Tertiary Structure of Oxidized Flavodoxin from an Eukaryotic Red Alga Chondrus crispus at 2.35-Å Resolution

LOCALIZATION OF CHARGED RESIDUES AND IMPLICATION FOR INTERACTION WITH ELECTRON TRANSFER PARTNERS

(Received for publication, February 12, 1990)

Ketlchi Fukuyama, Sadao Wakabayashi, Hiroshi Matsubara, and Lyndon J. Rogers

From the Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan and the Department of Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD, Wales, United Kingdom

The crystal structure of the oxidized form of a flavodoxin from an eukaryotic red alga, Chondrus crispus, has been determined by multiple isomorphous replacement and anomalous scattering methods. A model of the 173 residues and flavin mononucleotide (FMN) has been refined by a restrained least squares method to a crystallographic R-factor of 22.8% using 6236 reflections between 0.0 and 2.35 Å with F > 3σF.

This molecule has a sheet consisting of five parallel β-strands with two α-helices on one side of the sheet and three on the other side, and has a (βα)8 structure. The molecule incorporates a substantial insertion in β8, as in Anacystis nidulans flavodoxin, which distinguishes these flavodoxins from the short-chain type. The isoalloxazine ring of FMN is sandwiched between the side chains of Trp-56 and Tyr-98, with its C-7 and C-8 methyl groups being exposed to solvent. The phosphate group of FMN is located at the N-terminal end of α1, and forms extensive hydrogen bonds with the loop (T8-T13) between β1 and α1 of the protein. Six of the total 11 lysine residues are clustered at the opposing face to the FMN-binding site, while about two-thirds of the total 35 acidic residues are located in the half of the molecule which includes the FMN-binding site. Such localization of charged residues produces a dipole within the molecule, which may be important in its recognition of the other proteins participating in electron transfer reactions.

Flavodoxins are a group of proteins, possessing flavin mononucleotide (FMN) as a prosthetic group, which mediate electron transfer in prokaryotes and some eukaryotic algae (1, 2). They are believed to be functionally interchangeable with ferredoxins, and are active in the photoreduction of NADP+ in in vitro assays using chloroplasts (3, 4). Flavodoxin forms a 1:1 complex with ferredoxin-NADP+ reductase (EC 1.18.1.2) as does ferredoxin (5–7). This implies that in both flavodoxins and ferredoxins the surface features responsible for complex formation with ferredoxin-NADP+ reductase are similar. Although the models for the complexes between flavodoxin and cytochromes c and c3 have also been proposed (8, 9), most of these studies have been based on the structures of flavodoxins from nonphotosynthetic bacteria. Therefore it is questionable whether such models are applicable to complexes between the proteins from cyanobacteria and eukaryotic algae, because these organisms possess usually chloroplastic-type ferredoxins whose structures (10, 11) are quite different from those of bacterial ferredoxins (12–14).

The flavodoxins so far studied can be divided into two groups on the basis of their molecular size; either they are members of the short-chain type which consists of about 140 amino acid residues or the long-chain type with about 170 amino acid residues. The tertiary structures of two short-chain flavodoxins from Desulfovibrio vulgaris and Clostridium MP, and one long chain flavodoxin from Anacystis nidulans (Synechococcus PCC 6301) have been determined by x-ray crystallographic analysis (15–17). A. nidulans flavodoxin has a similar polypeptide folding to that exhibited by D. vulgaris and Clostridium MP flavodoxins and has an extra loop of about 20 amino acid residues in the fifth β-strand. However, the detailed tertiary structure of A. nidulans flavodoxin remains unclear, since only partial sequence information was taken into account in model building (17).

In order to clarify the structure-function relationships as well as the evolutionary aspect of flavodoxins, the tertiary structure of the flavodoxin from an eukaryotic red alga, Chondrus crispus, has been determined and is compared with those of other flavodoxins. This flavodoxin consists of 173 amino acid residues with a noncovalently bound FMN, and its physicochemical properties have been documented elsewhere (18, 19). Its amino acid sequence shows 35–40% sequence identity with other long-chain type flavodoxins and as low as 28% sequence identity with the short-chain type flavodoxins (20).

