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Structural Alignment of Ferredoxin and Flavodoxin Based on Electrostatic Potentials: Implications for Their Interactions With Photosystem I and Ferredoxin-NADP Reductase

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ABSTRACT **The two proteins ferredoxin and flavodoxin can replace each other in the photosynthetic electron transfer chain of cyanobacteria and algae. However, structure, size, and composition of ferredoxin and flavodoxin are completely different. Ferredoxin is a small iron-sulfur protein (~100 amino acids), whereas flavodoxin is a flavin-containing protein (~170 amino acids). The crystal structure of both proteins from the cyanobacteria *Anabeana* PCC 7120 is known. We used these two protein structures to investigate the structural basis of their functional equivalence. We apply the Hodgkin index to quantify the similarity of their electrostatic potentials. The technique has been applied successfully in indirect drug design for the alignment of small molecule and bioisosterism elucidation. It requires no predefined atom-atom correspondences. As is known from experiments, electrostatic interactions are most important for the association of ferredoxin and flavodoxin with their reaction partners photosystem I and ferredoxin-NADP reductase. Therefore, use of electrostatic potentials for the structural alignment is well justified. Our extensive search of the alignment space reveals two alignments with a high degree of similarity in the electrostatic potential. In both alignments, ferredoxin overlaps completely with flavodoxin. The active sites of ferredoxin and flavodoxin rather than their centers of mass coincide in both alignments. This is in agreement with electron microscopy investigations on photosystem I cross-linked to ferredoxin or flavodoxin. We identify residues that may have the same function in both proteins and relate our results to previous experimental data. Proteins 2000;38:301–309.** © 2000 Wiley-Liss, Inc.

Key words: photosynthesis; electrostatic alignment; similarity index; docking; electron transfer; physiologically equivalent proteins

INTRODUCTION

The [2Fe-2] protein ferredoxin (Fd) serves as a soluble electron carrier in the light phase of photosynthesis. It transports electrons between photosystem I (PSI) and ferredoxin-$NADP^+$ reductase (FNR) in the stroma of chloroplasts and in cyanobacteria.[1] FNR uses the electron received from two Fds to reduce $NADP^+$, which is required to synthesize carbohydrates in the dark reactions of photosynthesis. Ferredoxin is also involved in the cyclic electron transport, which leads to an increase of the pH gradient between the stroma and the thylakoid space and so finally to an increased production of ATP.[2] In addition, Fd delivers electrons to other proteins such as nitrite reductase, sulfate reductase, glutamate synthase, and ferredoxin-thioredoxin reductase.[1] Therefore, Fd plays a central role for many redox reactions in chloroplast and cyanobacteria and in the regulation of photosynthesis. Under conditions of iron deficiency, the flavin-containing protein flavodoxin (Fld) can replace the iron-sulfur protein Fd in some reactions in most cyanobacteria and some eukaryotic algae.[3] The electron transfer reactions of Fd with its reaction partners have been investigated extensively (for review see Knaff and Hirasawa[1]). Much less is studied on the electron-transfer reactions of Fld with its reaction partners (Navarro et al.[4] and references cited therein).

The structures of various plant-type Fds[5–10] and Flds[11,12] have been determined by X-ray crystallography and by nuclear magnetic resonance spectroscopy. For the cyanobacteria *Anabeana* PCC 7120, the structures of Fd[7] and Fld[12] are known. There are only minor structural variations among different plant-type Fds and among cyanobacterial and algal Flds. The structures of Fd and Fld from the same species differ, however, completely. In fact, they differ not only in structure and cofactor but also in size. Fd is with about 100 amino acids smaller than Fld with about 170 amino acids. Fd and Fld share neither a common secondary structure nor a common tertiary fold as shown in Figure 1, but both possess a negatively charged surface patch through which they interact electrostatically with positively charged surface areas at PSI and FNR.

The redox potential of Fd from *Anabeana* PCC 7120 for

Abbreviations: FNR, ferredoxin-NADP reductase; Fd, Ferredoxin; Fld, Flavodoxin; PSI, photosystem I.

