Structure of NADH

Boekema, Egbert J.; Breemen, Jan F.L. van; Keegstra, Wilko; Bruggen, Ernst F.J. van; Albracht, Simon P.J.

Published in:
Biochimica et Biophysica Acta - Bioenergetics

DOI:
10.1016/0005-2728(82)90248-1

Publication date:
1982

Biochimica et Biophysica Acta, 679 (1982) 7–11
Elsevier Biomedical Press

BBA 41001

STRUCTURE OF NADH:Q OXIDOREDUCTASE FROM BOVINE HEART MITOCHONDRIA STUDIED BY ELECTRON MICROSCOPY

EGBERT J. BOEKEMA [a], JAN F.L. VAN BREEMEN [a], WILKO KEEGSTRA [a], ERNST F.J. VAN BRUGGEN [a] and SIMON P.J. ALBRACHT [b]

[a] *Biochemisch Laboratorium, Rijksuniversiteit Groningen, Nijenborgh 16, 9747 AG Groningen and* [b] *Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam (The Netherlands)*

(Received June 30th, 1981)

Key words: NADH:Q oxidoreductase; Complex I; Crystallization; Electron microscopy; Respiratory chain; (Bovine heart mitochondria)

Two-dimensional crystalline arrays of NADH:Q oxidoreductase preparations have been obtained by microdiffusion of protein dissolved in detergent against a 15 mM sodium acetate buffer of pH 5.5 containing 10% (w/v) ammonium sulphate. Electron microscopy was used to study the structure of negatively stained crystals. Computer-reconstructed images were obtained by the Fourier peak filtering method. The crystals have p4 symmetry and a square unit cell with dimensions of 15.2 ± 0.5 nm. The four asymmetric units in the unit cell form a single tetrameric molecule with a dimension in the third direction of 8.2 nm. It is concluded on the basis of the estimated molecular mass that each tetramer cannot contain more than only one FMN molecule. This implies that the tetramers possibly are only a part of Complex I, since there is much evidence that one functional enzyme molecule of Complex I contains two FMN molecules.

Introduction

NADH:Q oxidoreductase (EC 1.6.99.3) is the most complicated enzyme of the mitochondrial respiratory chain. A purified form of the enzyme, that has been studied extensively, is Complex I from bovine heart which was first isolated by Hatefi et al. [1]. Complex I preparations are polydisperse at 5°C, containing equal amounts of two components, differing only in the state of dispersion [2]. In such preparations about 26 different polypeptide chains can be detected by SDS-polyacrylamide gel electrophoresis [3]. Based on polypeptide labelling experiments the group of Ragan [4,5] has proposed a model for the functional unit of the molecule, in which 19 of the polypeptide chains are placed in five groups differing in accessibility for labels. According to this group the flavoprotein fragment forms the core of the enzyme, buried in a shell of other more hydrophobic subunits. Another conclusion is that the iron-protein fragment is transmembranous but is excluded from direct interaction with the lipid phase of the membrane by the hydrophobic shell.

Despite much research in the last decade on the structure of NADH dehydrogenase [3], a picture of the overall structure is still lacking. Recently, electron microscopy has given much information on the structure of membrane-bound enzymes, such as the respiratory chain enzymes cytochrome *c* oxidase (EC 1.9.3.1) [6], QH_2:ferricytochrome *c* oxidoreductase (EC 1.10.2.2) [7] and bacterial rhodopsin of the purple membrane [8]. For more detailed analysis of the structure it is advantageous to order the enzymes in a regular two-dimensional array. This arrangement enables the improvement of low-contrast images with

Abbreviation: SDS, sodium dodecyl sulphate.

computer-aided noise-filtering techniques.

This paper deals with an electron microscopic study on crystallized NADH:Q oxidoreductase. Low-dose pictures of two-dimensional crystals were analyzed using computer Fourier peak filtering procedures.