We have previously reported crystallization of the oxidized flavodoxin, preparation of a platinum derivative, and the determination of the heavy atom site (21). However, the previous electron density map at 4-Å resolution based on the single isomorphous replacement phases gave only a little information on the molecular structure.

In this paper we describe the structure determined by the multiple isomorphous replacement and anomalous scattering methods followed by the restrained least squares method using the diffraction data to 2.35-Å resolution. In particular, characteristic distributions of charged residues of this flavodoxin are discussed in terms of the interactions with its electron transfer partners.

EXPERIMENTAL PROCEDURES

Preparation of Heavy Atom Derivatives—C. crispus flavodoxin was purified as described previously (20). The oxidized form are orthorhombic, the space group P212121, with unit cell dimen-

*This work was supported by Grant-in-Aid for Scientific Research 01470148 from the Ministry of Education, Science and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed. Tel.: 06 844 1151 (ext. 4297); Fax: 06-855-8139.

Printed in U.S.A.
Based on the intensities of 004 (0 ≤ φ < 360°). The resolution of the crystal soaked in CzHsHgCl solution showed a significant intensity with a Rigaku four-circle diffractometer, using nickel-filtered CuKα radiation from a rotating anode x-ray source. The air in the collimator and in the pathway from the crystal to the detector was evacuated. The folding of the polypeptide chain and the location of FMN were determined by the least squares method. In most cases, the values of a and b were greater than 0.94 and -2.0, respectively; i.e. the decay of a crystal was less than 16% in F at the highest resolution. The correlation for the decay was made such that the values of a and b decreased linearly starting from 1.0 and 0.6, respectively, as time elapsed. The data were scaled and merged by the method of Hamilton et al. (24). The results of the measurements are summarized in Table I.

### Location of Heavy Atoms and Phase Calculation
The heavy atom positions for the mercury derivative were determined from the isomorphous difference Patterson map. The common origin for the platinum and mercury derivatives was determined from the difference Fourier map with the coefficient of m (Fm - F) · exp(iφm). Here Fm and F are the structure factors of the mercury derivative and the native crystals, respectively; m is the figure of merit; and φm is the best phase angle derived from the single isomorphous replacement method for the platinum derivative. The best phase angle and the figure of merit were calculated on the basis of the probability treatment described by Blow and Crick (25). The parameters of the platinum and mercury sites were refined by the least squares method. In most cases, the values of a and b were greater than 0.94 and -2.0, respectively; i.e. the decay of a crystal was less than 16% in F at the highest resolution. The correlation for the decay was made such that the values of a and b decreased linearly starting from 1.0 and 0.6, respectively, as time elapsed. The data were scaled and merged by the method of Hamilton et al. (24). The results of the measurements are summarized in Table I.

| Table I
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum resolution</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>Pt-derivative</td>
</tr>
<tr>
<td>Hg-derivative</td>
</tr>
</tbody>
</table>

| Table II
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy atom parameters</td>
</tr>
<tr>
<td>Element</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Platinum</td>
</tr>
<tr>
<td>Mercury</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phasing power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Platinum</td>
</tr>
<tr>
<td>Mercury</td>
</tr>
</tbody>
</table>

---

*Include the intensities to 2.25-Å resolution for the hk0 zone.

**Intensities of Bijvoet pairs measured from each crystal were averaged first. The Rmerge defined as Σ(Fobs - Fcalc)/ΣFobs is for the sets of averaged intensities from different crystals.

---

Phasing power

---

Overall figure of merit was 0.67.

