron transfer ensured by collisional interactions of small connecting molecules (Coenzyme Q and cytochrome *c*) [7] has been recently revised when Schägger [1,8,9] found structural evidence by Blue-Native electrophoresis (BN-PAGE) of specific associations in yeast and mammalian mitochondria, and introduced the model of the "respirasome", confirming earlier observations in favor of specific inter-complex interactions (cf. [10] for an extended list of references). Concomitantly, biochemical evidence for homooligomeric ATP synthase competent for ATP hydrolytic activity has been provided [11–13] and recently specific associations of ATP synthase with other OXPHOS components building up "ATP synthasomes" have been proposed [14].

Respiratory supercomplexes were investigated in bovine heart mitochondria: Complex I–III interactions were apparent from the presence of a I_1III_2 supercomplex, which represents about 17% of total Complex I of mitochondria, that was found further assembled into two additional major supercomplexes (respirasomes) comprising different copy numbers of Complex IV ($I_1III_2IV_1$ and $I_1III_2IV_2$ contain 54% and 9% of total Complex I, respectively). Only 14–16% of total Complex I was found in free form in the presence of digitonin [1]; so it seems likely that all Complex I is bound to Complex III in physiological conditions (i.e. in the

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doi:10.1016/j.bbabio.2008.04.007

absence of detergents). Knowing the accurate stoichiometry of oxidative phosphorylation complexes according to Schägger and Pfeiffer [1], the average ratio I:III is 1.1:3, therefore it is plausible that approximately one-third of total Complex III in bovine mitochondria is not bound to monomeric Complex I. The fraction of Complex IV in free form represents >85% of total cytochrome oxidase of mitochondria. Associations of Complex II with other complexes of the OXPHOS system could not be identified under the conditions of BN-PAGE so far.

Based on this procedure, the existence of respirasome-like supercomplexes was also reported for bacteria [15], fungi [16] and higher plant mitochondria [17,18] as well as for rat [19] and human mitochondria [20]. However, the overall electrophoretic band pattern of the digitonin-solubilized mitochondria differs among the species and the tissues and the physiological conditions, showing high molecular weight assemblies that are identified as OXPHOS supercomplexes of different composition ($I_1III_2IV_{0-4}$ and III_2IV_{1-2}). Nevertheless, the I_1III_2 supercomplex proved to be especially stable and highly represented.

Fig. 1 exhibits a typical gel obtained from bovine heart mitochondria (A) and two gels from mitochondria isolated from human cultured cells derived from a papillary thyroid tumor (B) and from an oncocytic thyroid tumor (C). Note in A the confirmed presence in bovine mitochondria of several assemblies comprising complexes I, III and IV in different proportions, with most Complex I in associated form and large amounts of free Complex IV; a Complex V dimer co-migrates with a complex I_1III_2 assembly. The mitochondria from cultured cells are peculiar in showing no detectable associated form of