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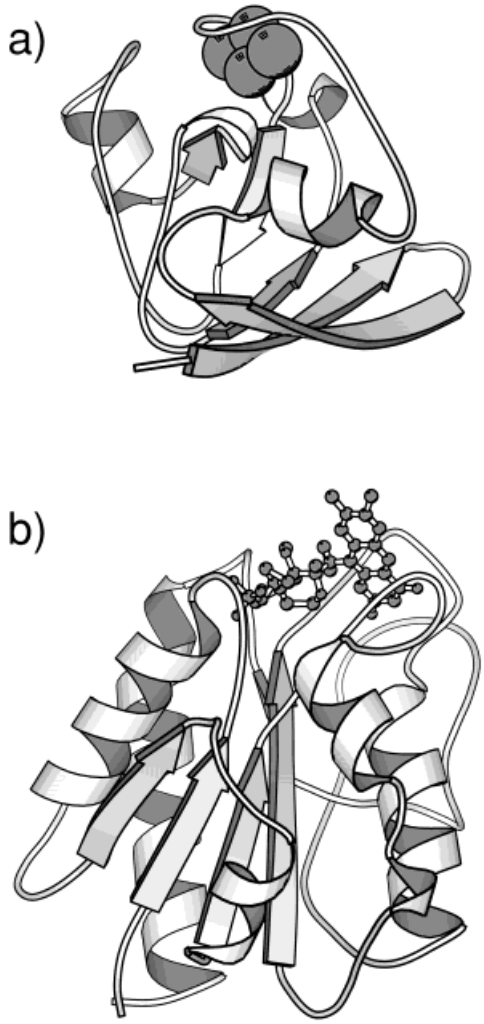

Fig. 1. Structures of ferredoxin and flavodoxin. Although both proteins differ in structure and size, they perform the same physiological function.

the redox couple $Fe^{II}Fe^{III}/Fe^{III}Fe^{III}$ is −430 m V.[13] The fully oxidized form of native Fd has never been detected[14] and is, therefore, believed to play no physiological role. The redox potential for a single oxidation step of the fully reduced form of Fld at pH = 7.0 is −436 m V, that of the semireduced form is −212 m V.[15] For the redox potential, it is believed that Fld alternates between the semireduced and fully reduced state in the photosynthetic electron transfer chain, although this has not been shown rigorously.[16] The flavin in Fld is sandwiched between Trp57 and Tyr94. The π-stacking interaction between these two aromatic rings of the amino acid side chains and the aromatic ring of the flavin causes a strong binding of the cofactor and also influences the redox potential of flavin.[17,18]

The similarity of proteins is usually defined on the basis of their primary, secondary, and tertiary structure. Sequence- and structure-based alignment algorithms have been developed to find the best structural correspondence between proteins and to reveal evolutionary relationships and common folding arrangements. Structurally conserved amino acids, if detected in active sites of proteins, allow deeper insights in the function and biological role of the biomolecules compared. It is well accepted that functional informations may also be derived from structural similarities.

Fd and Fld differ completely in their primary, secondary, and tertiary structure. Therefore, these characteristics cannot be used to compare these two nonhomologous, but physiologically equivalent proteins. It is surprising that identical biological function does not require similar structures to operate in vivo. However, common structural features might be present in each of the electron-carrier protein families. This raises the question, how structurally different proteins achieve equivalent biological function in practice. Molecular association processes are often steered by electrostatic interactions. Both Fd and Fld might, therefore, have equivalent electrostatic potentials to guide the association with their reaction partners. In this case, a similarity measure should be capable to quantify the electrostatic similarity of Fd and Fld. Furthermore, it should be possible to align the two proteins by optimizing their electrostatic similarity. These alignments can provide new insights into the molecular recognition of Fd and Fld by their reaction partners and allow a better interpretation of the electron transfer process.

We use the Hodgkin index as similarity measure. The Hodgkin index was originally introduced as a similarity measure for small molecules. It is used for the alignment of isofunctional drug molecules, for three-dimensional similarity search or pharmacophore analysis.[19–21] The Hodgkin index measures the similarity of two molecules by evaluating the normalized spatial overlap integral for property-based potentials. Because the importance of the electrostatic effects for molecular association of electron-transfer proteins has been pointed out earlier, we decided to use the electrostatic potential as distribution function. Recently, we used the Hodgkin index to measure and optimize the similarity of the Coulomb electrostatic potentials of two other electron-carrier proteins, namely the two functionally equivalent electron-carrier proteins plastocyanin and cytochrome c_6.[22] The obtained alignments could be interpreted in agreement with existing theoretical[23] and experimental[24–27] studies. Furthermore, we validated the alignment method and the usefulness of the similarity measure by aligning homologue and nonhomologue proteins.[22] Wade et al.[28,29] used also a similarity index to compare protein electrostatic potentials quantitatively. By using our implementation it is not only possible to compare protein electrostatic potentials quantitatively but also to compute optimal protein alignments. These alignments correspond to maxima on the similarity hypersurface. The accessible alignment space is carefully searched by applying a rigorous sampling method to ensure identification of all local Hodgkin index maxima.

