Structure of the Radical Form of Clostridial Flavodoxin: A New Molecular Model
(protein structure/flavoprotein/flavin mononucleotide/x-ray crystallography)


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ABSTRACT Interpretation of a new electron-density map at 3.25-Å resolution has led to a somewhat revised model for the free radical (semiquinone) structure of flavodoxin from Clostridium MP. Although the general conformation of the molecule is very similar to that of oxidized Desulfovibrio vulgaris flavodoxin, flavin mononucleotide-protein interactions are not identical in the two flavodoxins. In the Cl. MP semiquinone molecule, the isoalloxazine ring appears to retain the essentially planar conformation characteristic of oxidized flavins; within the limits imposed by the resolution of the data, the map shows no evidence for bending of the isoalloxazine ring about N5-N10.

Analysis of the structure of flavodoxin crystals is expected to provide a detailed picture of flavin mononucleotide-protein interactions, which account for the characteristic chemical properties of these model flavoproteins (1, 2). Earlier we reported an interpretation of an electron-density map of Clostridium MP flavodoxin semiquinone at 3.25-Å resolution. The model constructed on the basis of that map was considered tentative in several regions, and the orientation of flavin mononucleotide could not be assigned unequivocally (3).

Recently, we calculated another map of the semiquinone form of flavodoxin at 3.25-Å resolution, using three heavy-atom derivatives, Sm²⁺, Pt²⁺, and Au⁺, and giving more weight to anomalous scattering differences in the phasing. The resulting map had a much improved figure of merit, 0.80, and has proven easier to interpret. The new map clearly suggested a flavin mononucleotide position different from that proposed earlier and required some revisions in the chain tracing (Fig. 1). At about the same time, Watenpaugh et al. (4) succeeded in determining the structure of the oxidized form of a very similar protein from Desulfovibrio vulgaris at 2.5-Å resolution. Part of our current model of the semiquinone form was constructed after a preliminary sketch of the model of Watenpaugh et al. had been kindly sent to us by Dr. Jensen. We describe our new molecular model briefly here in order to facilitate comparison of the two structures (5).

Despite the greater chain length of flavodoxin from D. vulgaris (about 10 additional residues), the three-dimensional structures of the two molecules are clearly homologous. The prosthetic group is similarly situated in both proteins, but the flavin mononucleotide-protein interactions appear to differ in certain respects. Some differences are not unexpected in view of the known dissimilarities in amino-acid composi-

tion (3, 6), in optical and circular dichroic spectra (7), and in affinity for modified flavins (7, 8). Moreover, comparison of different oxidation states of the two species leaves open the possibility that some of the structural differences may result from changes in conformation as a function of oxidation state (9).

RESULTS AND DISCUSSION

The present model of the radical form of Cl. MP flavodoxin has a chain length of 138 residues, compared with the 140 predicted from amino-acid analysis. However, the chain length and sequence numbering cannot be regarded as certain until the chemical sequence and higher-resolution structures have been completed. For example, the addition of a residue between positions 58 and 59 is equally consistent with the observed electron density. Most of the residues participate in some form of secondary structure. As reported earlier (3), the molecule is composed of a central warped sheet of β-structure flanked on either side by helices. A comparison of the new with the previous model is presented in Fig. 1. In the matching regions, the average displacement of a total of 113 alpha carbons in constructing the new model was 1.7 Å; in the best-defined helix, H1, the change was only 0.5 Å. However, altered chain connections in the vicinity of the N-terminus and the flavin mononucleotide have led to a quite different ordering of the amino-acid sequence along the model. In the interpretation of Fig. 1b, the central sheet is composed entirely of parallel chains, and the N to C sense of helix H-2, which was poorly defined in the previous map, has been reversed.

The largest alterations in the model involve the binding site of flavin mononucleotide. The isoalloxazine ring and the phosphate group have now been built into regions that previously appeared to be part of the apoprotein; the phosphorus atom is now assigned, as it should be, to the position of maximum electron density. The present location of the isoalloxazine ring agrees nicely with the conclusions of W. A Eaton and M. W. Makinen (to be published), based on their polarized single-crystal absorption spectra of oxidized crystals and on theoretical predictions (10) of the transition moment directions for the 445-nm and 376-nm electronic transitions. It is still not possible, however, to distinguish clearly the benzene end from the pyrimidine end of the isoalloxazine ring in the electron-density map. The interpretation shown in Fig. 2 is favored by the spectroscopic results and also preserves the homology between the Cl. MP and D. vulgaris flavodoxins.

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Fig. 1. Stereo views of *Cl. MP* and *D. Vulgaris* flavodoxins. The drawings have been made as identical as possible by finding the orientations necessary to superimpose the homologous regions of the polypeptide chains. (a) The earlier model of flavodoxin semiquinone. Regions where the interpretation was considered tentative are designated by open bonds. (b) The present model with the helices labeled.
The three-dimensional homology between the structures of *D. vulgaris* and *Cl. MP* flavodoxins is demonstrated by comparison of Fig. 1b with c and with Fig. 2 of ref. 5. After superposition of the two models by least-squares procedures, the mean distance between the 132 a-carbons considered to be homologous was 1.9 Å. Some contrasts in the region of the prosthetic group are evident from examination of Fig. 2 of this paper and Fig. 4 of ref. 5. In the *D. vulgaris* structure, the flavin ring appears more "buried" in the protein. The angle between the flavin planes, after superposition of the two models, is about 30°. The loop bearing the aromatic residue approaches the isoalloxazine ring differently in the two structures; the aromatic residue itself extends over the dimethylbenzene end of the flavin ring in the *D. vulgaris* molecule, whereas in the *Cl. MP* radical map it covers what we assume to be the pyrimidine end of the isoalloxazine nucleus. Both the flavin mononucleotide and the adjacent aromatic residue are clearly visible in a recent 2.5-Å map of oxidized *Cl. MP* flavodoxin. Their positions are very similar to those found in the semiquinone structure. Thus, many of the differences between the flavin-binding sites of oxidized *D. vulgaris* and the semiquinone *Cl. MP* structures must be attributed to species variation.

A fuller description of the conformation of *Cl. MP* flavodoxin awaits the interpretation of electron-density maps at higher resolution. The orientation of the peptide planes and the residue numbering should then be established more precisely. A thorough comparison of the structures of *Cl. MP* and *D. vulgaris* flavodoxins at higher resolution, in conjunction with sequence analysis of both proteins, will permit a definitive study of species-dependent differences in conformation and in flavin mononucleotide–protein interactions.

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in order from the N-terminus. The present location of the N-terminus requires interruption of the chain near the top of a and continuity of the chain in the vicinity of residue 117. Near residue 60 in b, the backbone continues into helix H-2 rather than into H-3 as in a. Residue 113 was previously joined to 54, making 57 the C-terminus. (c) *D. vulgaris* flavodoxin rotated to show the homology with b.