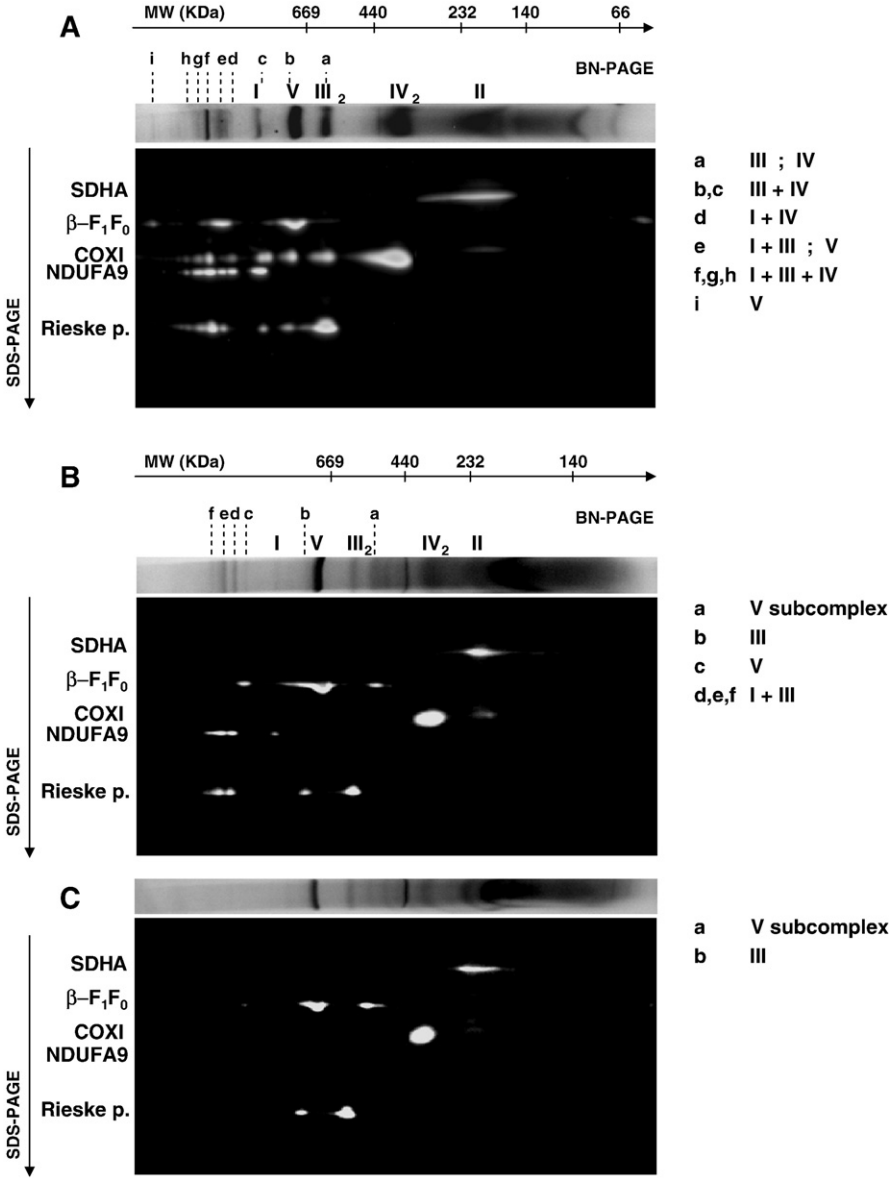

Fig. 1. Two-dimensional separation of OXPHOS supercomplexes of bovine and human mitochondria. Mitochondria were obtained from bovine heart mitochondria (A) and from two human thyroid cells lines: the non-oncocytic cell line TPC-1 derived from human papillary thyroid tumor (B) and the oncocytic carcinoma cell line XTC.UC1 derived from metastasis in the mammary gland of an oncocytic thyroid tumor (C), respectively. (see Ref. [21] for experimental details on the cell lines and preparation of mitochondria). Supercomplexes separation was achieved by 1D Blue-Native electrophoresis followed by 2D SDS-PAGE. Solubilization of membranes was accomplished by treatment of isolated mitochondria with digitonin (digitonin: protein weight ratio 6:1) at 4 °C. The 1D electrophoretic bands (upper lanes) were Coomassie blue stained, whereas the 2D gels were blotted onto nitrocellulose membrane and then exposed to a cocktail of monoclonal antibodies (MitoSciences Inc., Eugene, OR, USA) specific for single subunits of each OXPHOS complex, as follows: NDUFA9 (39 kDa) of Complex I, SDHA (70 kDa) of Complex II, Rieske protein (22 kDa, apparent molecular weight is 30 kDa) of Complex III, COX-I (57 kDa, apparent 45 kDa) of Complex IV, β-subunit (52 kDa) of the F_1F_0-ATPase. Detection of primary antibodies was achieved using a secondary goat anti-mouse IgG_{H+L} antibody labeled with horseradish peroxidase (Molecular Probes, Eugene, OR, USA) and a chemiluminescent technique based on the ECL™ Western Blotting Detection Reagent Kit (Amersham Biosciences, Piscataway, NJ, USA). The molecular mass scale of the 1D electrophoresis was drawn on the basis of standard proteins (HMW calibration kit for Native electrophoresis, Amersham Biosciences). The component complexes in the bands (a through i) are listed on the right of the figure; the list discriminates plausible co-migration (;) from assembly (+) on the basis of the molecular weights.