In this study, we superimpose Fd and Fld by optimizing the overlap of their electrostatic potentials without any prior assumptions of the binding mode. The obtained alignments are correlated with structural and other experi-

mental data that are available for the interaction of Fd and Fld with PSI and with FNR. The study gives new insights into the structural basis of the physiological equivalence of the two electron-transfer proteins Ferredoxin and Flavodoxin and may guide new experiments.

MATERIALS AND METHODS

Protein Structures

Properties of isofunctional proteins are likely to be most similar if they are from the same organism. Therefore, Fd and Fld from *Anabeana* PCC 7120 is best suited because the structures of both proteins are known.[7,12] We used the Protein Data Bank (PDB) entry 1fxa for Fd and the PDB entry 1rcf for Fld in our computations and added all hydrogen atoms to both proteins.

For the superposition of electrostatic potentials with the FAME algorithm,[22,30] we deleted all water molecules contained in the crystal structure. They may contribute nonspecifically to the overall electrostatic potential, because their position depends often on the crystalline environment and their orientation is uncertain. Only one of the two rotamers of the 16 disordered residues of Fld (those marked A in the PDB entry) were used. The PDB entry 1fxa includes two molecules in the unit cell. We used only chain A.

In the calculations of electron-transfer path, all crystal waters were considered. Moreover, we added solvent by placing Fd and Fld separately in the center of a water sphere with a diameter of 23 and 27 Å, respectively. We removed all water molecules that come in sterical conflict with the protein or water molecules in the crystal. The energy of the system created by this procedure was minimized by using the program CHARMM.[31] The water molecules were free, whereas all nonhydrogen atoms in the protein structures were fixed during the energy minimization. Waters are needed in these type of calculations, because empty hydrophilic cavities in the protein that are present in crystal structures are usually filled with water. These water molecules are often not seen in crystal structures but can participate in electron transfer paths.

Atomic Charges

The charges of most atoms were taken from the CHARMM22 parameters.[31,32] Atomic charges for the [2Fe-2S] center in Fd were taken from the literature.[33] Charges of titratable groups are those at pH 8.0 assuming standard pK_a values. The physiological pH value for Fd and Fld is 8.0. The structural superpositions were performed with the fully oxidized forms of Fd and Fld, in which the charge of both proteins is −17. Fully oxidized Fd interacts with PSI. Although it is likely that Fld interacts with PSI in its semireduced form, we used the fully oxidized form of Fld in the superposition. Because the charge of the additional electron of the semireduced form of Fld is neutralized by a proton,[34] the electrostatics of the fully oxidized and semireduced form of Fld are similar.

Electrostatic Potential-Based Matching of Protein Structures

We demonstrated previously[22] that similarity indices that are widely used as a similarity measure of molecules in drug design[19,20] can be useful in comparing isofunctional proteins having different tertiary structures. Here, we summarize only the key points of the method to superimpose proteins on the basis of their electrostatic similarity. For a detailed description of the so-called FAME method and additional features of the program see elsewhere.[30]

For defining the similarity of two molecules, we use the integral-based Hodgkin index H_{ab}^{elec}, defined in Eq. 1.

$$H_{ab}^{elec} = \frac{2\int \phi_a \phi_b dV}{\int \phi_a^2 dV + \int \phi_b^2 dV} \tag{1}$$

The Coulomb potentials ϕ of the structurally different molecules a and b are integrated over the whole volume. The numerator quantifies the spatial overlap of the electrostatic potentials ϕ, whereas the denominator normalizes this value. The resulting similarity index falls in the interval between −1 and +1. The value +1 corresponds to molecules with identical potentials, whereas −1 corresponds to electrostatic complementarity; this means potentials of the same magnitude but opposite sign. The electrostatic potential ϕ at the position $\mathbf{r}$ in a medium with the dielectric constant ε is calculated from point charges q_i assigned to each atom i at position $\mathbf{r_i}$ as given by Eq. 2.

$$\phi(\mathbf{r}) = \sum_{i=1}^{n} \frac{q_i}{\varepsilon|\mathbf{r} - \mathbf{r}_i|} \cong \sum_{i=1}^{n} \frac{q_i}{\varepsilon} \sum_{\mu=1}^{k} G_i^{\mu}(\mathbf{r}) \tag{2}$$

$$G_i^{\mu}(\mathbf{r}) = \gamma_{\mu} \exp(-\alpha_{\mu}(\mathbf{r} - \mathbf{r}_i)^2) \tag{3}$$