Materials and Methods

Complex I prepared from heart muscle particles [9], according to the method of Hatefi et al. [1], was dissolved in 50 mM Tris-HCl buffer (pH 8.0), 0.66 M sucrose and 0.5% (w/v) cholate in a final concentration of 58 mg protein/ml. The protein content was determined by the biuret method [1]. The FMN content, determined as described in Ref. 11, was 0.9 μmol/g protein.

Complex I was crystallized by means of equilibrium dialysis in glass capillaries (45 × 1 mm inner diameter) which were closed at the lower end with a dialysis membrane [12]. About 20 μl of the dilute protein solution (20 mg/ml) in 15 mM sodium acetate buffer of pH 5.0–6.0 or Tris-HCl buffer of pH 6.0–8.0 were brought into the capillaries, which were then placed into tubes containing 10 ml of the same buffer plus 10% (w/v) ammonium sulphate. After dialysis, the material was diluted with 1 or 2 vol. of the dialysis buffer and used without further purification.

Specimens were prepared for electron microscopy with the droplet technique using aqueous 1% unbuffered uranyl acetate as a negative stain and formvar-carbon as supporting film. Electron microscopy was carried out on Phillips EM 300 or EM 400 electron microscopes at 80 kV with an electron optical magnification of 50000. Minimum-dose microscopy was performed as described by Unwin and Henderson [8]. Images were recorded on Kodak electron image film 4463 with an electron dose of 100–500 electrons/nm^2.

Electron micrographs were selected by optical diffraction for lack of astigmatism, proper focussing and sharpness of reflections. The selected micrographs were scanned with an Optronics P1000 rotating-drum densitometer using a 25 × 25 μm sampling aperture (and grid), corresponding to 0.53 × 0.53 nm on the specimen scale. The digitized images were processed using the IMAGIC software system [13] on a Nord 10 minicomputer. The effect of noise due to statistical variation and radiation damage was minimized by the Fourier peak filtering method.

Results and Discussion

Two-dimensional crystals of NADH:Q oxidoreductase

Equilibrium dialysis turned out to be a very simple method of crystallization of Complex I. The best results were obtained at pH 5.5. Up to approx. 20% of the total protein material, adsorbed and stained in specimens, was packed in crystalline sheets as checked with the electron microscope. The highest yield was at pH 5.2, however, at this value crystalline sheets tend to become stacked. This behaviour was almost absent at pH 5.5. At higher pH values, i.e., pH 5.8–7.0, extensive crystals were seen sporadically. The crystals of NADH:Q oxidoreductase were ideally suited for study by electron microscopy. Fig. 1A shows a part of a single layered crystal. The rectangular edges of the sheets are characteristic and allow easy recognition of the crystals on grids at a low electron optical magnification. The interaction of the crystals with clots of the original detergent-lipoprotein complex was very small over the whole pH range 5.2–7.0.

Two-dimensional reconstruction

The lattice spacings were determined from the reciprocal lattice constants of several optical diffraction patterns. The crystals have a square lattice with spacings of $a = b = 15.2 \pm 0.5$ nm and $\alpha = 90°$, and the symmetry of the crystals is p4. In the optical transform Fig. 1B it can be seen that the odd reflections on both axes are missing, which might indicate p4g symmetry for the crystal [14]. For reconstruction we have used seven orders of diffraction falling within the first zone of the contrast transfer function. This corresponds to reliable structural details up to a resolution of 2.2 nm.

Further characterization of the crystals and their building blocks

The thickness of the crystals was determined on platinum-shadowed specimens (Fig. 2). Before shadowing, the specimens were slightly stained with 1% uranyl acetate which has a supporting effect. The