---

Model Building and Crystallographic Refinement

The map at 5-Å resolution showed clearly the molecular boundaries in the crystal. The folding of the polypeptide chain and the location of FMN were

---

Downloaded from http://www.jbc.org/ on October 22, 2017
immediately obvious in the best electron density map at 2.7Å resolution. The orientation of the isoalloxazine ring could be assigned from the shape of the electron density. It was also obvious that the arrangement of α-helices and β-strands in C. crispus flavodoxin corresponded to that in the flavodoxins from prokaryotes (15-17). The lobe interrupting the fifth β-strand, as found in A. nidulans flavodoxin, was clearly evident. We built the model with the graphics NEC N6970 using the program MOLFITG (28) with the aid of the amino acid sequence (20).

The model of the present flavodoxin was refined by alternate cycles of the restrained least squares calculations using the program PROLSQ (29) and manual revision of the model using PMAKER (30). All computations, except the manual revision of the model, were carried out with an ACOS-930 computer at the Institute for Protein Research, Osaka University. All conformational angles, ω, were restrained toward the trans (ω = 180°) positions. We gradually extended the maximum resolution from 2.7 to 2.35 Å. The individual temperature factors were refined with restraints on the differences of temperature factors between connected atoms. The occupancies of all the protein atoms were fixed to 1.0. We rebuilt the model based on a 2Fo-Fc omit map with a microcomputer NEC PC-9801. When the R-factor (R = \|Fo - |Fc|\|/\|Fo\|) reached 0.263 at 2.4 Å resolution, a 2Fo-Fc map was calculated to locate solvent molecules. Peaks were identified as water molecules when they were in the range of 2.5-3.2 Å from a polar protein group or another water molecule. They were included in the least squares calculation with unit occupancy. A total of 69 cycles of the least squares refinement interrupted 3 times for major manual readjustment of the model reduced the R-factor to 22.8% for 6236 reflections with F > 3σF in the 6.0-2.35 Å resolution range. The progress of the refinement is shown in Table III. Further refinement is in progress. The atomic coordinates and structure factors (codes 1FCR and R1FCRSF, respectively) have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, N.Y.

RESULTS

Accuracy of the Structure—The 2Fo-Fc omit map for the isoalloxazine ring of FMN is shown in Fig. 2, from which the quality of the model may be evaluated. For most residues the main chain conformations could be assigned from the bumps of the electron density of the carbonyl oxygen atoms in peptide bonds. The conformations of most side chains, in particular those of interior residues, could also be assigned from the shape of the electron density distribution. However, a few side chains faced to solvent have broad density distribution presumably due to their high mobility. The mean coordinate error is estimated to be about 0.3 Å from the Luzatti plot (31) (data not shown). The present analysis may permit unambiguous description of the surface feature and the environment of FMN, as well as the folding of the polypeptide backbone.

Protein Conformation—The primary, secondary, and tertiary structures of C. crispus flavodoxin are shown in Fig. 3.

![Fig. 1. A section (y = ½) of the anomalous difference Fourier map computed at 5Å resolution. The coefficient was m|Fhkl (hkl) - Fhkl (hkl)|/|Fhkl (hkl)|exp(i (φhkl - π/2)), where m and φhkl are the figure of merit and the best phase angle derived from the multiple isomorphous replacement method, respectively. The upper right peak is the shoulder of the peak at lower left generated by symmetry operation of the crystallographic 2-fold mirror symmetry.](image)

![Fig. 2. An omit map superimposed on the isoalloxazine ring of FMN. The Fourier coefficient was (2|Foa | - |Fc|)|e^ω |, where Foa and ω were derived from the atomic parameters of all atoms except those of FMN. The map is contoured at a peak height of 10 relative to a maximum peak height of 62. The figure was drawn with the program MOLFITG (28).](image)