The $1/r$ term of the Coulomb law can be approximated by a sum of spherical Gaussian functions (Eq. 3). This approximation avoids the singularities of the Coulomb potential and makes the computation approximately 100 times faster than those involving grid evaluation.[35] In general, two Gaussians are sufficient to fit a $1/r$ curve within a range of 12 Å. By applying standard least-squares fitting techniques with a spherical shell weighting function,[36] we obtained the following parameters: $\alpha_1 = 0.1247$ Å^{-2}, $\alpha_2 = 0.0065$ Å^{-2}, $\gamma_1 = 0.5168$ Å^{-1}, and $\gamma_2 = 0.1958$ Å^{-1}. With these parameters, the Hodgkin index H_{ab}^{elec} accounts mainly for the electrostatic potentials outside the two protein molecules which is relevant for protein association. The series of integrals reduces to spherical Gaussians (Eq. 4) with modified prefactors (Eq. 5) and exponents (Eq. 6), which depend only on the pairwise interatomic distances between the two proteins, i.e., on the relative orientation of these molecules.

$$G_{ij}^{\mu v}(\mathbf{r}_i, \mathbf{r}_j) = \int_V (G_i^{\mu} G_j^{v}) dV = \gamma_{ij}^{\mu v} \exp(-\alpha_{ij}^{\mu v}(\mathbf{r}_i - \mathbf{r}_j)^2) \tag{4}$$

$$\gamma_{ij}^{\mu v} = \gamma_i^{\mu} \gamma_j^{v} \left(\frac{\pi}{\alpha_i^{\mu} \alpha_j^{v}} \right)^{\frac{3}{2}} \tag{5}$$

$$\alpha_{ij}^{\mu v} = \frac{\alpha_i^{\mu}\alpha_j^{v}}{\alpha_i^{\mu} + \alpha_j^{v}} \quad (6)$$

Thus, the Hodgkin index can be expressed by Eq. 7 (μv is used as a united index in Eq. 8).

$$H_{ab}^{elec} = \frac{2S_{ab}}{S_{aa} + S_{bb}} \quad (7)$$

where

$$S_{ab} = \sum_{i=1}^{n_a}\sum_{j=1}^{n_b} q_i q_j \sum_{(\mu v)=1}^{k_a \times k_b} G_{ij}^{\mu v}(a,b). \quad (8)$$

The analytical formula for the Hodgkin index in Eq. 7 can be evaluated extremely rapidly, and no singularities exist in this approximation. In a homogeneous medium, the Hodgkin index based on the Coulomb electrostatic potential is independent of the dielectric constant, because the dielectric constant cancels in Eq. 1. Previously, we have shown that values of the Hodgkin index higher than 0.8 indicate a high similarity of electrostatic potentials, whereas values lower than 0.6 indicate a low similarity for proteins.[22]

We started from 100 different random initial orientations of the two proteins and maximized the Hodgkin index (Eq. 1) for translational and rotational degrees of freedom to ensure to find the largest maximum several times. Then the highest ranked alignment can be taken to represent the global maximum of the Hodgkin index. The rotations were parameterized in quaternions, as previously done in SEAL.[37] Quaternions behave correctly if the rotation matrix degenerates to the identity transformation, whereas Euler angles are undetermined in this case.[38] The FAME method uses the very efficient eigenvector-following algorithm[39] for optimizations, which takes advantage of analytic first and second derivatives of the function H_{ab}^{elec}. A maximization was considered as converged and was therefore terminated if the norm of the gradient became $<10^{-10}$.

Calculation of Electronic Couplings Between the Redox Sites and Surface Atoms

The theoretical basis and the algorithm of the Pathways method are described elsewhere.[40,41] Here, we only describe the parameters used in this study.

An electron-tunneling path is a trace of interacting covalent bonds, hydrogen bonds, and Van der Waals contacts (interactions through space) that connects the donor with the acceptor. The decay parameters for attenuation of electronic coupling via bonds and contacts are taken from the literature.[40] We neglected the attenuation within aromatic rings of flavin mononucleotid phosphate, histidine, phenylalanine, tyrosine, and tryptophane, within the guanidinium group of arginine, and within the iron-sulfur center of Fd by setting the decay parameter to 1.0 within these groups. The bonds between the iron atoms and cystein sulfur ligands were treated as a ususal bond, i.e., its decay parameter was set to 0.6. For crystal and solvent water, which were also considered in our calculation, we used the parameters described previously.[42]

RESULTS AND DISCUSSIONS

Electrostatic Alignment of Ferredoxin and Flavodoxin From *Anabaena* PCC 7120

We superimposed Fd and Fld by maximizing their Hodgkin index (Eq. 1) by using a detailed representation of their electrostatic potentials. Each of 100 optimizations started from a different random initial orientation. The search yielded four different alignments, which were found several times from different starting positions. The alignments with the same value for the Hodgkin index were identical within a root mean square deviation of 0.001 Å for all heavy atoms in the alignment. All of them have Hodgkin index values >0.9, indicating a high degree of similarity. Two of the alignments overlap only partially and are thus not interpretable. Namely, the acidic regions of Fd and Fld, i.e., the putative docking region, overlap, whereas the remainder of the proteins does not. These two alignments with poor Van der Waals overlap are related to each other by a rotation of about 180°. In the remaining two alignments, Fd and Fld overlap completely but not concentrically. The latter two alignments are also related to each other by a rotation of about 180°. Because a meaningful interpretation of the alignments that show only a poor Van der Waals overlap is not possible, we will restrict the following discussion to the two alignments that show a high Van der Waals overlap.

