Biochimica et Biophysica Acta, 298 (1973) 180–183

BBA 76230

OPTICAL ANALYSIS OF ELECTRON MICROGRAPHS OF CYTOCHROME OXIDASE MEMBRANES

JACK MANILOFF[a,*], GARRET VANDERKOOI[b], HIDEO HAYASHI[b] and RODERICK A. CAPALDI[b]

[a] *Departments of Microbiology and of Radiation Biology and Biophysics, University of Rochester, Rochester, N. Y. 14642 and* [b] *Institute for Enzyme Research, University of Wisconsin, Madison, Wisc. 53706 (U.S.A.)*

(Received September 21st, 1972)

SUMMARY

Optical analysis of electron micrographs of membranous cytochrome oxidase shows that the membrane lattice has the symmetry of the *pgg* two-dimensional space group. The unit cell size is 87 Å by 115 Å. After optical filtering, some details of the particle structure can be seen.

INTRODUCTION

Membranes formed by cytochrome oxidase and phospholipid have been reported by Vanderkooi *et al.*[1] to have a two-dimensional crystalline structure. Since these are interesting models for biological membranes, electron micrographs of these membranes have been investigated by optical analysis.

EXPERIMENTAL

Cytochrome oxidase membranes were prepared as previously described[1,2]. Electron micrographs of unfixed samples, negatively stained with uranyl acetate, were obtained[1,2]. These micrographs were analyzed by optical diffraction, and their optically filtered images prepared, using an apparatus similar to that described by Klug and De Rosier[3]. The details of the diffractometer and the procedures used in optical analysis have been reviewed by Horne and Markham[4].

RESULTS AND DISCUSSION

Fig. 1 is part of an electron micrograph of one of the preparations of membranous cytochrome oxidase examined. It has a regular two-dimensional lattice structure, as previously reported[1].

Fig. 2 is the optical transform of Fig. 1. Eight micrographs, of two different cytochrome oxidase preparations, were examined and gave similar transforms. No reflections were seen beyond 22 Å, which must, therefore, be the limit of resolution

* Visiting scientist, 1972–1973, John Innes Institute, Colney Lane, Norwich NOR 70F, England.

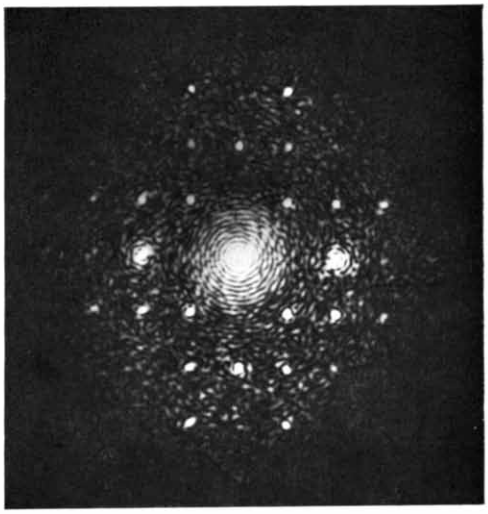

Fig. 1. Electron micrograph of a cytochrome oxidase membrane negatively stained with uranyl acetate. 530000×.

Fig. 2. Optical transform of Fig. 1. The equatorial reflection is at 1/115 Å and the meridianal one at 1/87 Å. The weak third-order reflections on the equator are believed to be spurious reflections; considering that this is the optical transform of an electron micrograph of negatively stained material, it has been suggested that such extra reflections are due to either multiple scattering or to non-linear information transfer in the photographic process[4,6].

in these studies. The odd reflections on the axes are missing, which means that Fig. 2 is the transform of the *pgg* two-dimensional space group[5]. Vanderkooi *et al.*[1] had assigned the cytochrome oxidase lattice to the *pg* space group, on the basis of their examination of electron micrographs; but the optical analysis described here cleary shows the additional *pgg* lattice symmetry elements. The *pgg* unit cell (outlined in Fig. 3) is rectangular and contains two asymmetric particles[5]: there are identical particles at each corner (each contributing a quarter particle to the unit cell) and one in the center, related to the others by a glide line of symmetry.

Optical reconstruction was carried out utilizing filtration to reduce noise[3,4]. For optical filtering, a mask with holes at the diffraction reflection positions was put in the diffraction plane. This filtered out the noise and transmitted the transform. The filtered diffraction pattern was optically recombined to produce an image with decreased noise for that part of the original micrograph which produced the transform. Fig. 3 is the optically filtered and reconstructed image of Fig. 1. The *pgg* space group symmetry is clearly seen in the reconstruction: the particles on alternate rows have the same orientation and are related to the particles on the interleaving rows by a glide symmetry operation. Hence, the particles at the four corners of the unit cell are on alternate rows, with a particle on the interleaving row in the center of the unit cell.