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of cycles</th>
<th>Resolution range</th>
<th>No. of reflections</th>
<th>No. of water molecules</th>
<th>Temperature factors</th>
<th>R-factor</th>
<th>Distances</th>
<th>Planar groups</th>
<th>Chiral volumes</th>
<th>Omegas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Bond</td>
<td>Angle</td>
<td>1-4</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>8.0-2.7</td>
<td>4173</td>
<td>0</td>
<td>10.0°</td>
<td>0.413°</td>
<td>0.328</td>
<td>0.012</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>7.0-2.5</td>
<td>5418</td>
<td>0</td>
<td>9.3°</td>
<td>0.355°</td>
<td>0.294</td>
<td>0.014</td>
<td>0.041</td>
<td>0.048</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>6.0-2.4</td>
<td>5943</td>
<td>0</td>
<td>9.4°</td>
<td>0.309°</td>
<td>0.263</td>
<td>0.015</td>
<td>0.040</td>
<td>0.050</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>6.0-2.35</td>
<td>6236</td>
<td>68</td>
<td>9.8°</td>
<td>0.285°</td>
<td>0.226</td>
<td>0.013</td>
<td>0.029</td>
<td>0.040</td>
</tr>
</tbody>
</table>

*a* Root mean square deviations from the standard geometry.

*b* Overall temperature factors.

*c* R-factor for the model built based on the MIR map.

*d* Average of individual temperature factors.
The polypeptide chain folds in such a way that β-strands and α-helices occur alternately to form the typical α/β structure (32). The five parallel β strands form a β sheet, which is surrounded by five α-helices. C. crispus flavodoxin has a one-turn helix between β₂ and β₃, in contrast to the other flavodoxins (15-17). Here we have given the numbers of α-helices in sequential order from the N terminus; α₃, α₄, and α₅ in C. crispus flavodoxin correspond to α₂, α₃, and α₄, respectively, in other flavodoxins. In the current model, the locations of α₁, α₂, α₃, α₄, and α₅ in the peptide chain are 12-24, 40-45,
Structure of C. crispus Flavodoxin

69–75, 104–113, and 158–171, respectively, whereas those of β₁, β₂, and β₃ are 2–8, 28–32, 48–55, and 85–94, respectively. Referring to other flavodoxins (15–17), β₁ is divided into three pieces; viz, βₐ (G122–N125), βₐ (G140–L143), and βₐ (L145–D148). The strands βₐ and βₐ are antiparallel and between them there is a inserted sequence of 14 amino acid residues. Except for these differences, C. crispus flavodoxin has substantially the same secondary structures in the same spatial arrangement as D. vulgaris, Clostridium MP, and A. nidulans flavodoxins. To one side of the β-sheet is located two α-helices (α₁ and α₃) and on the other side three α-helices (α₂, α₃, and α₄).

The distribution of the side chains in α-helices is shown in Fig. 4. Clear compartmentation in the distribution of hydrophobic and hydrophilic side chains is observed; the hydrophilic side chains are exposed to solvent, whereas the hydrophobic side chains are buried within the molecule. The β-sheet, in particular the central β₁, β₂, and β₃, consists mainly of hydrophobic residues (see Fig. 3 c).

**FMN Binding Site**—A close-up view of the protein-FMN interaction is shown in Fig. 5, and possible hydrogen bonds between FMN and the protein are listed in Table IV. The phosphate group of FMN is located at the N-terminal end of α₁. As is well established, an α-helix has a dipole moment with its N-terminal end having a net positive charge and the C-terminal end a negative charge (33–35). The interaction of the dipole moment of α₁ and the negative charge of the phosphate group contributes in binding FMN to the protein. In addition, extensive hydrogen bonds between the loop (T8–T13) and the phosphate group also contribute to bind FMN to the protein (Table IV).

The isoalloxazine ring is located at the periphery of the molecule. The two methyl groups of the ring are exposed to solvent, while the pyrimidine portion of the ring is buried in the protein. The bulky side chain of Trp-56 is located at the upper side of the ring, and that of Tyr-98 at the lower side of the ring. The side chain of Tyr-98 and the isoalloxazine ring are very close and almost parallel to each other, while that of Trp-56 is tilted by 35° in respect to the isoalloxazine ring.

Except for O₃ and O₄ atoms in the ribityl group, most of the hydrophilic portion of FMN is enclosed by the protein, while the hydrophobic portion of FMN is exposed to solvent. The C₅ atom of Thr-10, the benzene ring of Trp-56, and the side chain of Tyr-98 are also exposed. Thus the surface area closest to the FMN-binding site is relatively hydrophobic.