The values of the Hodgkin index for the two alignments that show a high Van der Waals overlap are 0.94 and 0.92. In the following we will call these alignments, alignment 1 and alignment 2, respectively. The large values of the Hodgkin index indicate a high degree of similarity of the two proteins. Out of 100 optimizations, 31 and 30 minimizations ended at alignment 1 and alignment 2, respectively. Hence, the highly ranked alignments had also the largest convergence radii. The two alignments of Fd and Fld, the electrostatic potentials of Fd and Fld, and their relative electronic coupling are shown in Figure 2. The residues in these two proteins that may have a similar function in the recognition of the reaction partners are listed in Table I and are depicted in Figure 3. Two residues are considered to be isofunctional, if the distance of their acidic groups in the alignments is <6.5 Å. This distance seems large at the first glance but can easily be bridged by a reorientation of the amino acid side chains. Also, the redox centers of Fd and Fld overlap in both alignments. Namely, the iron sulfur center is at the same position as residue Trp57, which makes a π-stacking interaction with the flavin of Fld.

The first alignment shows an interesting feature. The α-helix (68–73) in Fd superimposes with the α-helix (149–166) in Fld. Both α-helices have the same orientation. The negative pole of the dipole moment of the α-helices points, however, away from the putative docking site and does thus not contribute constructively to the negative electrostatic potential at the docking site. Acidic residues that are part of the α-helix in Fd have been indicated to be involved in the association of Fd to FNR.[43] Possibly the shape of the α-helix is important for the

