Review

Mitochondrial respiratory chain super-complex I–III in physiology and pathology

Giorgio Lenaz [a], Alessandra Baracca [a], Giovanna Barbero [a], Christian Bergamini [a], Maria Elena Dalmonte [a], Marianna Del Sole [a], Marco Faccioli [a], Anna Falasca [b], Romana Fato [a], Maria Luisa Genova [a,*], Gianluca Sgarbi [a], Giancarlo Solaini [a]

[a] Dipartimento di Biochimica, Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy
[b] Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche applicate, Università di Parma, V.le Usberti 27/A, 43100 Parma, Italy

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ABSTRACT

Recent investigations by native gel electrophoresis showed the existence of supramolecular associations of the respiratory complexes, confirmed by electron microscopy analysis and single particle image processing. Flux control analysis demonstrated that Complex I and Complex III in mammalian mitochondria kinetically behave as a single unit with control coefficients approaching unity for each component, suggesting the existence of substrate channeling within the super-complex. The formation of this supramolecular unit largely depends on the lipid content and composition of the inner mitochondrial membrane. The function of the super-complexes appears not to be restricted to kinetic advantages in electron transfer: we discuss evidence on their role in the stability and assembly of the individual complexes, particularly Complex I, and in preventing excess oxygen radical formation. There is increasing evidence that disruption of the super-complex organization leads to functional derangements responsible for pathological changes, as we have found in *K-ras*-transformed fibroblasts.

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1. Introduction

The mitochondrial respiratory chain was first described as a sequence of prosthetic groups (flavins and cytochromes) embedded in a protein matrix in the inner mitochondrial membrane, transferring electrons in the order of increasing redox potential [1], and subsequently as the functional sequence of four major multi-subunit complexes, randomly dispersed in the membrane, and designated as NADH–Coenzyme Q reductase (Complex I, C_I), succinate–CoQ reductase (Complex II, C_{II}), ubiquinol–cytochrome c reductase (Complex III, C_{III}) and Cytochrome c oxidase (Complex IV, C_{IV}); in this latter view, the enzyme complexes are connected by two mobile redox-active molecules, i.e. a lipophilic quinone, designated Coenzyme Q (CoQ) or ubiquinone, embedded in the membrane lipid bilayer, and a hydrophilic heme protein, cytochrome c (cyt. c), localized on the external surface of the inner membrane [2].

Some evidence against a random distribution of respiratory complexes was already available by analysis of the early investigations reporting isolation of Complex I–Complex III [3] and Complex II–Complex III units [4,5] and suggesting that such units may be preferentially associated in the native membrane. Further data on the presence

of stable associations of Complex III and IV isolated from some bacteria [6–8] as well as circumstantial evidence on the existence of OXPHOS assemblies [9,10] was usually overlooked by subsequent studies.

Much more recently, new evidence of multi-complex units in yeast and mammalian mitochondria was produced by Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) for the isolation of membrane protein complexes [11–13].

In bovine heart mitochondria (BHM) Complex I–III interactions were apparent from the presence of about 17% of total Complex I arranged in the form of the I_1III_2 super-complex while the majority of Complex I (67%) was found assembled into super-complexes (respirasomes) comprising dimeric Complex III and different copy numbers of Complex IV.

Only 14–16% of total Complex I was found in free form in the presence of digitonin [13], so it seems likely that all Complex I is bound to Complex III in physiological conditions. Moreover, since the two complexes occur in a 1:3 average stoichiometry in mammalian mitochondria [13], it is plausible that approximately one-third of total Complex III is not bound to monomeric Complex I. Two-dimensional gels of mitochondria from beef heart, rat liver and potato tuber (Fig. 1) show the presence of respiratory super-complexes in both animal and plant mitochondria.

