Biochimica et Biophysica Acta, 460 (1977) 375—378

BBA Report

BBA 41294

UNCOUPLER-INDUCED CHANGES IN MITOCHONDRIAL STRUCTURE DETECTED BY SMALL-ANGLE X-RAY SCATTERING

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(Received December 22nd, 1976)

Summary

Small-angle X-ray scattering data suggest that major but reversible rearrangements of mitochondrial inner membrane structure are induced by uncouplers. Low levels of 2,4-dinitrophenol (10 μM) cause a perceptible wide-angle shift of the 20 mrad X-ray scattering maximum characteristic of intact liver mitochondria. Higher dinitrophenol concentrations (> 25 μM) reduce this scattering maximum to one-third its initial intensity. In terms of mitochondrial function, the former scattering change appears to correlate with the uncoupling of oxidative phosphorylation while the latter occurs in the course of dinitrophenol stimulation of mitochondrial ATPase activity.

The small-angle scattering of Cu K_α X-rays from dense suspensions of rat liver mitochondria has been described in a previous report [1]. The X-ray scattering from mitochondria in the condensed state is intense and monotonically decreasing in the region 0.5—5 mrad, with a diffuse maximum centered near 20 mrad. The continuous scattering at very small angle appears to arise predominantly from vectors in the order of thousands of Ångstroms, associated with inner membrane shape. Deconvolution of the cristae by low-scale swelling causes a 90% decrease in the scattered intensity in this region. The scattering maximum at larger angle, on the other hand, has been shown to arise from smaller (80—100 Å) vectors associated with the mitochondrial cristae. While

Abbreviations: DNP, 2,4-dinitrophenol; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone.

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the latter vectors are as yet unidentified, the intensity of the 20 mrad maximum suggests that they constitute a prominent structural feature of the cristae. Significant changes in the X-ray scattering in this region should, therefore, be indicative of fundamental reorganizations of the inner mitochondrial membrane.

An example is the reversible change in the X-ray scattering from rat liver mitochondria induced by 2,4-dinitrophenol (DNP). As illustrated in Fig. 1, preincubation of the mitochondria in DNP diminishes by two-thirds the integrated intensity of the diffuse maximum at 85 Å, while a single wash largely restores both the scattering maximum and respiratory control of the mitochondria. (Respiratory control ratios during succinate oxidation: 4.6

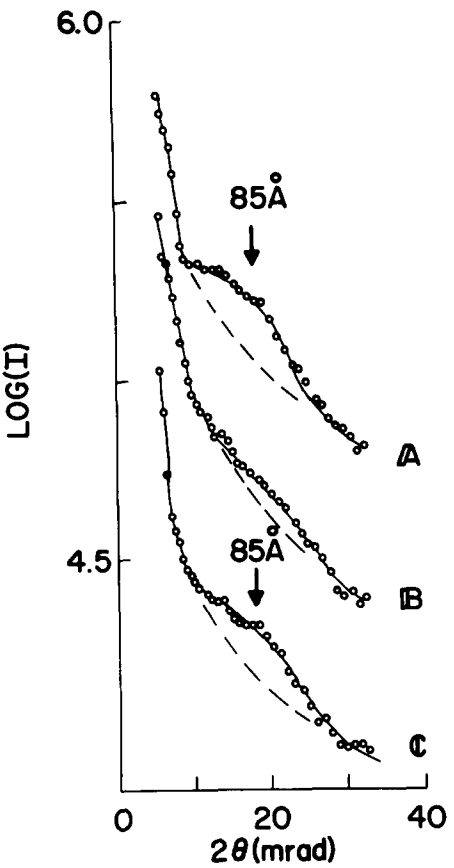

Fig. 1. Effect of DNP on small-angle X-ray scattering from mitochondria, (A) Suspension of rat liver mitochondria (approx. 150 mg protein/ml) prepared by 10 min centrifugation at 12 000 × g in 0.3 osM mannitol-sucrose + 0.1 mM EGTA (MSE medium) at 4°C. (B) Mitochondria from the same preparation pelleted in MSE + 100 μM DNP. (C) The same DNP-treated mitochondria after resuspension in 100 vols. MSE and recentrifugation. Mitochondria prepared by the procedure of Parsons et al. [2] and scanned with a Siemens X-ray diffractometer, including a Kratky slit-collimated camera and proportional counter detection system. Scattering data were corrected for slit geometry according to Lin et al. [3]. Peak positions (given in these figures in terms of $1/r^* = \lambda/2\sin\theta$, where λ is the wavelength of the Cu K_α X-rays, 1.54 Å, and 2θ is the scattering angle) are established by subtracting the smoothly varying components of the curves from the maxima and determining the centers of the resulting approximately Gaussian curves. Successive curves in this figure are shifted down by 0.5 log units.

