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Review

Flavodoxins: sequence, folding, binding, function and beyond

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Abstract. Flavodoxins are electron-transfer proteins involved in a variety of photosynthetic and non-photosynthetic reactions in bacteria, whereas, in eukaryotes, a descendant of the flavodoxin gene helps build multidomain proteins. The redox activity of flavodoxin derives from its bound flavin mononucleotide cofactor (FMN), whose intrinsic properties are profoundly modified by the host apoprotein. This review covers the very exciting last decade of flavodoxin research, in which the folding pathway, the structure and stability of the apoprotein, the mechanism of FMN recognition, the interactions that stabilize the functional complex and tailor the redox potentials, and many details of the binding and electron transfer to partner proteins have been revealed. The next decade should witness an even deeper understanding of the flavodoxin molecule and a greater comprehension of its many physiological roles. The fact that flavodoxin is essential for the survival of some human pathogens could make it a drug target on its own.

Keywords. Flavodoxin, protein folding and stability, protein ligand interaction, electron transfer, ferredoxin, FNR, *Helicobacter pylori*, drug target.

Introduction

Flavodoxins are small electron transfer proteins that contain one molecule of non-covalently but tightly bound flavin mononucleotide (FMN) as the redox active component. They were discovered in the 1960s in cyanobacteria [1] and clostridia [2] growing in low-iron conditions, where they replaced the iron-containing ferredoxin in reactions leading to $NADP^+$ and N_2 reduction, respectively. Although these remain perhaps their better-known physiological roles and those that have fuelled the bulk of flavodoxin research, it should be emphasized that flavodoxins are involved in a variety of reactions and that in some organisms they are essential, constitutive proteins. The early studies, centred in characterizing flavodoxin physicochemical properties, including its peculiar redox potentials, its interaction with partners such as ferredoxin(flavodoxin)-$NADP^+$ reductase (FNR), the reversible removal of FMN, and the first three-dimensional structures, are reviewed in [3–4]. Since then, two reviews specifically devoted to the flavodoxins from *Anabaena* PCC 7119 [5] and *Azotobacter vinelandii* [6] have appeared. The revolution brought about by the invention of site-directed mutagenesis has provided the essential tool needed to investigate many aspects of flavodoxin structure and function at atomic detail, and the old issues have been vigorously revisited during the 1990s and in this century. In addition, the renewed interest of the biophysical community in protein stability, protein folding and protein ligand recognition has stimulated the use of several flavodoxins as models to understand the specific details of the pathway by which the synthesized flavodoxin polypeptide becomes functional and also the fundamentals of these processes, which are common to every protein. Finally, some flavodoxins have found practical applications in diverse fields, such as in the determination of iron deficiencies in phytoplankton or the design of mixed protein/metal nanostructures [7–8], while those from pathogenic bacteria are being investigated as potential drug targets [9].