**Distribution of Proline Residues**—C. crispus flavodoxin has an unusually high proline content (10 residues compared to 3 residues in A. nidulans flavodoxin). Their distribution is shown in Fig. 6. Most of the proline residues are located at

---

**Fig. 4.** Schematic arrangement of α-helices and β-strands in C. crispus flavodoxin. All β-strands go down and all α-helices come up to the figure. The α-helices are represented by helical wheels (41), where hydrophobic side chains are shown with bold letters, and hydrophilic side chains are boxed.

**Fig. 5.** Close-up view of the environment of the FMN in C. crispus flavodoxin. The view direction is the same as in Fig. 3a. The bonds of the FMN are enhanced. Residue numbers are given near the C₅ positions.
the junctions of the secondary structures and the loops.

**Heavy Atom-binding Sites**—Soaking the crystal in a solution of K₂PtCl₄ gave a heavy atom derivative with a single heavy atom site/molecule (21). The exact species of the heavy atom complex is not known, since in ammonium sulfate solution PtCl₂⁻ complex is modified by substitution of Cl⁻ by NH₃. In other proteins the complex is known to bind frequently to methionine and in some cases to histidine residues (36). Here the platinum complex binds to methionine residues, M₁₄⁹ and M₁₅₆, which are located at the C-terminal end of β₅ and the N-terminal end of α₁, respectively. Both side chains are accessible to solvent, and are closely located (the distance between the two Sₐ atoms is about 5 Å).

The reagent C₂H₅HgCl also binds to a single site with higher occupancy. The site corresponds to C₁₁₂ in α₁, which is exposed to solvent.

**DISCUSSION**

**Structural Comparisons of Flavodoxins**—The tertiary structure of the oxidized flavodoxin from *C. crispus* has been compared with those from two species of nonphotosynthetic bacteria, *D. vulgaris* (15) and Clostridium MP (16), and from the cyanobacterium, *A. nidulans* (Synecocoece PCC 6301) (17). The orthogonal coordinates for *D. vulgaris* and Clostridium MP flavodoxins were taken from the Protein Data Bank for the present comparisons. Since the coordinates for *A. nidulans* flavodoxin are not deposited yet, the structural comparison for this flavodoxin is less quantitative.

Two sets of superpositions of the C₅ atoms are shown in Fig. 7. *C. crispus* flavodoxin belongs to the long-chain type as does *A. nidulans* flavodoxin. The long-chain type flavodoxins have substantially the same secondary structures, in similar arrangement, as the short-chain flavodoxins from *D. vulgaris* and Clostridium MP. However, the long-chain flavodoxins have an insertion of about 20 residues in β₅ compared to the short-chain flavodoxins; these residues, none of which is in contact with FMN, form a loop beneath the β-sheet. Besides the substantial insertion in β₅, Fig. 7 shows that insertion/deletion of residues occur frequently in the loops between the β-strands and α-helices. The characteristic difference in the secondary structure which is found in *C. crispus* flavodoxin is that this has a one-turn α-helix between β₂ and β₃.

