Bioenergetics (1972) 3, 339-344

Conformational Changes in Membranous Preparations of Cytochrome Oxidase

T. Wakabayashi,* A. E. Senior, O. Hatase,† H. Hayashi‡ and D. E. Green

Institute for Enzyme Research
University of Wisconsin
Madison, Wisconsin 53706

Received 15 November 1971

Several years ago J. Asai in our laboratory observed that purified oxidized preparations of Complex III (QH_2 cytochrome *c* reductase [1]) of the mitochondrial electron transfer chain appeared as crystalline arrays in surface view of negatively stained specimens, and that reduction of the complex led to significant changes in the crystal patterns.

Seki, Hayashi and Oda [2] more recently have described a preparation of what they called the "green membrane". This fraction derived from the mitochondrial inner membrane contains a mixture of complexes, but Complex IV (cytochrome oxidase) is the major component. The fraction shows a very regular crystalline structure in surface views of negatively stained specimens.

We found that pure membranous cytochrome oxidase, prepared by a method involving extraction with Triton X-100, after Sun *et al.* [3] also showed a crystalline structure in surface view of negatively stained specimens. This crystalline pattern was quite reproducible and very striking. On reduction of the membranous cytochrome oxidase preparation, the regular crystalline pattern was completely replaced by a randomized surface structure. In this paper we wish to describe and

* On leave of absence from the Department of Pathology, Nagoya University School of Medicine, Nagoya, Japan.

† On leave of absence from the Department of Microbiology, Okayama University Medical School, Okayama, Japan.

‡ On leave of absence from the Department of Biochemistry, Cancer Institute, Okayama University Medical School, Okayama, Japan.

document these findings, and to discuss them in relation to their importance as evidence that reduction of a membranous electron transfer complex leads to quaternary conformational changes in the membrane.

Methods

Preparation of membranous cytochrome oxidase. Membranous cytochrome oxidase was prepared by the method of Sun *et al.* [3] with the following modifications. Beef heart mitochondria were used as source, and were prepared by the method of Crane, Glenn and Green [4] with 10 mM Tris-Cl replacing phosphate buffer. The purification of the oxidase was similarly carried out using 10 mM Tris-Cl instead of phosphate throughout. Thorough homogenization was achieved by the use of a Potter-Elvehjem-type homogenizer rather than by sonic irradiation. The final preparation was twice washed in a mixture 0.25 M in sucrose and 10 mM in Tris-Cl of pH 7.8. The preparation was membranous and contained 22% by weight phospholipid and 8.25 nmoles heme *a*/mg protein.

Analytical methods. Phospholipids were estimated by the method of Chen *et al.* [5], protein by the method of Miller [6], and heme *a* by the alkaline pyridine hemochromogen method.

Preparation of the oxidized and the reduced forms of membranous cytochrome oxidase. The oxidized form was prepared by aeration of a sample. The reduced form of the oxidase was generated by the addition of sodium dithionite; and KCN or NaN_3 was present in the medium to prevent re-oxidation. In some experiments, reduced cytochrome *c* was used as reductant instead of dithionite, and in other experiments dithionite alone, without KCN or NaN_3 was used. Essentially the same results were obtained regardless of the method of reduction. The reduced and the oxidized forms of membranous cytochrome oxidase thus obtained were immediately fixed with glutaraldehyde and prepared for electron microscopy.

Procedure for electron microscopy. The oxidized and the reduced materials specified above were immediately fixed with a solution 0.05% in glutaraldehyde, 0.05 M in K-cacodylate of pH 7.5, and 0.25 M in sucrose. After 1 min of fixation with glutaraldehyde, one drop of a sample was placed on a grid and negatively stained with 1% phosphotungstic acid (PTA).

Results

Figure 1A shows an electron micrograph in which the oxidized form of membranous cytochrome oxidase was negatively stained with PTA after