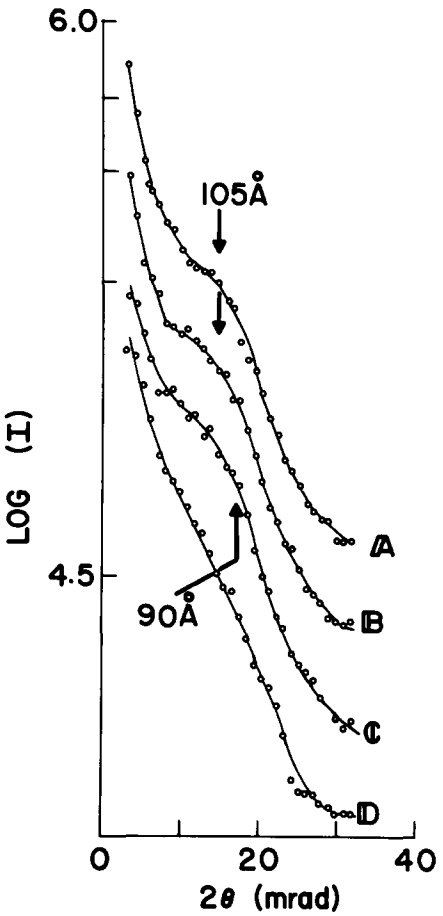

Fig. 2. Titration of the DNP-induced X-ray scattering changes. Mitochondria pelleted after 10 min incubation at 4°C in 0.15 M mannitol + 0.1 mM EGTA + (A) 0, (B) 2.5, (C) 10 or (D) 40 μM DNP. Succesive curves shifted down by amounts indicated by hash marks at top of ordinate axis. Note that the X-ray scattering maximum in curve A occurs at smaller angle than that of the corresponding curve in Fig. 1. This shift is characteristic of the condensed-to-orthodox conversion of liver mitochondria [1], induced in this instance by the low osmolarity of the suspension media.

prior to DNP treatment, 1.0 in the presence of 100 μM DNP and 3.2. after washing.) Titration of the DNP-induced X-ray scattering changes, shown in Fig. 2 for orthodox mitochondria, indicates that these changes are complete only after DNP levels are reached which completely uncouple the mitochondria (> 25 μM). In particular, the change in mitochondrial X-ray scattering induced by 10 μM DNP is slight, a 2-mrad shift of the maximum to larger angle (Fig. 2, curve C), although this same DNP concentration is sufficient to reduce the respiratory control ratio of the mitochondria from 4.7 to 1.7 during succinate oxidation. Similar changes in the small-angle X-ray scattering from liver mitochondria are also elicited by the uncoupler CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone), but again the scattering maximum is significantly diminished only at levels CCCP above those at which oxidative phosphorylation is completely uncoupled (0.5—1 μM).

There are several biochemical events that occur in mitochondria at these relatively high uncoupler concentrations. One is the stimulation of mitochondrial ATPase. While 25 μM DNP is sufficient to uncouple rat liver mitochondria completely, half-maximal ATPase activation requires up to 80 μM DNP [4]. Another effect of high uncoupler concentrations is the inhibition of respiratory rates. However, the succinate oxidation rates of these rat liver mitochondria increase continuously with DNP concentration up to 75 μM, i.e. well-above the levels that produce the X-ray scattering changes.

Correlation of the loss of the 20 mrad X-ray scattering maximum of liver mitochondria with ATPase activation would be consistent with another observation. Namely, well-coupled mitochondria isolated from transplanted Reuber H-35 hepatomas display all the X-ray scattering characteristics described above for normal liver mitochondria except the loss of the scattering maximum at high levels of DNP. The absence of uncoupler-stimulated ATPase activity is a well-documented defect of non-ascites hepatoma mitochondria [4—7].

Formal interpretation of these uncoupler-induced X-ray scattering changes is not possible since the identity of the mitochondrial structures responsible for the 20 mrad scattering maximum is not known. There is some evidence (Ref. 1 and Mannella and Parsons, unpublished results) to suggest that this maximum arises from interference among the F_1-ATPase subunits of the cristae. Speculating along the lines of such a model, the 2-mrad wide-angle shift of the scattering maximum evidenced at low DNP levels would imply as much as a 25% increase in the packing density of these subunits. The absence of a concomitant change in the slope of the scattering curve at very small angle appears to rule out any major change in inner membrane convolution accompanying this lateral compression of membrane components. The loss of intensity from the X-ray scattering maximum observed at higher DNP concentrations could come about in several ways, including decreases in either the number of subunits present in the tightly-packed arrays of F_1-ATPase or in the electron density contrast between the subunits and their microenvironment. The latter case would arise if, for example, the subunits were to move from lipid to aqueous surroundings, i.e., from the vicinity of the hydrocarbon core of the inner mitochondrial membrane to the membrane surface [8]. If such movement of the F_1-ATPase were in fact induced by high levels of uncoupler, it could readily account for the observed increase in the hydrolytic activity of the enzyme complex.

C.A.M. was supported by Fellowship Number 5 F22 CA00785, awarded by the National Cancer Institute, DHEW.

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