2. Molecular structure of super-complex I–III

A well defined architecture was observed for all super-complexes that were investigated, supporting the idea of highly ordered associations of the respiratory super-complexes and not of random aggregates, thus
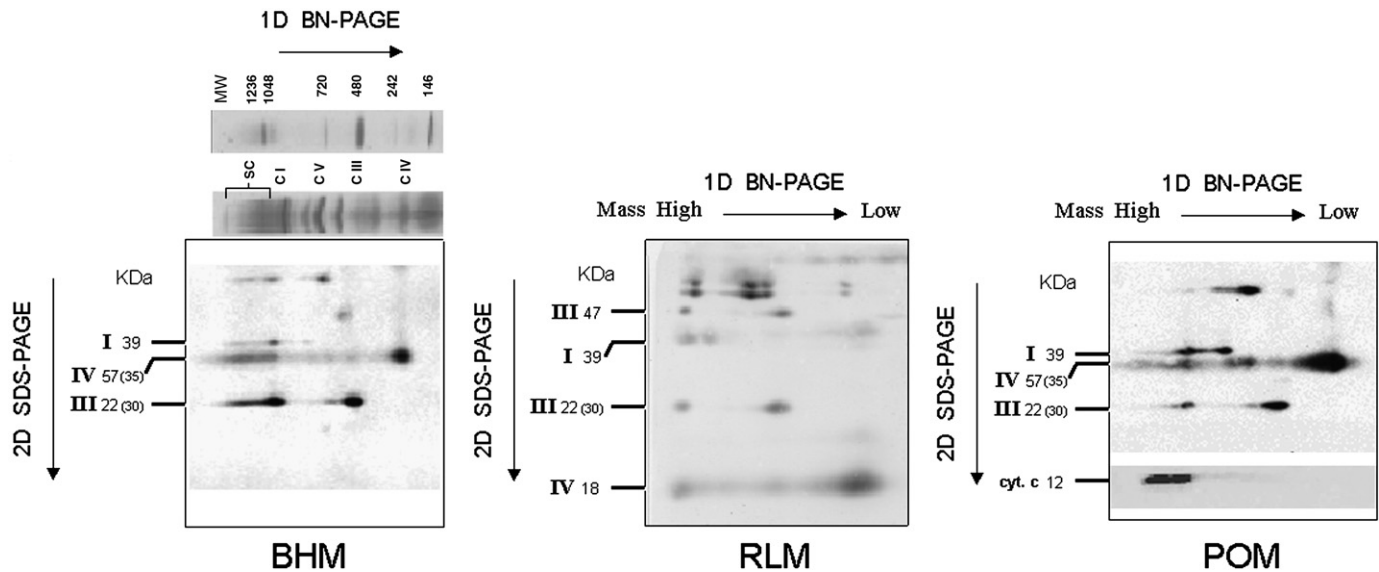
Fig. 1. Two-dimensional electrophoretic separation of OXPHOS supercomplexes. Digitonin-solubilized mitochondrial proteins from beef heart (left), rat liver (center) and potato tuber (right) were previously resolved by 1D BN-PAGE (shown only for BHM, upper lane) and then loaded on 2D SDS-PAGE gel. After electrophoresis, the whole gel was transferred onto nitrocellulose and submitted to immunoblotting with monoclonal antibodies specific for single subunits of the OXPHOS complexes (MitoSciences Inc., Eugene, OR, USA) and to chemiluminescent detection (ECL™ Detection Kit, GE Healthcare Europe GmbH), as highlighted in the picture: NDUFA9 (39 kDa) of Complex I, Rieske protein (22 kDa, apparent molecular weight is 30 kDa) and Core I (47 kDa) of Complex III, COX-I (57 kDa, apparent molecular weight is 35 kDa) and COX-IV (18 kDa) of Complex IV, cytochrome *c* (12 kDa). The Coomassie blue-stained bands in the 1D lane of BHM were indentified on the basis of the molecular mass scale, labeled by numbers 146–1236 (Native Mark™, LC0725, Invitrogen), and of their protein subunit composition as assessed by the corresponding spots in the immunoblot. SC, high molecular weight assemblies, super-complexes.

discarding most doubts on artificial interactions. From the limited data available to date, it appears that such interactions may be species- or kingdom-specific [14].

Three-dimensional models of the I_1III_2 super-complex isolated from plant and mammalian mitochondria [15–17] were generated by comparison of the 2D projection map of the super-complex, as revealed by Electron Microscopy analysis (EM) and single particle image processing, with known EM and X-ray structures of Complex I and Complex III. The specific orientation observed for the two respiratory-chain complexes indicates an interaction within the plane of the membrane whereas the matrix-exposed protein domains are in one another's vicinity but probably do not (strongly) interact. However, the apparent interaction between the two complexes may be different in *Arabidopsis* [15] and in a bovine super-complex consisting of Complex I, dimeric Complex III and Complex IV ($I_1III_2IV_1$) where positions and orientations of all the individual complexes were determined in detail [17]; in the plant structure Complex III attaches to the end of the membrane arm of Complex I, whereas Schäfer et al. [17,18] suggested that the surface interaction is more extensive in the bovine super-complex showing Complex III and IV respectively associated with the middle and the terminal portion of the membrane arm of Complex I, and in contact with each other. On the basis of the structural information gained from the 3D map [17], the putative mobile electron carrier (CoQ or cytochrome *c*) binding site of each complex is facing the corresponding binding site of the succeeding complex in the respiratory chain, supporting the notion of a more efficient electron transfer through the super-complex due to the short diffusion distances of substrates. Two-dimensional projection structures were also reported for super-complexes III–IV from yeast [19].

It has been also proposed [19,20] that the OXPHOS complexes may be further organized in (row-like) mega-complexes or respiratory strings composed by super-complexes as building blocks, which seem to be important for the morphology of the inner mitochondrial membrane.

3. Role of super-complexes in substrate channeling

A recent study [21] confirmed the presence of different forms of super-complexes in mouse liver mitochondria, some also containing Complex II and ATP synthase. Respiration was measured with a Clark electrode in protein bands of high molecular weight excised from BN-PAGE stripes, showing full respiratory activity from either NADH or succinate, which was sensitive to the specific respiratory inhibitors of all involved complexes. Interestingly, excised bands of individual complexes were unable to support integrated electron transfer when mixed together.

The demonstration that super-complexes are capable of respiration is not however sufficient to demonstrate if they are a necessary prerequisite for respiratory activity and if they provide a kinetic advantage over electron transfer based on random collisions. Our laboratory has produced evidence for a functional role of the I–III super-complex in substrate channeling in the CoQ region [22–24].

Metabolic flux control analysis allows a quantitative measurement of the control exerted on a composite pathway by its individual enzymatic steps [25,26]. It is assumed by the theory of this analysis that the control would be differently exerted by one or more steps in the pathway, and that the sum of the control coefficients of all steps would approach 1, and not overcome unity, if the individual steps consist of separate enzymes joined by the diffusion of common intermediates.

The situation would be different, however, in enzymatic super-complexes where the interactions between active sites are fixed and substrates and intermediates are channeled from one defined site to another one without leaving the protein environment; in such a super-complex, the metabolic pathway would behave as a single enzyme unit, and inhibition of any one of the enzyme components would elicit the same flux control. In a system in which the respiratory chain is totally dissociated from other components of the OXPHOS apparatus (ATP synthase, membrane potential, and carriers), such as open non-phosphorylating submitochondrial particles, the existence of a super-complex would elicit a flux control coefficient near unity at any of the respiratory complexes, and the sum of all apparent coefficients would be times above 1, depending on the number of addends [27].

The flux control coefficients of the respiratory complexes were investigated in bovine heart mitochondria and submitochondrial particles devoid of substrate permeability barriers by performing