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*

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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 22.6% 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 (ββα)4 structure. The molecule incorporates a substantial insertion in βs, 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 phosphate group of FMN is located at the N-terminal end of a1, and forms extensive hydrogen bonds with the half of the molecule which includes the FMN-binding site, while about two-thirds of the total 35 acidic residues are located in the 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 chloroplast-type ferredoxins whose structures (10, 11) are quite different from those of bacterial ferredoxins (12–14).

The flavodoxin 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 25% 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-
STRUCTURE OF C. CRISPUS FLAVODOXIN

TABLE I

Summary of intensity measurements

|                      | Maximum resolution | No. of crystals | No. of measurements* | No. of independent reflections | Rmerge  | Reflections with F > 3σF | Σ|F_{platinum} - \langle F_{platinum} \rangle | Σ|F_{mercury} - \langle F_{mercury} \rangle |
|----------------------|--------------------|-----------------|----------------------|--------------------------------|---------|-------------------------|-----------------------------|-----------------------------|
| A                    | 2.35               | 5               | 26,800               | 7,768                          | 5.3     | 87                      | 11                          | 19                          |
| B                    | 2.70               | 4               | 12,500               | 5,175                          | 5.0     | 85                      | 11                          | 19                          |
| C                    | 2.70               | 4               | 15,300               | 5,194                          | 5.9     | 90                      | 19                          | 24                          |

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

**Intensities of Bijvoet pairs measured from each crystal were averaged first. The R_{merge} defined as Σ |F_{platinum} - \langle F_{platinum} \rangle | / Σ |F_{platinum}| is for the sets of averaged intensities from different crystals.

**The reflections with F > 3σF were used.

TABLE II

Heavy atom parameters and multiple isomorphous replacement (MIR) statistics

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R

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Phasing power

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<td>The phasing power of a derivative is the calculated strength of the heavy atom contribution divided by the estimate of the error in the analysis.</td>
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Intensity Measurements—Diffraction data for the native crystals and those of the platinum and mercury derivatives were measured 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. Each crystal was mounted on the diffractometer with its c axis parallel to the 002 axis of the diffractometer. The unit cell parameters and setting parameters were determined by a least squares method based on setting angles of 10 reflections (17° < 2θ < 20°). The reciprocal lattice points to 2.35 Å resolution for the native crystal and to 2.7 Å resolution for both derivative crystals were divided into five and four shells, respectively, each of which included several hundred overlapping points with adjacent shells. The intensities of hkl and hkl within a given shell were measured from one crystal by the ω-scan technique with 0.2° < 2θ < 20°. In addition, the intensities of hkl in the 20-2.25 Å resolution range were measured before and after the data collection. The intensities of three standard reflections were measured every 100 reflections to monitor crystal damage and/or alteration in the crystal setting.

Absorption correction was made by the method of North et al. (93) based on the intensities of 004 (0 ≤ θ < 360°). The resolution-depending decay for each crystal was estimated from the ratios of the structure factors of hkl measured at both the start and end points of data collection; the ratios were assumed to follow the equation of a + b(sinθ/λ)^2, and the two variables, a and b, 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 correction for the decay was made such that the values of a and b decreased linearly starting from 1.0 and 0.0, 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 possible origin for the platinum and mercury derivatives was determined from the difference Fourier map with the coefficient of m (F_{platinum} - F_{mercury} - \langle F_{platinum} \rangle ) - \text{exp}(iαN). Here F_{platinum} and F_{mercury} are the structure factors of the mercury derivative and the native crystals, respectively; m is the figure of merit; and αN 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 of Dickerson et al. (26). The occupancies were fixed, but the temperature factors were fixed at reasonable values. The heavy atom parameters and statistics at the final stage of the refinement are given in Table II. No significant peak due to the minor heavy atom site was observed in the isomorphous difference Fourier maps. The overall figure of merit for the data with 15° > d > 2.7 Å was 0.67.

The absolute configuration was determined by the anomalous scattering data for the mercury derivative (27). One of the two anomalous difference Fourier maps showed significant peaks at the mercury sites (Fig. 1). Contribution of the anomalous scattering for the mercury was included in the calculation of phase probability. However, the resolution was limited to 4.3 Å, because at higher resolution the signal of the anomalous scattering was less than the noise level as seen in the anomalous difference Fourier map.

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...
Structure of C. crispus Flavodoxin

immediately obvious in the best electron density map at 2.7Å resolution. The orientation of the isalloxazine 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 2F o-F o omit map with a microcomputer NEC PC-9801. When the R-factor (R = Σ[|F o| - |F c|]/Σ|F o|) reached 0.263 at 2.4Å resolution, a 2F o-F o 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 > 3σ(F) in the 6.0-2.35Å 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 RICRCSF, respectively) have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, N.Y.

