PRELIMINARY NOTES

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Target size of components in oxidative phosphorylation. Studies with a linear accelerator

The actual size and arrangement of the components of oxidative phosphorylation in the mitochondrial membrane *in situ* is still largely unknown, despite several theories on the presence of morphological and functional units in the membrane such as "respiratory assembly" (mol. wt. $1.4 \cdot 10^6$)[1] or "elementary particle" (mol. wt. $1.3 \cdot 10^6$)[2], which are composed of stoichiometric amounts of electron-transport carriers. On the other hand, particles of 87 Å diameter lining the inner mitochondrial membrane were discovered by FERNÁNDEZ-MORÁN[3]. These were initially suggested to represent elementary particles[2,3], but were then found to be identical to coupling factor 1 (F_1), *i.e.* mitochondrial ATPase (ATP phosphohydrase, EC 3.6.1.4)[4,5]. However, the inner membrane observed after fixation is too thin to accommodate these latter units, whereas one observed without fixation might be deformed by artifacts[6].

The aim of this work was to measure the size of these components *in situ* by irradiating mitochondria with high-energy electrons and applying the target theory[7] to deduce the molecular weight of the various mitochondrial enzymes.

A suspension of fresh rat-liver mitochondria (protein: 20 mg/ml in 0.25 M sucrose) was prepared as described[8], and 1 ml of the suspension was pipetted into a glass dish (diameter 2.2 cm, depth 0.3 cm) which was placed into the field of irradiation. In some cases the mitochondrial suspension was lyophilized and an amount equivalent to 1 ml (0.107 g) of lyophilized powder was put into the dish. The dishes were covered with parafilm to improve dose distribution. The temperature was kept constant at 0° for unfrozen samples or at −78.5° for frozen or lyophilized ones. 16–20 dishes were then irradiated with a 6-MeV electron beam emitted uniformly from a linear accelerator (Nippon Electric Co., Tokyo, Model NELAC 1006) at a dose rate of about 1 Mrad/min, until the total dose reached the desired value. The dosimeter was calibrated with $FeSO_4$ solution as described elsewhere[9]. After the irradiation, samples were assayed for enzyme activity as indicated in Table I or for electron-transport activities as described[10].

The molecular weight of each enzyme was calculated from this statistical ultramicrometry with the equation below. As the density of enzymes is unknown, while the radiation effect is proportional to density, the unit of the target size in this report is g/mole or g/target of N_{Avogadro} (*i.e.* molecular or target weight): it is not possible to express the size as cm^3/mole. There are two conditions applying to this equation: (1) activity diminishes exponentially with increased dose irrespective of dose rate (single-hit irreversible inactivation); (2) indirect effects, *i.e.* inactivation by radicals produced from irradiated H_2O, are negligible (direct-hit inactivation). The equation is:

$$D_{37} \times \text{molecular weight} = 0.72 \cdot 10^{12}$$

D_{37} (expressed in rad) is the dose that diminishes enzyme activity to 37 % (e^{-1}) of that of the unirradiated sample[7].

The results are summarized in Table I. The target size of mitochondrial ATPase was close to the molecular weight of F_1 prepared from beef heart[11]: 284000. It was necessary to use an ATP-regenerating system[19] and to disrupt mitochondria hypotonically to assay ATPase. The indirect effect mentioned above is apparently negligible, since lyophilization, change in temperature or addition of glutathione did not affect the results. Essentially the same results were obtained when beef heart and kidney mitochondria were irradiated. F_1 purified from rat liver was cold-labile, like F_1 from beef heart[12]. Oligomycin sensitivity was not lost until 5 Mrad.

TABLE I

TARGET SIZE AND 50% INACTIVATION DOSE OF ENZYMIC ACTIVITIES IN MITOCHONDRIA *in situ*

Enzymic activity or property	*Assay methods (ref. No.)*	*Conditions of irradiation*	*50% inactivation dose (Mrad)*	*Computed target size (mol. wt.)*
ATPase	(11)*	unfrozen	1.7–2.2	260 000 ± 43 000
ATPase	(11)*	frozen	1.8–2.0	290 000 ± 37 000
ATPase	(11)*	lyophilized	1.8–2.1	270 000 ± 32 000
NADH oxidase	(10)	frozen	5.4–8.3	77 000 ± 24 000
NADH ferricyanide reductase	(10)	frozen	9.3–14.1	40 000 ± 11 000
Succinate oxidase	(10)	frozen	5.7–7.9	74 000 ± 18 000
Cytochrome oxidase	**	frozen	7.1–12.5	50 000 ± 16 000
Cytochrome oxidase (purified)	**	frozen	7.0–7.8	67 000 ± 8 000
Respiratory control ratio*** (succinate)	(13)	unfrozen	0.1–0.2	—
$^{32}P_i$–ATP exchange reaction	(14)	unfrozen	0.2–0.3	—
^{45}Ca accumulation (succinate or ATP)	(15)	unfrozen	0.3–0.5	—
P:O ratio (succinate)	(16)	unfrozen	0.4–0.6	—
Swelling contraction	(17) §	unfrozen	0.6–0.8	—

* After irradiation, mitochondria were disrupted with 9 vol. of 0.02 M Tris–HCl (pH 7.4) and aliquots of 0.1 ml (0.2 mg protein) were assayed for ATPase by described methods except at 37°.

** The activity was assayed with an oxygen electrode in a closed 2.5-ml reaction chamber containing 1.25 μatoms O, 10 μmoles sodium ascorbate (pH 7.4), 1 mg cytochrome *c*, and 50 μmoles Tris–HCl (pH 7.4), at 24°.

*** Ratio of respiratory rate after addition of ADP (State 3) to that before its addition (State 4).

§ The mitochondrial swelling was induced by 0.01 M P_i (pH 7.4), and then reversed by the addition of Mg-ATP and albumin as described, and the absorbance at 520 mμ was recorded automatically.

Electron-transport activity was much more resistant to irradiation than F_1 and titration up to 30 Mrad was necessary. Owing to the coagulation of the mitochondrial suspension irradiated with high doses, probably caused by the increased denatured protein, the results may be inaccurate.

Those activities that depend on the conservation of a high-energy state showed high sensitivity to irradiation, and the inactivation was not exponential. Freezing of the samples was avoided to prevent loss of respiratory control and energy-linked functions. The 50% inactivation doses of energy-linked processes, of ATPase and of electron-transport reactions are compared in Table I.

Electron micrographs revealed the intact outline of whole mitochondria up to 10 Mrad, but inner membrane particles[3] were lost or deformed at 5 Mrad.

In conclusion, F_1 was inactivated by a single direct hit; its dissociation into 11 subunits[11] *in situ* is thus improbable. On the other hand, the "elementary particle" of electron transport may not exist as a separate morphological entity, since its probable molecular weight[2] would be inconsistent with the small target size of the components of electron transport. Recently, the "elementary particle" concept was reconsidered by GREEN AND TZAGOLOFF[18]. Considering the high sensitivity of energy-conserving activities to irradiation, any structure necessary for these functions should be much larger than the hypothetical "elementary particle".

Determination of the shape and arrangement of these enzyme systems in oriented mitochondrial membranes with heavy particles emitted from the cyclotron is now in progress.

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