**TABLE IV**

Possible hydrogen bonds between FMN and the protein

<table>
<thead>
<tr>
<th>FMN atom</th>
<th>Protein atom</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>D94 N</td>
<td>3.3</td>
</tr>
<tr>
<td>O₂</td>
<td>C103 N</td>
<td>2.7</td>
</tr>
<tr>
<td>N₃</td>
<td>N101 O</td>
<td>2.7</td>
</tr>
<tr>
<td>O₄</td>
<td>T₅₈ O₁₁</td>
<td>2.9</td>
</tr>
<tr>
<td>N₅</td>
<td>T₅₈ O₁₁</td>
<td>3.1</td>
</tr>
<tr>
<td>O₂*</td>
<td>T₅₅ O</td>
<td>2.9</td>
</tr>
<tr>
<td>O₆*</td>
<td>T₁₀ O₁₁</td>
<td>2.4</td>
</tr>
<tr>
<td>O₆*</td>
<td>G₁₁ N</td>
<td>3.0</td>
</tr>
<tr>
<td>O₆*</td>
<td>N₁₂ N</td>
<td>2.7</td>
</tr>
<tr>
<td>O₆*</td>
<td>N₁₂ N₁₂</td>
<td>3.1</td>
</tr>
<tr>
<td>O₇*</td>
<td>T₈ O₁₁</td>
<td>9.4</td>
</tr>
<tr>
<td>O₇*</td>
<td>T₁₃ O₁₅</td>
<td>3.0</td>
</tr>
<tr>
<td>O₇*</td>
<td>S₉ N</td>
<td>2.7</td>
</tr>
<tr>
<td>O₈*</td>
<td>T₁₀ N</td>
<td>2.8</td>
</tr>
<tr>
<td>O₈*</td>
<td>W₅₆ N₁₁</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The alignment of the sequences (Fig. 2 in Ref. 20) shows that invariant and semi-invariant residues are concentrated in distinct regions. These are residues 1–13, 47–56, 85–91, 98–102, and 145–152 in the sequence for *C. crispus* flavodoxin. In contrast, the sequences corresponding to α-helices show little sequence identity. As described earlier, the α-helices surround the β-sheet, and it is clear (see Fig. 4) that whereas the internal residues are well conserved, the surface residues have changed rapidly in the course of the evolution of flavodoxins. This may explain why the constraint is placed on the sizes of the side chains of the internal residues that are accommodated by the organization of the secondary structure.

Fig. 7 shows that the conformation of the phosphate-binding site is similar to each other in various flavodoxins. The phosphate group is located at the N-terminal end of the α₁-helix. The pattern of hydrogen bonds connecting the loop region (T₈–T₁₃ in *C. crispus* flavodoxin) and the phosphate group are well conserved in all flavodoxins whose tertiary structures are known (15–17). The amino acid residues at positions 8, 10, 11, and 13 are conserved to be Thr/Ser, Thr, Gly, and Thr, respectively. All of the O₅ atoms of the residues at positions 8, 10, and 13 participate in hydrogen bonds to the phosphate group in *C. crispus*, *A. nidulans*, Clostridium MP, and *D. vulgaris* flavodoxins. The α and β angles of G₁₁...
FIG. 7. Superposition of the Cα and FMN atoms. Two structures are overlayed so that the root mean square deviation of the corresponding Cα atoms in the β-strands is minimum. a, C. crispus flavodoxin (thick bonds) versus D. vulgaris flavodoxin (thin bonds); b, C. crispus flavodoxin (thick bonds) versus Clostridium MP flavodoxin (thin bonds).

The isoalloxazine ring in all flavodoxins whose structures have been elucidated is bound to the protein in such a way that the two methyl groups are exposed to solvent and the pyrimidine ring is buried in the protein. The orientation of the ring relative to the protein moiety in C. crispus flavodoxin is similar to those in D. vulgaris and A. nidulans flavodoxins, but is tilted by 24° in Clostridium MP flavodoxin. The residue at the upper side of the ring is tryptophan in C. crispus, A. nidulans (37), and D. vulgaris flavodoxins, but methionine in Clostridium MP flavodoxin. The lower residue is tyrosine in the former three flavodoxins, but is tryptophan in the latter flavodoxin, where the side chain is approximately parallel to the isoalloxazine ring.

The distributions of basic and acidic residues in C. crispus flavodoxin are shown in Fig. 8. These figures clearly show that both basic and acidic residues are localized on the surface of the molecule. Out of 11 lysine residues, 6 (K1, K27, K44, K83, K115, and K119) are concentrated on the opposite face of the molecule to the FMN-binding site (around the N-terminal end, shown at the upper right in Fig. 8a). This region will be referred to the lysine-rich region. The remaining 5 lysines and 3 arginines are randomly distributed on the molecular surface between the lysine-rich and the FMN-binding regions. No basic residue is located near the FMN-binding site. The side chain of R64, whose Cα position is rather close to the FMN-binding site, points toward the side chain of E107 in α4 (rightward in Fig. 8a, data not shown) and is remote from the FMN-binding site.