In the reconstructions (*e.g.* Fig. 3), each negatively stained particle was seen to be in contact with its six nearest neighbors, touching the ones in front and behind and connected to the two above and the two below by interconnections ("bridges"). The particles also seem to have some fine structure in the reconstructions, but the limit of resolution does not allow any detailed conclusions to be drawn.

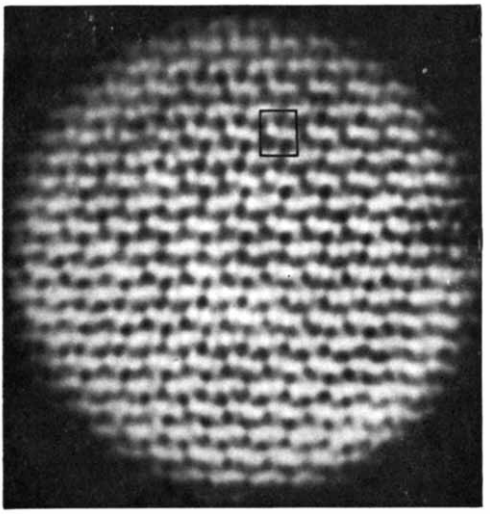

Fig. 3. Optically filtered and reconstructed image of Fig. 1. One *pgg* unit cell is outlined. 530000×.

TABLE I

CYTOCHROME OXIDASE MEMBRANE LATTICE PARAMETERS CALCULATED FROM OPTICAL TRANSFORMS

The samples were all suspended in 10 mM Tris–HCl buffer at pH 7.4–7.6; for Sample 8, 5 mM tetramethylammonium chloride was also added. Preparation 1 was the same preparation as Preparation 5 of Vanderkooi *et al.*[1], and contained 42% phospholipid. Preparation 2 did not appear in ref. 1; it contained 27–28% lipid. Mean ± S.D. calculated for Samples 1–7.

Sample	*Preparation No.*	*Unit cell axes*	
		x-axis (Å)	*y-axis (Å)*
1	1	83	116
2	1	89	117
3	1	89	115
4	2	91	118
5	2	84	110
6	2	91	118
7	2	83	108
8	2	78	130
Mean ± SD.		87 ± 4	115 ± 4

The lattice parameters calculated from the optical transforms (Table I) give the cytochrome oxidase cell size as 87±4 Å × 115±4 Å. No significant difference was found between the two preparations for which micrographs were analyzed. Addition of 5 mM tetramethylammonium chloride caused a distortion of the lattice (Sample 8) but did not change the space group or the unit cell area; the area of Sample 8 is 10140 Å^2 as compared to 10005 Å^2 for the average of Samples 1–7.

The method of determining lattice constants employed in this work is in principal superior to that used in the earlier work[1], since in the optical analysis averaging over the entire lattice examined is automatically carried out. The average unit cell size obtained by direct measurement on the micrographs[1] was 88 ± 4 Å $\times$ 124 ± 7 Å for 4 preparations, none of which were used in the present study. Thus the two methods gave similar but not identical results, with the present method yielding a somewhat smaller cell size than the former.

In summary, cytochrome oxidase membranes form two-dimensional lattices having *pgg* space group symmetry. The unit cell size was the same in the two preparations examined. The constancy of the lattice parameters may reflect the necessity for retention of the "bridges" between particles, which may provide mechanical stability for membrane formation.

ACKNOWLEDGEMENTS

We wish to thank Drs Robert Collins, Robert Horne and Roy Markham for many helpful discussions on diffraction. These studies were supported in part by U.S.P.H.S. Grant No. AI-07939 from the National Institute of Allergy and Infectious Diseases and by U.S.P.H.S. Grant No. GM-12847 from the National Institute of General Medical Sciences. J. M. is the recipient of a U.S.P.H.S. Research Career Development Award (No. AI-17480). R.A.C. is grateful to the Wellcome Trustees for the award of a Wellcome Research Travel Grant.

REFERENCES

1 Vanderkooi, G., Senior, A. E., Capaldi, R. A. and Hayashi, H. (1972) *Biochim. Biophys. Acta* 274, 38–38
2 Hayashi, H., Vanderkooi, G. and Capaldi, R. A. (1972) *Biochem. Biophys. Res. Commun.* 49, 92–98
3 Klug, A. and De Rosier, D. J. (1966) *Nature* 212, 29–32
4 Horne, R. W. and Markham, R. (1972) in *Practical Methods in Electron Microscopy* (Glauert, A. M., ed.), Vol. 1. pp. 327–43, North-Holland Publishing Company, Amsterdam
5 Henry, N. F. M. and Lonsdale, K. (1952) *International Tables for X-Ray Crystallography*, Vol. 1, p. 63, The Kynoch Press, Birmingham, England
6 Finch, J. T., Klug, A. and Nermut, M. V. (1967) *J. Cell Sci.* 2, 587–90