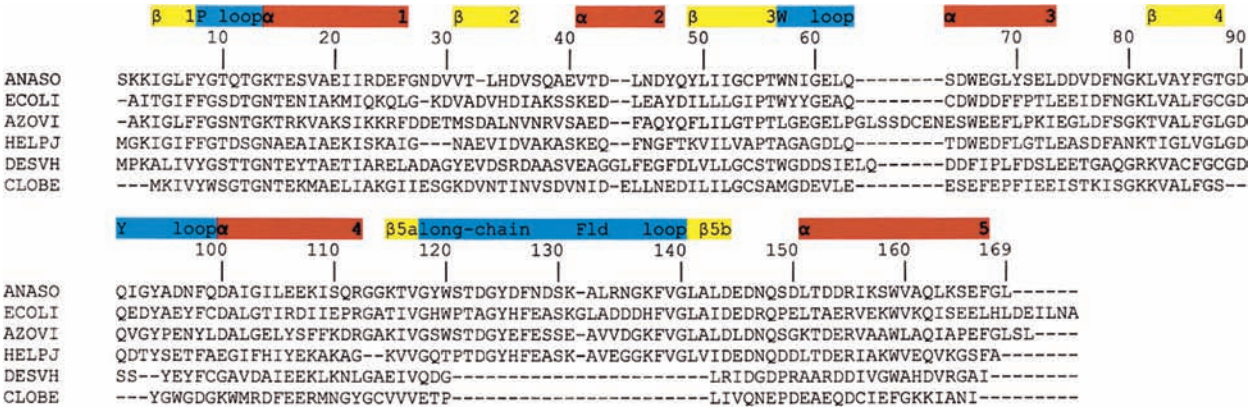

Figure 1. Amino acid sequences of a representative selection of flavodoxins from different species covered in this review aligned with *Clustal W (1.82)*: ANASO (*Anabaena PCC7119*), ECOLI (*Escherichia coli*), AZOVI (*Azotobacter vinelandii*), HELPJ (*Helicobacter pylori*), DESVH (*Desulfovibrio vulgaris*) and CLOBE (*Clostridium beijerinckii*). The numbering follows the sequence of the *Anabaena* PCC 7119 protein, whose α-helices and β-strands are highlighted in red and yellow, respectively. Distinct structural or functional loops of the flavodoxin molecule are highlighted in cyan: the phosphate binding loop (P loop), the isoalloxazine binding loops (W loop and Y loop) and the loop specific to long-chain flavodoxins, which is possibly involved in the binding of partner proteins.

This review focuses on research done since 1995, which is presented so as to reflect the several steps by which the information contained in the flavodoxin gene unfolds to give rise to a functional protein that is able to interact with several other proteins and perform a variety of physiological functions. Potentially interesting areas for future research are then summarized.

Sequence, distribution and phylogeny

All known flavodoxins are highly acidic proteins. They roughly contain between 140 and 180 residues. From sequence alignment and structural considerations, they can be divided into two groups, short-chain and long-chain flavodoxins, that differ in the presence of a 20-residue loop of a so far unknown function. Throughout this review, the numbering of key flavodoxin residues will follow the *Anabaena* PCC 7119 sequence (Fig. 1). With the only reported exceptions of some eukaryotic green and red algae [10–11], flavodoxins seem to be present only in bacteria, where they are widely distributed among many different groups. A thorough phylogenic analysis of the flavodoxin gene is still missing. Our preliminary analysis [M. Bueno and J. Sancho, unpublished] indicates that any long flavodoxin is more similar to any other long one than to short-chain flavodoxins, which suggests that the two groups have diverged only once. It is unclear which group is older, and the phylogenic analysis is complicated by clear evidence of frequent horizontal transfer events. Based in structural considerations it has been proposed that the long-chain flavodoxins may have preceded the shorter ones [12]. Although flavodoxin is not present in higher eukaryotes, a descendant of the flavodoxin gene appears fused in eukaryotic genes that encode multidomain proteins [13–14].

Folding, stability and three-dimensional structure of apoflavodoxin

To acquire its functional conformation, the flavodoxin polypeptide must fold and bind to FMN. These two processes, which could in principle be coupled, are sequential, and the cofactor only binds the apoprotein when it is already folded. Apoflavodoxin folding has been investigated *in vitro* using the long-chain *Anabaena* PCC7119 [15] and *Azotobacter vinelandii* [16] proteins. Although some differences in the folding mechanism of

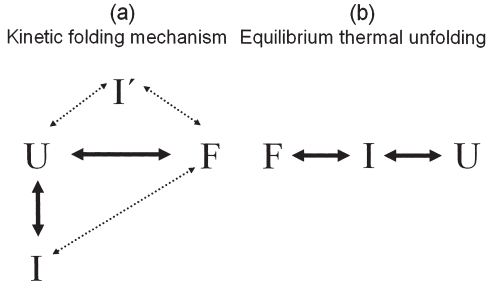

Figure 2. Schemes representing: (*a*) The kinetic folding mechanism of apoflavodoxin (based in those proposed for the *Anabaena* and *Azotobacter* proteins). The similarities found in the kinetic folding mechanisms of the two apoflavodoxins so far studied are shown in solid arrows, while the differences found are depicted in open ones. (*b*) The equilibrium thermal unfolding mechanism (based in those from *Azotobacter*, *Anabaena* and *Desulfovibrio).* The equilibrium and transient intermediates do not need to be similar. At present, only the equilibrium thermal unfolding intermediate of *Anabaena* has been structurally characterized.

