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DEFICIENCY OF SUBUNITS IN HEART MITOCHONDRIAL NADH-UBIQUINONE OXIDOREDUCTASE OF A PATIENT WITH MITOCHONDRIAL ENCEPHALOMYOPATHY AND CARDIOMYOPATHY

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Summary: The heart mitochondria isolated from a patient with hypertrophic cardiomyopathy associated with mitochondrial encephalomyopathy were analyzed by immunoblotting using specific antibody against each of the purified mitochondrial energy transducing complexes from beef heart. Subunits of NADH-ubiquinone oxidoreductase (Complex I) were markedly decreased and those of cytochrome c oxidase (Complex IV) were decreased to some extent, but the deficiency of any of these subunits was only partial. On the other hand, the contents of subunits of ubiquinol-cytochrome c oxidoreductase (Complex III) were normal. These results suggest that the decreased levels of some of the Complex I subunits might be the primary cause of disorder in this patient.

The mitochondrial respiratory chain is a machinery that transduces the redox energy into a driving force for ATP synthesis (1). Defects in the respiratory chain result in dysfunction of the affected tissue, giving rise to clinically heterogeneous disorders, such as mitochondrial myopathy, cardiomyopathy, encephalomyopathy, or mitochondrial cytopathy (2-4). The classification of these disorders has been attempted on the basis of biochemical analyses. However, either enzymic activity measurement or spectral analysis alone has not lead to the understanding of the relationship between the clinical and biochemical entities. In order to elucidate the molecular basis of these disorders, we have developed immunochemical methods to analyze the mitochondrial polypeptides from a small amount of tissues, and have started to identify the molecular defects in the cases of mitochondrial myopathies (5, 6).

Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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In the present communication, we report the analysis of the subunits of NADH-ubiquinone oxidoreductase in heart mitochondria isolated from a patient who died from congestive heart failure due to cardiomyopathy associated with mitochodrial encephalomyopathy.

Materials and Methods

Patient: A 16-year-old girl with a mitochondrial encephalomyopathy died of congestive heart failure. The clinical features of this patient and the histological and biochemical studies on her postmortem heart muscle will be reported elsewhere.

Preparation of Mitochondria: Heart muscle was obtained at autopsy 2 h after death. Mitochondria were isolated from postomortem human control and from patient heart muscles as reported (7).

Activity Measurements: Activities of NADH-ferricyanide reductase (8), NADH-coenzyme Q_1 reductase (9), succinate-cytochrome c reductase (10), and cytochrome c oxidase (11) were determined as described.

Purification of Mitochondrial Electron-Transfer Complexes: NADH-ubiquinone oxidoreductase (9), ubiquinol-cytochrome c oxidoreductase (12), cytochrome c oxidase (13), and F_1-ATPase (14) were isolated from beef heart mitochondria as described.

Immunization: Antibodies against the mitochondrial enzymes were raised in rabbits by intradermal injections of 0.5 - 1 mg of enzyme protein in 1 ml of saline emulsified with 1 ml of Freund's complete adjuvant. Booster injections of the same mixture were administered 3, 5, and 7 weeks later. One week after the last injection, blood was withdrawn from the carotid.

Electrophoresis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 9.38-18.75% acrylamide-gradient gels (13.5 x 12 x 0.1 cm) essentially as described previously (6).

Electroblotting: Mitochondrial proteins separated by SDS-PAGE were electrophoretically transferred to Durapore GVHP filter (Millipore, Bedfore, MA) essentially according to the method of Towbin et al. (15) with addition of 0.1% SDS to the electrode buffer. The binding of antibodies to the subunits of the mitochondrial enzymes on the filter was detected by the peroxidase-anti-peroxidase method as described previously (6).

Results and Discussion

Fig. 1 shows the immunoblots using anti-Complex I antibody of purified Complex I (lane 1), of beef heart mitochondria (lane 2), of normal human heart mitochondria from two different individuals (lanes 3 and 4, and lanes 5 and 6), and of patient heart mitochondria (lanes 7 and 8). Most of the Complex I subunits were decreased in the patient mitochondria, but the degree of deficiency was severe in subunits 1 and 10 as indicated by arrows. The specific activity on protein basis of NADH-ferricyanide reductase in the patient mitochondria was 56% of the control value, whereas the specific activity of NADH-coenzyme Q_1 reductase was 19% of the control. The former

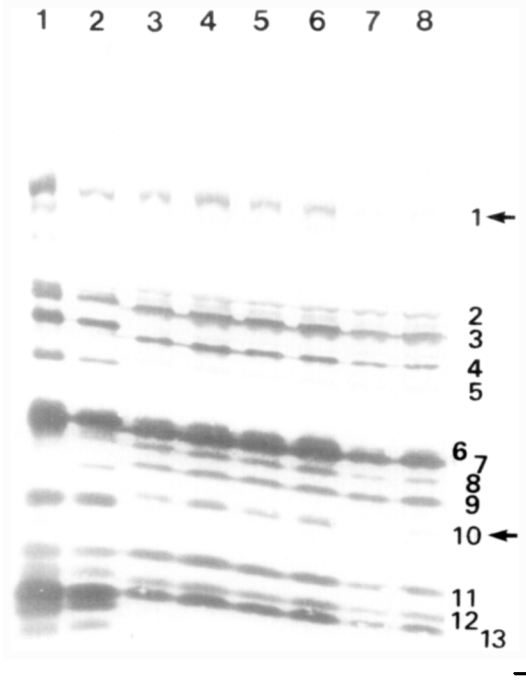

Fig. 1. Immunochemical detection of subunits in NADH-ubiquinone oxidoreductase (Complex I). Immunoblotting using anti-Complex I antibody was conducted as described under Materials and Methods. Lane 1, 2 µg of purified Complex I; lane 2, 25 µg of beef heart mitochondria; lanes 3 and 4, normal human heart mitochondria; lanes 5 and 6, mitochondria from another normal human heart; lanes 7 and 8, patient heart mitochondria. The amounts of protein applied are 37.5 µg in lanes 3, 5, and 7, and 75 µg in lanes 4, 6, and 8. The subunits are numbered tentatively on the right side of the figure.

