EFFECTS OF DIVALENT METAL IONS AND CHELATORS ON THE STRUCTURE OF OUTER MITOCHONDRIAL MEMBRANES FROM *NEUROSPORA CRASSA*

CARMEN A. MANNELLA AND JOACHIM FRANK
Ultrastructure Analysis Section, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201 U.S.A.

There is good in vitro evidence that the intact outer mitochondrial membrane is usually permeable to metabolites, excluding only large molecules such as holocytochrome *c*. A physical basis for this high permeability was first suggested by electron microscopic observations of close-packed 3-nm-Diam "pits" in outer membranes of plant mitochondria (1). The hypothesis that these sites of negative-stain accumulation might represent the openings of aqueous channels through the membranes (1) has been supported by recent reports of pore-forming activity in outer membrane fractions from various mitochondria (2, 3).

RESULTS AND DISCUSSION

We have isolated outer membranes from mitochondria of *Neurospora crassa* (Slime mutant, ATCC 32360) by hypotonic shock and sucrose step-gradient centrifugation (4). These membranes display a single prominent polypeptide band when electrophoresed on 12% polyacrylamide gels containing 1% sodium dodecylsulfate (data not shown). The apparent molecular weight of this polypeptide (31,000) is close to the reported values for the pore-forming polypeptides of outer mitochondrial membranes of both liver (32,000 mol wt) and higher plants (30,000 mol wt) (2, 3).

When spread and negatively stained, the surfaces of the fungal outer mitochondrial membranes contain stain-accumulating subunits which are like those seen in the higher plant membranes but which in 10–20% of the membranes are present in extended ordered arrays. The predominant type of lattice in these membranes is oblique (parameters given in Table I), each unit cell containing six negative-stain centers (2.5–3 nm diam) arranged in a hexagon with vertices related by a two-fold rotation axis. Fig. 1 *A* is a high magnification image of one such outer mitochondrial membrane, obtained by computer filtration using the SPIDER image processing system (5), negatively stained with phosphotungstate, a heavy-metal complex anion. When the complex cation uranyl is instead used as the negative stain (Fig. 1 *B*), there are several differences in the membrane images. While the lattice symmetry is the same as with phosphotungstate, there is a small (~ 4%) increase in the unit cell dimensions (Table I, specimen 3) and a decrease in diameter of the negative-stain accumulating sites to 2–2.5 nm. There are also differences in the distribution of the anionic and cationic stains outside the opaque centers. Phosphotungstate tends to accumulate in the region between adjacent stain centers, so that there is an appearance of "cross-bridging." Uranyl is excluded from the area immediately around each stain center and the "cross-bridging" appears with reversed contrast. A possible explanation for this staining behavior is that the membrane components which define the stain-accumulating centers have a positive surface charge, which leads to their positive staining by phosphotungstate and true negative staining with uranyl.

These outer mitochondrial membranes are routinely dialyzed overnight against 1 mM Tris-HCl (pH 7.0) prior

TABLE I
LATTICE PARAMETERS OF *NEUROSPORA* OUTER MITOCHONDRIAL MEMBRANES*

Specimen	Addition to dialysis buffer	Negative stain‡	Lattice parameter§			N
			θ (deg)	a (nm)	b (nm)	
1	None	KPT	109 ± 1	12.6 ± 0.3	11.2 ± 0.1	11
2	0.1 mM EDTA	KPT	109 ± 1	$12.5^{+0.1}_{-0.2}$	$11.1^{+0.1}_{-0.2}$	12
3	0.1 mM EDTA	UA	109 ± 1	13.0 ± 0.2	11.7 ± 0.2	10
4	0.1 mM $CaCl_2$	KPT	91^{+3}_{-1}	$8.7^{+0.4}_{-0.3}$	5.1 ± 0.1	11

*Membranes were dialyzed, pelleted, and resuspended in 1 mM Tris-HCl (pH 7.0). One drop of the suspension was mixed on a glow-discharged carbon-formavar-coated specimen grid with one drop of stain solution. After one or two min the specimen was washed twice with stain and air dried.

‡Stain solutions: KPT, 2% phosphotungstic acid (Ernest F. Fullam, Inc., Schenectady NY), adjusted to pH 7.0 with KOH, containing 0.01% bovine serum albumin (Sigma Chemical Co, St. Louis MO), crystallized, lyophilized, essentially fatty acid-free). UA, 1% uranyl acetate (Fullam).