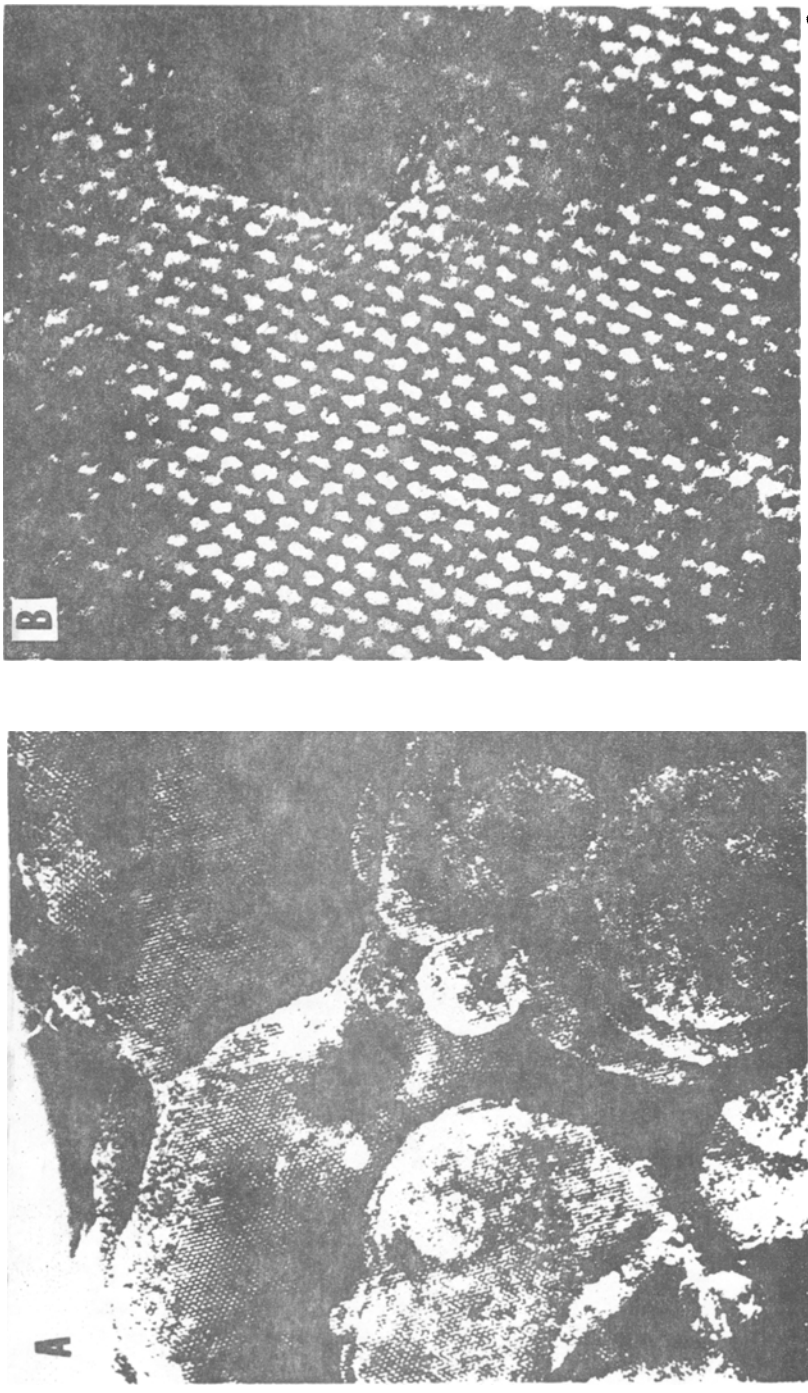

Figure 1. Electron micrographs of oxidized membranous oxidase. For technical details see under "Methods".
A. Low magnification. B. High magnification.