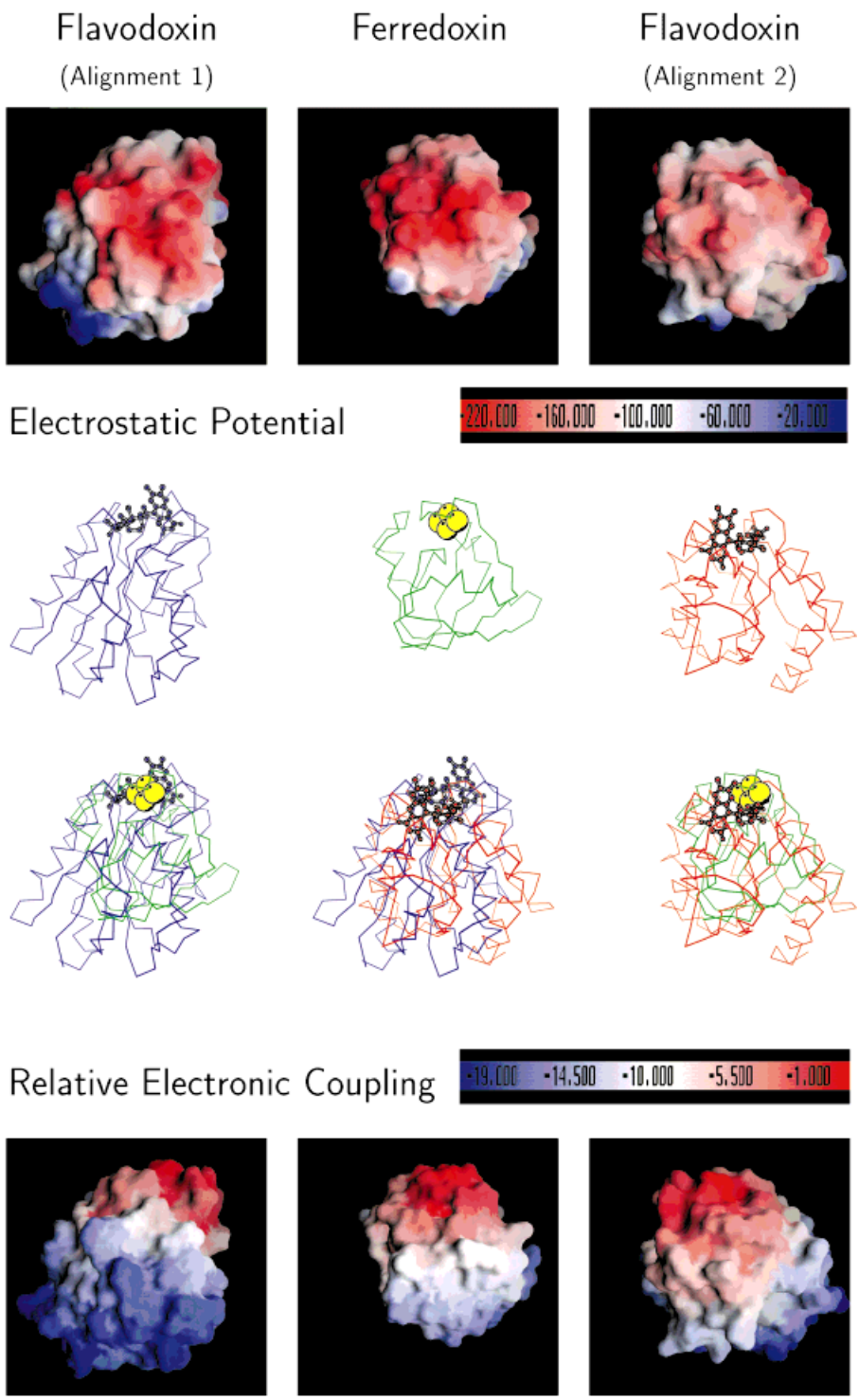

Fig. 2. Properties of ferredoxin and flavodoxin from *Anabeana* PCC 7210 that are relevant to the interprotein electron-transfer reaction. The alignments of the two proteins that correspond to the best match of their electrostatic potentials is shown in the middle of the figure. The separate proteins are kept in the orientation defined by the optimal structural alignment. **First row:** electrostatic energy of a unit test charge mapped to the protein surface. The electrostatic energy was calculated with the uniform dielectric constant of 4. The color is calibrated in the units of k_BT, $T = 298$ K. **Second row:** C_α-traces of ferredoxin and flavodoxin. The redox centers are high-lighted. **Third row:** Superpostion of ferredoxin and flavodoxin. In the left and right picture alignment 1 and alignment 2 are shown, respectively. Ferredoxin (green) is show in the same orientation in both pictures. In the middle picture, the orientation of flavodoxin for the two alignments is shown. Ferredoxin is omitted for the sake of clarity. **Fourth row:** Electronic coupling between surface amino acid residues and the active centers mapped to the protein surface. The decadic logarithm of the square of the relative couplings, $\log_{10}((\gamma_{DL}^2 \Pi \varepsilon_i)^2)$, is mapped onto the molecular surface of the proteins. Strongest coupling is shown in red and the weakest in dark blue.

TABLE I. Putatively Corresponding Residues in Ferredoxin and Flavodoxin From *Anabaena* PCC 7120 Identified in Two Superpositions Obtained by Optimizing the Match of Electrostatic Potentials.[†]

	Flavodoxin	
Ferredoxin	Alignment 1	Alignment 2
Glu19	—	**Asp123**
Asp22, Asp23, Glu24	Glu72, Asp43, Glu40	**Asp126, Asp129**
Glu94, Glu95	Asp90, Asp96, Asp129	Asp35, Glu16
Asp28, **Glu31,** Glu32	Asp65, **Glu67**	**Asp144, Glu145, Asp146**
Asp67, Asp68, Asp69, Glu72	**Glu145,** Asp150, Asp153, Asp154	**Glu67,** Glu72
C-Terminus	**Glu126**	—
Fe_2S_2	**Trp57**	**Trp57**
Ser47	Tyr94	Thr10
Arg42	—	Lys14
Helix 68–73	Helix 149–166	—

[†]Residues for which an involvement in the association reaction was implied experimentally are marked in bold face.