these two apoflavodoxins have been stressed [17], the fact is that the two proteins share a similar folding mechanism where a transient intermediate accumulates, that is essentially off-pathway (Fig. 2a) and slows down the reaction [15–16]. Nevertheless, apoflavodoxin folds fast: in dilute solution and under strong native conditions most molecules are folded in less than a second [15], and the presence of FMN in an apoflavodoxin refolding solution does not speed up the reaction [18]. It has been suggested that the occurrence of the transient intermediate could be related to the presence of the long-chain specific loop [19], but no detailed study of the folding mechanism of a short-chain apoflavodoxin is yet available. The folding of apoflavodoxin *in vivo* has not been specifically studied. There is, nevertheless, good evidence indicating that the FMN cofactor is not required: when the wild-type *Anabaena* protein is overexpressed and the cells are quickly harvested (in FMN limiting conditions), or when FMN-binding defective apoflavodoxin variants are expressed, large amounts of well-folded apoprotein are recovered (our unpublished observations and [19]).

The stability of apoflavodoxin has been investigated using, in addition to the *Anabaena* [20–21] and *Azotobacter* [22–23] proteins, those of several *Desulfovibrio* strains and species [24–26]. It seems clear that the equilibrium thermal unfolding is three-state, so that a partly unfolded intermediate accumulates at moderately high temperatures (Fig. 2b). The structure of this equilibrium intermediate has been investigated by equilibrium ϕ-analysis and nuclear magnetic resonance (NMR) [27]. Most of it displays a native-like conformation, but some of the FMN binding loops, one β-strand and, more significantly, the long loop characteristic of long-chain flavodoxins, appear unfolded. These regions, which are clustered in the three-dimensional structure, do not seem to contribute very much to apoflavodoxin stability, and the 20-residue long loop can be removed from the *Anabaena* wild-type protein with minor impact on its conformational stability [19]. The stability of apoflavodoxin has also been investigated by chemical denaturation, but in this case no common scheme has emerged: while for some apoflavodoxins an intermediate has been described [22], for others, a simple two-state mechanism can explain the chemical unfolding [20]. The apoflavodoxin from *Anabaena* has additionally been used as a model protein to investigate general stability principles (such as the contribution of cation/pi interactions [28], charge/charge interactions [29–30] and surface-exposed hydrogen bonds [31] to protein stability), to put forward new concepts such as those of the relevant and residual stabilities of proteins with equilibrium intermediates [32], and to obtain structural and thermodynamic information on molten globule intermediates [33].

The first three-dimensional structure of an apoflavodoxin (that from *Anabaena*) [34] revealed that the fold of the apoprotein was essentially identical to that of the functional holoflavodoxin complex [35], the only significant differences being traced to one of the FMN binding loops (57–63) that appears displaced so as to close the gap left by the absent cofactor (Fig. 3). Apoflavodoxin is thus, as flavodoxin, a typical three-layered α/β protein, with a central five-stranded β-sheet surrounded by two helical layers. In the X-ray structure of *Anabaena* apoflavodoxin (crystallized in high ammonium sulfate concentration), a sulfate ion is bound, mimicking the FMN phosphate, which opens the possibility that the native conformation in this region is a consequence of the binding of the ion. In this respect, NMR data on the apoflavodoxin from *Azotobacter vine-*