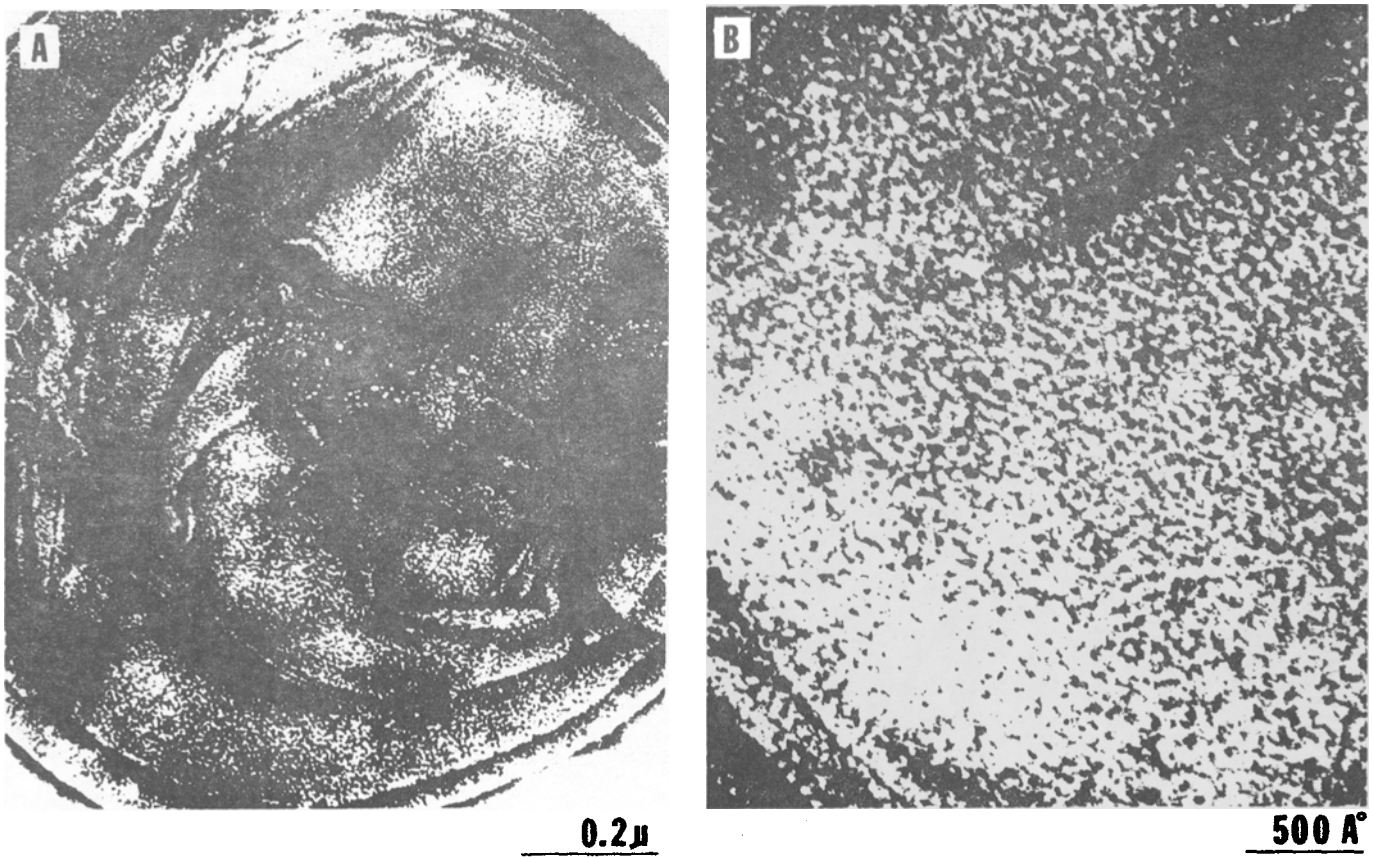

Figure 2. Electron micrograph of reduced membranous cytochrome oxidase. For technical details see under "Methods".
A. Low magnification. B. High magnification.

fixation with glutaraldehyde. It can be seen that the entire surface of each of many vesicles displays very regular lattice structures. This pattern can be seen more clearly in the high magnification electron micrograph shown in Fig. 1B. The size of each particle is about 50 Å in width and 60-65 Å in length.

Figure 2 shows two electron micrographs in which the reduced form of membranous cytochrome oxidase was negatively stained with PTA after fixation with glutaraldehyde. The size of each particle remained the same as that seen in the oxidized form but the particles lost their regular arrangement one with respect to the others and the pattern of arrangement became random. Figures 2A and 2B are at low and high magnifications respectively.

Discussion

Many biological membranes have been shown to form crystalline arrays when examined electron microscopically in negatively stained preparations [7]. This crystalline pattern has been interpreted in terms of the protein and lipid of membranes forming separate and highly regular domains [7]. The most stable form of the membrane at least under the conditions of visualization would then correspond to the state in which there is regular alternation of protein and lipid domains. In the case of crystalline cytochrome oxidase, the protein domain corresponds to a single multimeric complex. Hence each multimeric complex is oriented in a highly regular manner in respect to other such complexes and to lipid. When the complex is reduced by dithionite or reduced cytochrome *c*, this regularity of pattern disappears. The orientation of the particles with respect to one another and to lipid becomes randomized. This change may be described as a quaternary conformational change in the membrane continuum, involving a rearrangement in the orientation of the individual protein domains with respect to one another and with respect to the lipid domains. According to the electromechanochemical model of the mitochondrion [8], electron transfer generates an electric potential and field in the complex. The creation of charge centers in the individual protein domains would lead to electrostatic repulsion or attraction between the particles depending whether the paired charges are like or unlike. This would lead to a metastable rearrangement of the particles in adjustment to these coulombic interparticle interactions. The fact that such a rearrangement does occur is suggestive of charge separation in cytochrome oxidase as predicted by the electromechanochemical model.

On close inspection of the randomized pattern shown by negatively stained specimens of reduced cytochrome oxidase, it would appear that the geometry of the individual particles has also changed during

reduction although the details are too blurred to permit an exact description of the geometric change. If the geometry of the particles has also changed in consequence of electron transfer, such change would be a token of tertiary changes in the proteins of the multimeric unit of cytochrome oxidase.

Acknowledgements

We gratefully acknowledge the expert technical assistance of Mrs. Sandra Winder. This work was supported by training grant GM-88 and program project grant GM-12847 from the National Institute of General Medical Sciences (USPHS).

References

1. Y. Hatefi, A. G. Haavik and D. E. Griffiths, *J. Biol. Chem.*, 237 (1962) 1681.
2. S. Seki, H. Hayashi and T. Oda, *Arch. Biochem. Biophys.*, 138, (1970) 110.
3. F. F. Sun, K. S. Prezbindowski, F. L. Crane and E. F. Jacobs, *Biochim. Biophys. Acta*, 153 (1968) 804.
4. F. L. Crane, J. L. Glenn and D. E. Green, *Biochim. Biophys. Acta*, 22 (1956) 475.
5. R. S. Chen, T. Y. Toribara and H. Warner, *Anal. Chem.*, 28, (1956) 1756.
6. G. L. Miller, *Anal. Chem.*, 31 (1959) 964.
7. D. E. Green and R. F. Brucker, *Bio Science*, 22, (1972) 13.
8. D. E. Green and S. Ji, *Bioenergetics*, special issue on membrane function, 1972.