Abstract for Moscow Colloquium

1. Youssef Hatefi, Yves M. Galante, and Reinhold Kiehl

2. Organization of the mitochondrial respiratory chain

3. In mitochondria, the machinery for oxidative phosphorylation is located in the inner membrane in the form of five enzyme complexes. Complexes I, II, III and IV (plus cytochrome *c* and ubiquinone) make up the respiratory chain, while Complex V is responsible for ATP synthesis and hydrolysis. Complex I catalyzes electron transfer from NAD(P)H to ubiquinone. It is composed of 16-18 polypeptides, FMN, and 5 iron-sulfur centers. A soluble iron-sulfur flavoprotein with a mol. wt. of 75,000 ± 6% (three subunits) is the primary NAD(P)H dehydrogenase. Complex II catalyzes electron transfer from succinate to ubiquinone. It is composed of 4 polypeptides. Two polypeptides belong to succinate dehydrogenase, which is an iron-sulfur flavoprotein of mol. wt. 97,000 ± 4%. In addition to succinate dehydrogenase, Complex II contains a low potential cytochrome *b* whose reduced form at 77°K exhibits absorption maxima at 557.5, 550, 531, 523 and 422 nm. Complex III catalyzes electron transfer from reduced ubiquinone to cytochrome *c*. It is composed of 7-8 polypeptides. The identified electron carriers of Complex III are two *b*-type cytochromes, cytochrome c_1 an iron-sulfur protein, and a component with cytochrome *b*-like electron transfer properties and an absorption peak when reduced at 77°K at 558 nm. Complex IV catalyzes electron transfer from ferrocytochrome *c* to molecular oxygen. It is composed of 7 polypeptides, and contains hemes *a*, a_3 and two atoms of copper per mole. In addition to the above, each enzyme complex also contains

20-30% phospholipids by dry weight, and Complexes I and III contain bound ubiquinone. Complexes I, II, III and IV have been physically and functionally recombined in the presence of cytochrome c (and added ubiquinone where necessary) to reconstitute the entire electron transport system ($\frac{I}{II}$ > III-c-IV) or segments thereof (I-III, II-III, $\frac{I}{II}$ > III, I-III-c-IV, II-III-c-IV) with the expected overall activities of the participating complexes. In mitochondria, Complexes I, III and IV contain the energy coupling sites 1, 2 and 3, respectively. The oxidation energy captured by these complexes is transferred to Complex V for ATP synthesis. Recently, the latter complex has been obtained in a highly purified form in our laboratory. It is composed of 11 polypeptides, of which 10 have been identified as follows: the five F_1 subunits, the oligomycin-sensitivity-conferring protein, the dicyclohexylcarbodiimide-binding protein, the uncoupler-binding protein, coupling factor F_6, and provisionally coupling factor B. Complexes I to V are present in the mitochondrial inner membrane in the approximate ratio of 1 I:2 II: 3 III: 7 IV: 3 V, and together they make up about 50% of the inner membrane protein.

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COMPOSITION OF THE MITOCHONDRIAL UNCOUPLER- AND OLIGOMYCIN-SENSITIVE ATP-Pi EXCHANGE COMPLEX (COMPLEX V). Y. M. Galante,* R. Kiehl,* and S. Y. Wong* (SPON: J. Spizizen). Dept. of Biochem., Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Removal of minor Complex V contaminants by chromatography on Agarose A5M in the presence of cholate has yielded a highly purified preparation with 11 polypeptides and ATPase and ATP-Pi exchange activities of 8-10 x 10^5 and 110-120 (or 210-230 when corrected for ATP hydrolysis during exchange) nanomol/min/mg protein, respectively. As isolated, the preparation contains only about 0.1 µmol phospholipids per mg protein, and requires added phospholipids for activity. Thus, its ATPase activity is stimulated 15-20 fold upon addition of sonicated phospholipids (∿20 µg phospholipid phosphorus per mg protein) directly to a reaction mixture containing enzyme and substrate. Purification of Complex V involves also the loss of some F_1-ATPase, which should be added back to attain maximal ATP-Pi exchange activity. Ten polypeptides of Complex V have been identified by comparison of M_r values and coelectrophoresis with pure, authentic preparations, as well as by affinity labeling with appropriate radioactive reagents. They are: the 5 subunits of F_1, the oligomycin-sensitivity-conferring protein, the dicyclohexylcarbodiimide-binding protein, the uncoupler-binding protein, coupling factor F_6, and provisionally coupling factor B (M_r 11-12 x 10^5). In collaboration with Y. Hatefi (This work was supported by USPHS grant AM08126 and NSF grant PCM 76-01378 to Y. H.)

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