In contrast with the distribution of basic residues, the acidic residues are located mainly on the opposite surface of the molecule to the lysine-rich region. No acidic residue is located near the FMN-binding site. The side chain of R64, whose Cα position is rather close to the FMN-binding site, points toward the side chain of E107 in α4 (rightward in Fig. 8a, data not shown) and is remote from the FMN-binding site.

Distribution of Charged Residues and Electron Transfer—
It has been discussed that interactions between negatively charged residues at the vicinity of the FMN binding sites in flavodoxins and positively charged residues around the heme edges in cytochromes are involved in complex formation between these proteins (8, 9). Based on computer graphics analysis it has been proposed that D58, E62, E65, and E120 in Clostridium MP flavodoxin form salt linkages with basic residues in cytochrome c, and correspondingly that D62, E66, D69, D95, and D129 in D. vulgaris flavodoxin interact in cytochrome c5. However, the peptide segments including these acidic residues have a few conserved amino acid residues even when the sequences of the flavodoxins from bacteria are compared (Fig. 2 in Ref. 20).

Alternatively, we speculate that the molecular dipole is primarily important in alignment of the protein partners. Such a localization of charged residues is also observed for other flavodoxins, suggesting that the molecular dipole might be commonly present in flavodoxins. The acidic residues discussed in the Clostridium MP flavodoxin-cytochrome c complex (8) or D. vulgaris flavodoxin-cytochrome c5 complex (9) might be more or less important in individual cases. In the case of the cytochrome c-cytochrome-c peroxidase system, the cytochrome c molecules are disordered in the co-crystals (38). There appears no requirement for a restricted mode of complex for each system of flavodoxin and a given partner protein. Role of the molecular dipole and/or individual charged residues in the flavodoxin should be clarified in more detail with the methods of chemical modifications and site-directed mutagenesis as well as quantitative analysis of the charge on the molecular surface. For horse cytochrome c, results of reaction kinetics between its various derivatives and the partner proteins have been interpreted in terms of the molecular dipole of this cytochrome c (39).

It is noteworthy that a localization of charged residues are also observed in the chloroplast-type ferredoxins. The 2 lysine residues (K4 and K50 in Spirulina platensis ferredoxin, and K4 and K50 in Aphanothece sacrum ferredoxin) on the β-sheet at the opposite face of the iron-sulfur cluster are spatially conserved in all chloroplast-type ferredoxins. An electrostatic potential map of the molecular surface of A. sacrum ferredoxin has shown that the region around the cluster is rather negative and its opposite face is positively charged (40).
the surface area closest to the cluster is relatively hydrophobic. So these features may be common to ferredoxins and flavodoxins. Since they are functionally interchangeable, they may be similar interactions with partner proteins.

Acknowledgments—We thank Prof. Yukiteru Katsube for valuable discussions, Drs. Yoshiki Matsuura, Yasuo Hata, Masami Kusunoki, and Mamoru Sato for their kind help in executing computations, and Tohru Kimura for assistance in preparing the mercury derivative. We also thank the staff of the Research Center for Protein Engineering, Institute for Protein Research, Osaka University for the use of the x-ray equipment and the ACOS-300 computer.

REFERENCES

23. Iga, Y. (1966) MOLFIT, Institute for Protein Research, Osaka University
28. Iga, Y. (1966) MOLFIT, Institute for Protein Research, Osaka University
Tertiary structure of oxidized flavodoxin from an eukaryotic red alga Chondrus crispus at 2.35-A resolution. Localization of charged residues and implication for interaction with electron transfer partners.

K Fukuyama, S Wakabayashi, H Matsubara and L J Rogers