§Parameters are the average unit cell angle, θ, and lengths a and b, measured (by optical diffraction from the electron image plates) for the indicated number of randomly selected lattices, N. The dimensions of the oblique lattices (specimens 1–3) are those associated with the smallest of the three primitive-lattice obtuse angles which can be specified. In specimen 4, in addition to the 11 rectangular lattices, there were seven oblique lattices (with parameters like those of specimens 1–3) and five lattices with intermediate symmetry (θ = 96 ± 1°, a = 9.6 ± 0.4 nm, b = 4.9 ± 0.1 nm).

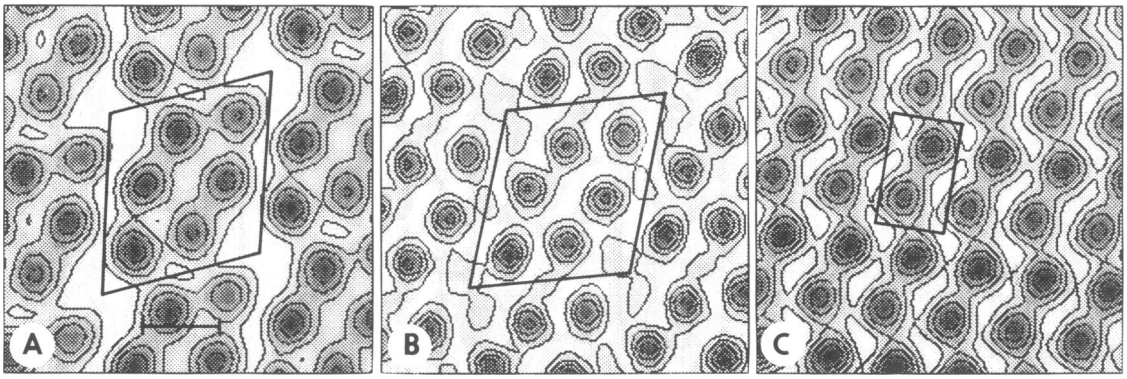

FIGURE 1 Computer filtered images of outer mitochondrial membranes containing oblique (*A*, *B*) and rectangular (*C*) lattices, stained with potassium phosphotungstate (*A*, *C*) or uranyl acetate (*B*) as described in footnote of Table I. Micrographs taken with a Philips 301 (Philips Electronic Instruments, Inc., Mahwah, NJ) operated at 100 kV (*A*, *B*) or a JEOL 200 at 150 kV (*C*) (JEOL USA, Electron Optics Div., Cranford, NJ). Each image was reconstructed by inverse transformation of the discrete Fourier spectrum from a single membrane layer. The scale in each image is the same; the bar in *A* represents 5 nm. Contour lines and unit cells are superimposed.

to negative-stain specimen preparation (details of which are presented in footnote to Table I). When 0.1 mM EDTA is included in the dialysis buffer, the lattice parameters are unaffected (Table I, specimen 2) although there are indications in the filtered images of decreased phosphotungstate accumulation by the subunits (data not shown). On the other hand, when the dialysis medium contains 0.1 mM $CaCl_2$, the predominant lattice is rectangular (or near-rectangular) with unit cells large enough to accommodate only two stain-accumulating sites (Table I, specimen 4). With phosphotungstate, the stain centers in the new lattice display the same diameters and nearest-neighbor distances, 4.5 to 5 nm, as in the oblique lattice, although their packing is now nearly hexagonal (Fig. 1 *C*). The change in ordering of the outer mitochondrial membrane subunits induced by calcium pretreatment is interesting in light of evidence that similar treatment alters the accessibility of substrate molecules to enzymes in the intermembrane space of intact plant mitochondria (6, 4). The calcium-induced symmetry change may, for example, reflect a permeability-related conformation change in the outer membrane subunits. Alternatively, the lattice changes associated with calcium exposure may be lipid-related, e.g., the result of endogenous phospholipase activation. These two possibilities are currently under investigation.

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PHOTOACOUSTIC CALORIMETRY OF PURPLE MEMBRANE

JANE LEGRANGE, DAVID CAHEN, AND S. ROY CAPLAN
Departments of Membrane Research and Structural Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel.

Photoacoustic measurements on suspensions of purple membrane fragments at optical density 38, extracted from the halophilic bacterium *Halobacterium halobium* were used to determine the thermal energy changes associated with the photochemically-induced intermediates and conformational states of bacteriorhodopsin. Bacteriorho-

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