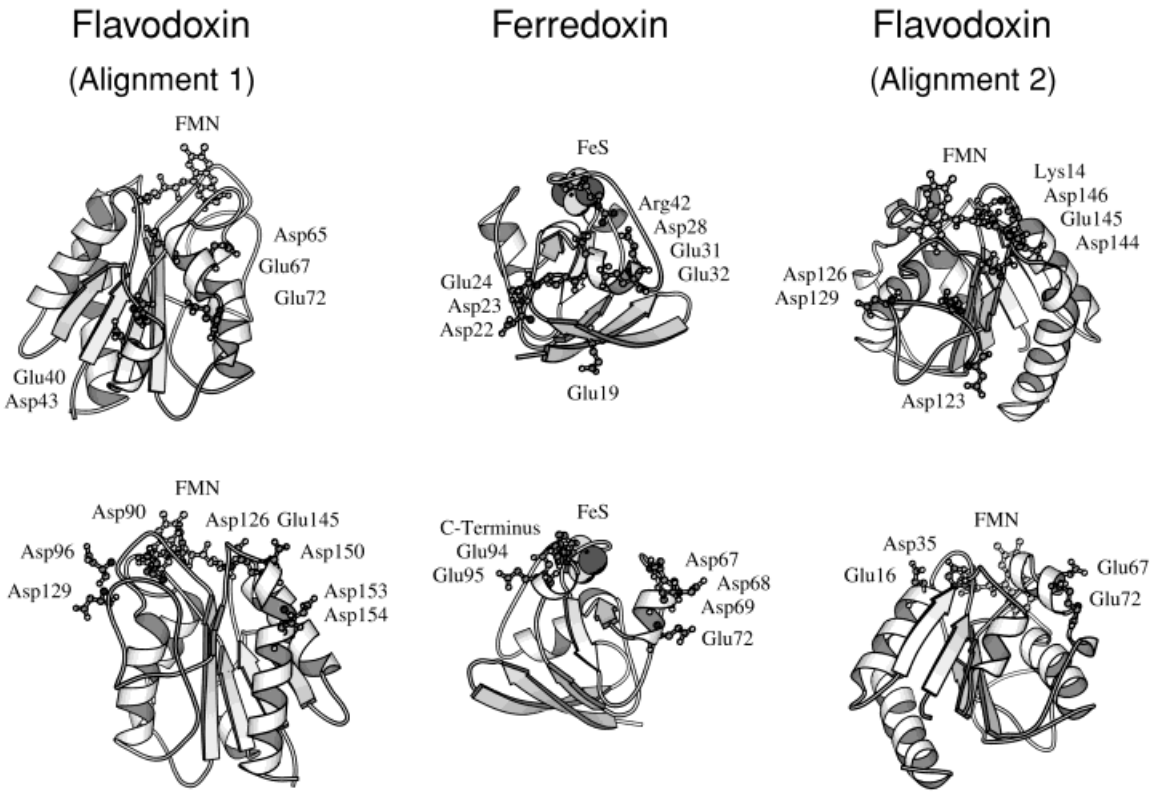

Fig. 3. Similarity between the acidic patches of flavodoxin and ferredoxin. The molecules in the lower row are rotated by 180° for the molecules in the upper row. Residues that are putatively important are highlighted.

recognition rather than its contribution to the electrostatic potential at the binding site.

Arg42 of Fd is a conserved, positively charged residue within a negatively charged region. This residue may influence the redox properties of the iron-sulfur cluster, but it may also take care of a specific recognition mechanism of the reaction partners. It is interesting that Arg42 superimposes with Lys14 of Fld in alignment 2. Lys14 of Fld is the only positively charged residue within a negatively charged region. In other sequences of Fld, this lysine is replaced by a asparagine, which can also work as a hydrogen bond donor. Possibly, a hydrogen-bond donor is required at this position to provide specific recognition of Fd and Fld by the redox partners.

Relation to Experimental Studies on the Interaction of Fd and Fld With PSI

Fd and Fld can be chemically cross-linked to PSI.[44,45] The cross-linked complexes have been investigated by electron microscopy.[46,47] Because the electron-transfer rates of the cross-linked complexes are similar to those of

the free electrostatic complexes, the cross-linked complexes are likely to resemble these electrostatic complexes.[46,47] The binding site identified by electron microscopy is in agreement with a previously proposed structural model[48] of Fd docked to PSI that is based on the 6 Å electron density map[49] of PSI from *Synechococcus elongatus.* It is, however, not possible to decide by using the electron microscopy pictures whether alignment 1 or alignment 2 is correct. Fd and Fld dock at the same binding site and the electron densities of both proteins in the electron microscopy pictures overlap almost completely. However, their electron densities are not arranged concentrically, which may indicate that both proteins try to arrange their prosthetic groups as close as possible to the terminal electron acceptor in PSI.[47] This interpretation is in agreement with the orientation found in our alignment of Fd and Fld where Fd and Fld superimpose also not concentrically. Nevertheless, their active sites superimpose in our alignments. Apparently, Fd and Fld superimpose in the same manner in the electron microscopy pictures and in our alignments.

The cross-linking of Fd to PS I takes place between Glu93 of Fd from *Synechocystis* (Glu95 in Fd from *Anabaena* PCC 7120) and Lys 106 of subunit PsaD of PSI.[50] Mutation studies on Fd from *Anabaena* PCC 7120 reveal that Glu31, Arg42, Thr48, Asp67, Asp68, Asp69, Glu94, and Glu95 influence the bimolecular electron transfer from PSI to Fd and are thus involved either in the binding process or in the electron transfer.[4] The residues Trp57, Glu61, Glu67, Asp126, and Glu145 of Fld influence its electron transfer reaction with PSI[4] and are thus believed to be involved in the interaction with PSI. These residues of Fd and Fld superimpose in the alignment found by optimizing the Hodgkin index (see Table I and Fig. 3).