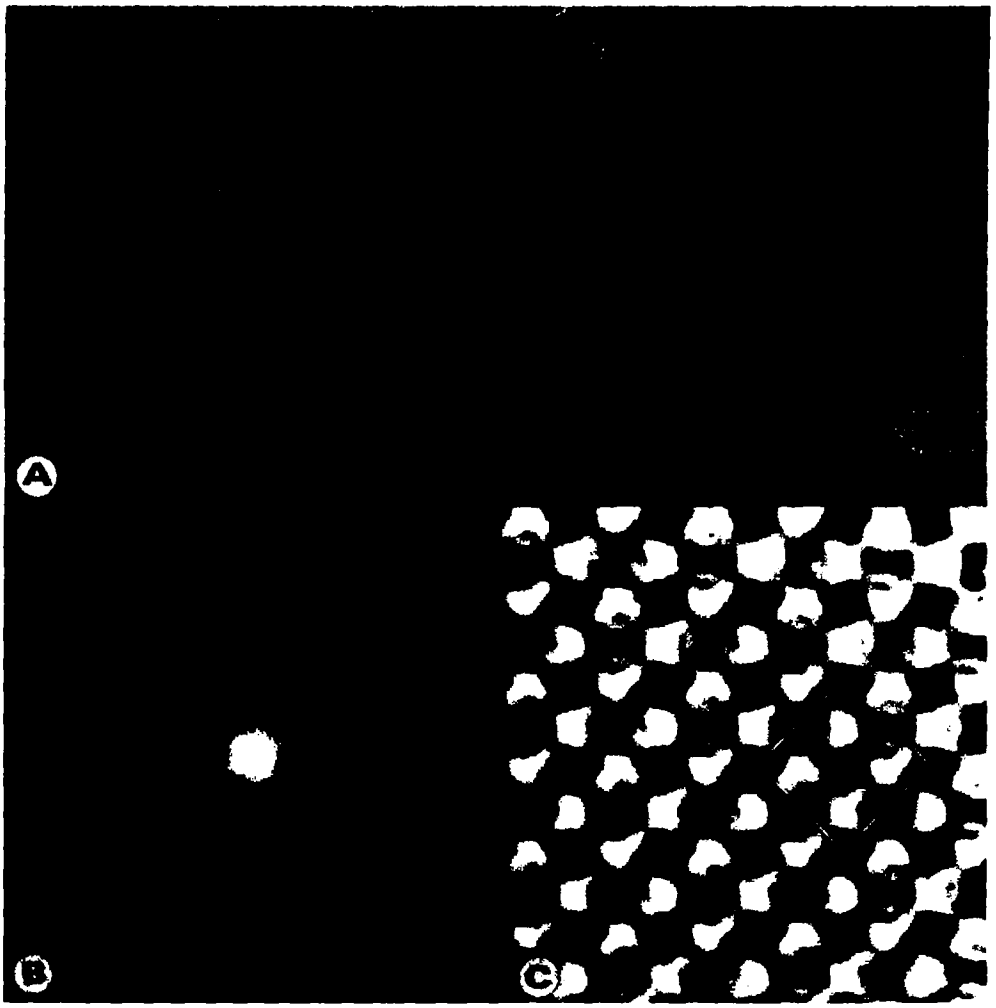

Fig. 1. Electron microscopy of NADH:Q oxidoreductase crystals from bovine heart mitochondria. (A) After negative staining with uranyl acetate. The bar represents 100 nm. (B) Optical diffraction pattern from a crystal similar to that in A, but the micrograph was taken under low-dose conditions. The bar represents 0.175 nm^{-1}. (C) Filtered image corresponding to the optical diffraction figure of B. One unit cell (15.2 X 15.2 nm) has been drawn.

thickness of the crystals as determined from the shadow length was 8.0 ± 0.6 nm (S.D., n = 10). Fig. 3 demonstrates the building blocks of the crystal. In most solutions obtained by equilibrium dialysis single tetrameric molecules were present, which were nearly absent in the original batch of protein. The dimensions of this square tetramer are 15.2 ± 1.1 nm (S.D., n = 65) in both directions. This value is in good agreement with the 15.2 ± 0.5 nm of the largest repeating unit in the crystal. The visibility of the single tetramers on the micrographs is often poor, possibly because the surrounding lipid excludes the uranyl acetate stain. Fig. 3 also shows oblong particles, presumably side views of tetramers because they have a length of about 15 nm or 2- or 3-times this value. These side views have a mean thickness of 8.4 ± 1.0 nm (S.D., n = 33). In some specimens the side views are rather angular with a maximal thickness of 11.0 nm. However, we cannot rule out the possibility that these side views are not perpendicularly fixed

Fig. 2. NADH:Q oxidoreductase crystal, slightly negatively stained with uranyl acetate and shadowed with platinum from an angle of 26°. The direction of shadowing is indicated by the arrow. The bar represents 100 nm.