activity requires only the three-subunit flavoprotein fragment of Complex I, while the latter the whole structure of Complex I. The difference between these activities in the patient mitochondria may be due to the differential deficiency of subunits in Complex I.

The immunoblotting with antibody against ubiquinol-cytochrome c oxidoreductase (Complex III) demonstrated that the contents of the subunits in Complex III in the patient heart mitochondria were comparable to those of the subunits in the control mitochondria (Fig. 2A). The contents of subunits α and β in mitochondrial F_1-ATPase were also normal in the patient mitochondria (data not shown). In contrast, the contents of the subunits in Complex IV were somewhat decreased in the patient mitochondria as compared to those in the control mitochondria (Fig. 2B). These findings are consistent with the

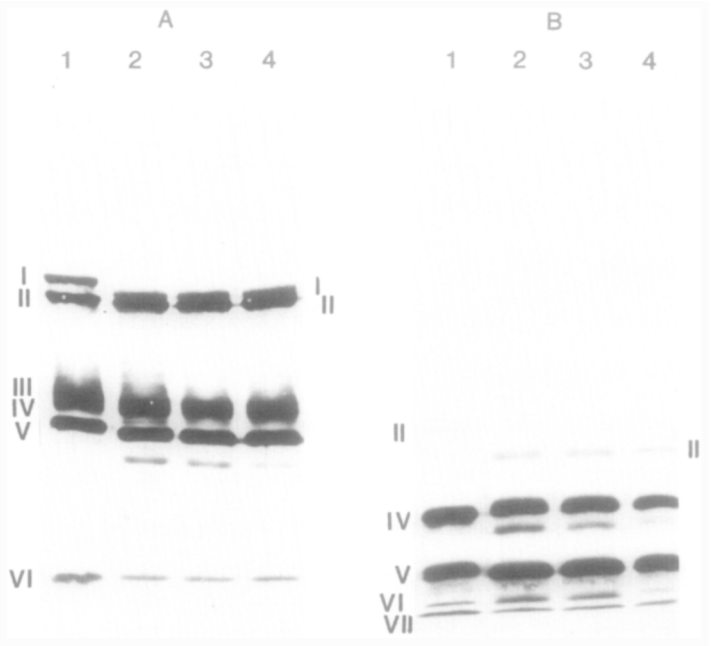

Fig. 2. Immunochemical detection of subunits in ubiquinol-cytochrome *c* oxidoreductase (A) and in cytochrome *c* oxidase (B) in beef heart, normal human heart, and patient heart mitochondria. Mitochondrial proteins were separated by SDS-PAGE, transferred to Durapore filter, and incubated with the specific antibody against each enzyme. The antibody binding was detected by the peroxidase-anti-peroxidase method. Lane 1, 25 µg of beef heart mitochondria; lanes 2 and 3, 75 µg of normal human heart mitochondria; lane 4, 75 µg of patient heart mitochondria. Roman numerals indicate the subunit numbers. In A, I and II, core proteins; III, cytochrome *b*; IV, cytochrome c_1; V, Rieske iron-sulfur protein; and VI, ubiquinone-binding protein. Note that the electrophoretic mobilities of subunit I of ubiquinol-cytochrome *c* oxidoreductase and of subunit II of cytochrome *c* oxidase are different between beef heart mitochondria and human heart mitochondria.

observations that succinate-cytochrome *c* reductase activity was normal while cytochrome *c* oxidase activity was decreased to 68% of the control value in the patient mitochondria.

The present analysis on the mitochondria from a patient with encephalomyopathy and cardiomyopathy has revealed partial deficiency of subunits in Complex I. The decrease in the enzymic activity of Complex I was not due to the complete defect of a single subunit, but due to a general deficiency of several subunits. Furthermore, no abnormalities in the electrophoretic mobilities of the immunochemically detectable subunits were observed. These results suggest that structural genes for these subunits are normal.

We previously found that in a patient with muscle-type Complex IV deficiency (5), subunit II in Complex IV was hardly detectable and the levels of other subunits of this complex were markedly reduced. The study on Chinese hamster mutant cells demonstrated that impaired synthesis of mitochondrially-encoded subunits, I, II, and III, leads to decreases in the levels of most of the subunits encoded by the nuclear genes (16). In the cases of Complex III (17) and Complex V (18), mitochondrially-encoded subunits were also shown to be essential for proper assembly of the complexes. Therefore, it is reasonable to consider that in the muscle-type Complex IV deficiency, the absence of subunits II impaired the assembly of other subunits. Recently, several subunits of Complex I have been demonstrated to be encoded by the mitochondrial genes (19), so it seems likely that the deficiency of Complex I subunits observed in the present case is likewise due to impaired synthesis of its mitochondrially-encoded subunits.

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