RESULTS

Accuracy of the Structure—The 2F o-F c omit map for the isalloxazine 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 = z/2) of the anomalous difference Fourier map computed at 5Å resolution. The coefficient was m[F o(hkl) - F c(hkl)]exp[i(ωMN - ω/2)], where m and ωMN 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 21 at y = z/2.](http://www.jbc.org/)

![Fig. 2. An omit map superimposed on the isalloxazine ring of FMN. The Fourier coefficient was (2 |F o| - |F c|)exp[i(ωMN - ω/2)], where F o and ωMN 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).](http://www.jbc.org/)

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<td>0.013</td>
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- "Root mean square deviations from the standard geometry.
- Overall temperature factors.
- R-factor for the model built based on the MIR map.
- Average of individual temperature factors.
The polypeptide chain folds in such a way that $\beta$-strands and $\alpha$-helices occur alternately to form the typical $\alpha/\beta$ structure (32). The five parallel $\beta$ strands form a $\beta$-sheet, which is surrounded by five $\alpha$-helices. *C. crispus* flavodoxin has a one-turn helix between $\beta_2$ and $\beta_3$, in contrast to the other flavodoxins (15–17). Here we have given the numbers of $\alpha$-helices in sequential order from the $N$ terminus; $\alpha_3$, $\alpha_4$, and $\alpha_5$ in *C. crispus* flavodoxin correspond to $\alpha_4$, $\alpha_3$, and $\alpha_2$, respectively, in other flavodoxins. In the current model, the locations of $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_4$, and $\alpha_5$ in the peptide chain are 12–24, 40–45,
69–75, 104–113, and 158–171, respectively, whereas those of
\( \beta_1, \beta_2, \beta_3, \) and \( \beta_4 \) are 2–8, 28–32, 48–55, and 85–94, respectively.
Referring to other flavodoxins (15–17), \( \beta_1 \) is divided into three
pieces; viz. \( \beta_{\alpha} \) (G122-N125), \( \beta_{\beta} \) (G140-L143), and \( \beta_{\gamma} \) (L145-
D148). The strands \( \beta_{\alpha} \) and \( \beta_{\beta} \) are antiparallel and between
them there is a inserted sequence of 14 amino acid residues.
Except for these differences, \( \textit{C. crispus} \) flavodoxin has sub-
stantially the same secondary structures in the same spatial
arrangement as \( \textit{D. vulgaris}, \textit{Clostridium MP}, \) and \( \textit{A. nidulans} \)
flavodoxins. To one side of the \( \beta \)-sheet is located two \( \alpha \)-helices
(\( \alpha_1 \) and \( \alpha_3 \)) and on the other side three \( \alpha \)-helices (\( \alpha_2, \alpha_3, \) and \( \alpha_4 \)).

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

The distribution of the side chains in \( \alpha \)-helices is shown in
Fig. 4. Clear compartmentation in the distribution of hydro-
phobic and hydrophilic side chains is observed; the hydro-
philic side chains are exposed to solvent, whereas the hydro-
phobic side chains are buried within the molecule. The \( \beta \)-
sheet, in particular the central \( \beta_1, \beta_2, \beta_3, \) and \( \beta_4 \), 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
\( \alpha_1 \). As is well established, an \( \alpha \)-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 \( \alpha_1 \) 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 C6 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**—\( \textit{C. crispus} \) flavodoxin has
an unusually high proline content (10 residues compared to 3
residues in \( \textit{A. nidulans} \) flavodoxin). Their distribution is
shown in Fig. 6. Most of the proline residues are located at

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

Heavy Atom-binding Sites—Soaking the crystal in a solution of K$_2$PtCl$_4$ 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 PtCl$_2^-$ complex is modified by substitution of Cl by NH$_3$. 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 $\beta_5$ and the N-terminal end of $\alpha_6$, respectively. Both side chains are accessible to solvent, and are closely located (the distance between the two S$_\text{a}$ atoms is about 5 Å).

The reagent C$_2$H$_5$HgCl also binds to a single site with high occupancy. The site corresponds to C112 in $\alpha_6$, 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 (Synechococcus 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 C$_\alpha$ 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 $\beta_5$ compared to the short-chain flavodoxins; these residues, none of which is in contact with FMN, form a loop beneath the $\beta$-sheet. Besides the substantial insertion in $\beta_5$, Fig. 7 shows that insertion/deletion of residues occur frequently in the loops between the $\beta$-strands and $\alpha$-helices. The characteristic difference in the secondary structure which is found in C. crispus flavodoxin is that this has a one-turn $\alpha$-helix between $\beta_2$ and $\beta_3$.

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. These regions correspond to the strands of $\beta_1$, $\beta_2$, $\beta_4$, and $\beta_5$ and are in the vicinity of the phosphate group of FMN. In contrast, the sequences corresponding to $\alpha$-helices show little sequence identity. As described earlier, the $\alpha$-helices surround the $\beta$-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 tertiary 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 $\alpha_6$-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 O$_\text{a}$ 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 $\phi$ and $\psi$ angles of G11

![Fig. 6. Distribution of proline residues in C. crispus flavodoxin.](http://example.com/fig6.png)
FIG. 7. Superposition of the C, and FMN atoms. Two structures are overlayed so that the root mean square deviation of the corresponding C, 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).

are 108 ° and −11 °, respectively, in C. crispus flavodoxin, a conformation which allows only for a glycine residue. This may be why residue 11 is conserved as glycine in all these flavodoxins. On the other hand, the residues at positions 9 and 12 are not conserved; for these, only the NH of the main chain participates in hydrogen bonding. Thus a change of amino acid at these positions will not primarily affect the binding between the protein and FMN.

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.

Distribution of Charged Residues and Electron Transfer—

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 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 lysine 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 C, position is rather close to the FMN-binding site, points toward the side chain of E107 in α (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. When the molecule is divided into two halves, the half including the FMN-binding site, points toward the side chain of E107 in α (rightward in Fig. 8b, data not shown) and is remote from the FMN-binding site.
ization of basic and acidic residues may produce a molecular dipole.

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 K90 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). In addition,
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.

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