Relation to Experimental Studies on the Interaction of Fd and Fld With FNR

The proteins Fd and FNR can be chemically cross-linked mediated by carbodiimides. The residues Glu92 of spinach Fd (Glu94 in *Anabaena* Fd) and the residue Lys85 or Lys86 of spinach FNR (Lys69 or Lys72 in FNR from *Anabaena* PCC 7120) were identified as cross-linking sites.[51] Differential chemical modification studies on spinach Fd suggest that Asp26 (Asp28 in Fd from *Anabaena* PCC 7120), Glu29 (Glu31), Glu30 (Glu32), Asp34 (Asp36), Asp65 (Asp67), and Asp66 (Asp68) are buried at the interface, because these residues are protected against chemical modification in the associated complex.[52] The same approach revealed that in spinach FNR, Lys18 (not present in FNR from *Anabaena* PCC 7119), Lys33 (Arg16), Lys35 (not present), and Lys153 (Lys138) are buried in the interface of the protein complex.[53] Also, arginines are involved in the association of Fd and FNR.[54] Concluding from differential chemical modification studies and from a modeling study it was proposed that Asp26 (Asp28), Glu29 (Glu31), Glu30 (Glu32), and Asp34 (Asp36) of spinach Fd interact with Lys304 (Lys293) and Lys305 (Lys294) of spinach FNR and that Asp65 (Asp67) and Asp66 (Asp68) of spinach Fd interact with Lys33 (Arg16), Lys35 (not present), Lys91 (Lys75) and Arg93 (Arg77) of spinach FNR.[52] Earlier studies also imply that these residues of Fd participate in the association.[55] The modifications of the residues Asp67, Asp68, Asp69, Thr48, and Arg42 also affect the second-order rate constant of the electron-transfer reaction.[13,56] The mutants Asp62Lys, Asp68Lys, Gln70Lys, Glu94Asp, Glu95Lys, Phe65Tyr, and Ser47Thr modulate the second-order rate constant of the electron transfer reaction.[57] The mutants Glu94Lys, Phe65Ile, Phe65Ala, Glu94Gln, and Ser47Ala are virtually not able to transfer electrons to FNR.[13,57,58] It was shown that Fd requires an aromatic amino acid at position 65 for an efficient electron transfer.[58]

Fld and FNR from *Anabaena* PCC 7119 can be cross-linked in 1:1 stoichiometry.[59] Chemical modification studies on Fld from *Anabaena* PCC 7119 suggest that the residues Asp123, Asp126, Asp129, Asp144, Asp145, and Asp146 of Fld interact with FNR.[60] Also, arginine residues of Fld are involved in the interaction with FNR.[61] Mutation studies reveal the involvement of residues Asp126 and Glu67 in the association reaction.[4] The redox potential of Fld and of FNR are affected because of the association.[15]

The residues of Fd and Fld that have been identified to be involved in the interaction with FNR superimpose in both alignments found in this study. The residues are listed and compared in Table I and are shown in Figure 1.

CONCLUSIONS

In this study, we have compared the two isofunctional electron-transfer proteins ferredoxin and flavodoxin on the basis of their electrostatic potentials. An algorithm usually applied to align pharmacologically equivalent but structurally dissimilar drugs has been used to superimpose two proteins. We used the Hodgkin index as a quantitative measure of the similarity of both proteins. We found two different structural alignments that showed both a high degree of similarity. The two alignments are related to each other by an approximately 180° rotation. Most likely, only one alignment is physiologically relevant. It is, however, also possible that one alignment represents the superposition of Fd and Fld at the binding site of one reaction partner, whereas the other alignment represents the superposition at the binding site of another reaction partner. On the basis of these alignments, we proposed residues that may play a similar role in the function of these proteins.

Residues that are known from experiments to be involved either in the docking reaction or in the electron transfer reaction superimpose in both alignments. Furthermore, in alignment 1 an α-helix in Fd superimposes with an α-helix in Fld. The shape of the α-helix may be important for the association of Fld or Fd with it reaction partners. In alignment 2, two positively charged residues, namely Arg42 of Fd and Lys14 of Fld, superimpose. They are the only positively charged residues in a broad acidic patch. Thus, it may be possible that these residues are important for the specific recognition of Fd and Fld by its reaction partners.

We hope that the present study stimulates new experi-

ments to investigate the functional equivalence of Fd and Fld. Hopefully, our predictions that residues putatively correspond to each other in Fd and Fld will be tested experimentally by systematic mutation studies in the future.

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