Fig. 3. Single molecules of NADH:Q oxidoreductase: the thick arrow indicates tetrameric front views, the thin arrow corresponds to side views of molecules. The bar represents 100 nm. In the lower half of the figure selected front and side views are shown.

on the support film. If our interpretation of the mean value of the side views of 8.4 nm is correct, it is in good agreement with the value of 8.0 ± 0.6 nm for the shadowed crystal.

On the basis of dimensions of 15.2 X 15.2 X 8.2 nm for the crystal unit cell and assuming a mean density for protein crystals corresponding to 0.0025 nm^3/dalton [15], the relative molecular mass of the protein within the unit cell is estimated as 750 000. The flavin content of the Complex I used was 0.9 μmol/g protein. Hatefi et al. [1] and Galante and Hatefi [16] reported values of 1.3–1.5 μmol FMN/g protein, indicating a minimum molecular weight of about 700 000. From these numbers we conclude that each tetramer cannot contain more than only one molecule of FMN. Dooijewaard et al. [2] have determined the molecular weight of Complex I from sedimentation velocity measurements and concluded that one enzyme molecule must contain two FMN molecules. This is in line with the finding [17,18] that the amount of NADH-reducible Fe-S cluster I in the enzyme complex is only half that of FMN. This implies that the tetrameric arrangement in the unit cell represents possibly only a part of Complex I.

Acknowledgements

We wish to thank K. Gillissen for printing and mounting of the photographs and Miss E. Hendriks for typing of the manuscript. This study was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

References

1 Hatefi, Y., Haavik, A.G. and Griffiths, D.E. (1962) J. Biol. Chem. 237, 1676–1680
2 Dooijewaard, G., De Bruin, G.J.M., Van Dijk, P.J. and Slater, E.C. (1978) Biochim. Biophys. Acta 501, 458–469
3 Ragan, C.I. (1980) in Subcellular Biochemistry (Roodyn, D.B., ed.), vol. 7, pp. 267–307 Plenum Press, New York
4 Smith, S. and Ragan, C.I. (1980) Biochem. J. 185, 315–326
5 Early, F.G.P. and Ragan, C.I. (1980) Biochem. J. 191, 429–436
6 Fuller, S.D., Capaldi, R.A. and Henderson, R. (1979) J. Mol. Biol. 134, 305–327
7 Hovmöller, S., Leonard, K. and Weiss, H. (1981) FEBS Lett. 123, 118–122
8 Unwin, P.N.T. and R. Henderson (1975) J. Mol. Biol. 94, 425–440
9 Keilin, D. and Hartree, E.F. (1947) Biochem. J. 41, 500–502
10 Cleland, K.W. and Slater, E.C. (1953) Biochem. J. 53, 547–556
11 Dooijewaard, G. and Slater, E.C. (1976) Biochim. Biophys. Acta 440, 1–15
12 Zeppezauer, M., Eklund, H. and Zeppezauer, E.S. (1968) Arch. Biochem. Biophys. 126, 564–573
13 Van Heel, M.G. and Keegstra, W. (1981) Ultramicroscopy, in the press
14 Henry, N.F.M. and Lonsdale, K. (1952) International Tables for X-ray Crystallography, vol. 1, p. 63, Kynoch Press, Birmingham
15 Matthews, B.W. (1968) J. Mol. Biol. 33, 491–497
16 Galante,Y.M. and Hatefi, Y. (1979) Arch. Biochem. Biophys. 192, 559–568
17 Albracht, S.P.J., Dooijewaard, G., Leeuwerik, F.J. and Van Swol, B. (1977) Biochim. Biophys. Acta 459, 300–317
18 Albracht, S.P.J., Leeuwerik, F.J. and Van Swol, B. (1979) FEBS Lett. 104, 197–200