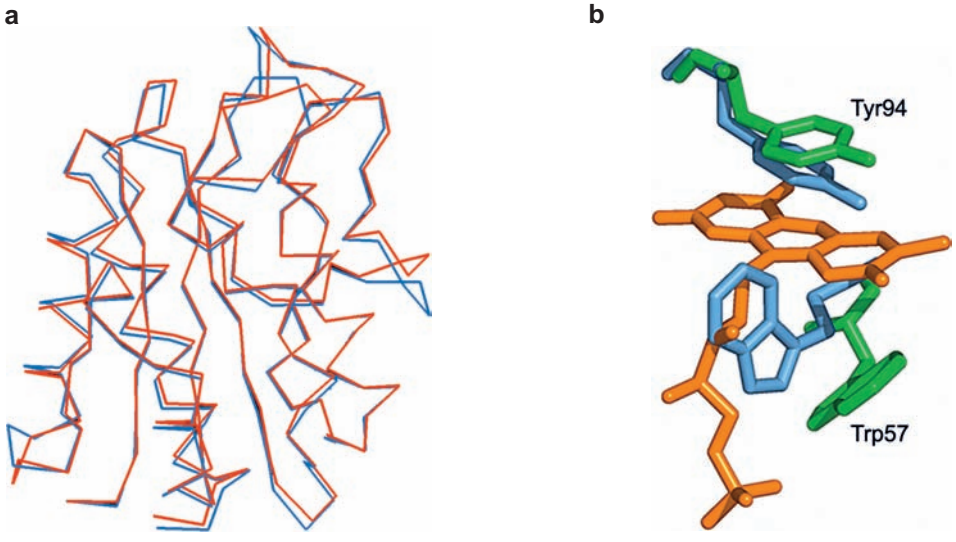

Figure 3. Superposition of the X-ray structures of the holo and apoflavodoxins from *Anabaena*. (*a*) α-Carbon trace of the holo (orange) and apo (blue) forms. (*b*) Differences between the two structures at the FMN binding site: holoflavodoxin residues and FMN (1flv) in green and gold, respectively, and apoflavodoxin residues (1ftg) in blue.

landii [36] and *Anabaena* PCC 7119 [37] suggest that the three FMN binding loops are quite flexible in solution. Recently, the X-ray structure of another apoflavodoxin (from *Helicobacter pylori*) was solved from crystals obtained in a buffer lacking sulfate and phosphate ions [38], and was compared with the corresponding holoprotein [39]. Interestingly, this second apoflavodoxin displays exactly the same features as that from *Anabaena*: the loop equivalent to the 57–63 loop in *Anabaena* moves towards the gap left by the cofactor, and a chloride ion appears bound at the FMN phosphate site. It seems thus that a general characteristic of apoflavodoxins is the ability to recruit anions at the phosphate binding site together with that of filling the empty space left by the missing cofactor by bringing one of the FMN isoalloxazine binding loops in contact with the other one, which poses an interesting mechanistic problem for FMN recognition.

FMN recognition, stability of the complex and its influence on flavodoxin stability

The FMN molecule is made of a fused, aromatic, triple ring (the isoalloxazine), a phosphate group and a linking ribityl chain. A comparison of the available flavodoxin structures indicates that the ribityl binding site, unlike the isoalloxazine and phosphate ones, is not conserved, which suggests that the primary recognizing event between apoflavodoxin and FMN leading to complex formation must take place at either the phosphate or isoalloxazine sites. The fact that electrostatic interactions have sometimes been found to play important roles in molecular recognition events would point to the phosphate site, but in flavodoxin no positively charged residues interact with the phosphate, which is instead bound by a network of hydrogen bonds. Besides, the overall electrostatic field of apoflavodoxin is negative, as is that of the approaching phosphate group in FMN. That the isoalloxazine site in the *Anabaena* X-ray structure appears closed while the phosphate site is in a native conformation (with a bound sulphate ion) suggested that the recognition could take place at the phosphate site [34]. However, the apparent promiscuity of the phosphate site in recruiting anions from solution, as judged by the similarities observed in the two apoflavodoxin X-ray structures solved [34, 38], indicates that, although for different reasons, neither the isoalloxazine nor the phosphate sites in apoflavodoxin are readily available for FMN recognition. The primary site for FMN/apoflavodoxin recognition has been determined by mutational analysis of the transition state of complex formation in the *Anabaena* protein [40]. While mutating the tryptophane and tyrosine residues that sandwich the isoalloxazine ring in the holoprotein lowers the rate of complex formation, mutating some of the threonines at the phosphate site does not change the binding rate constant. This indicates that the binding is likely driven by hydrophobic/aromatic interactions at the isoalloxazine site. Some evidence of a similar mechanism has been obtained for *Desulfovibrio desulfuricans* flavodoxin [41]. The fact that riboflavin (non-phosphorylated FMN) binds to the apoflavodoxins from *Anabaena* PCC 7119 and *Desulfovibrio vulgaris* faster than FMN [40, 42] is also consistent with the proposed mechanism. A potential communication between the phosphate and isoalloxazine sites that would facilitate the binding at the latter in the presence of bound phosphate has been proposed [43–44]. Once the binding event has taken place, a very tight complex is formed. The contribution of the three FMN moieties and of specific amino acid residues to the strength of the complex has been studied in *Anabaena* flavodoxin [45]. The phosphate group contributes most, through its many hydrogen bonds with several threonine side chains and with main chain NH groups. In contrast, the ribityl hardly makes the complex more stable (Fig. 4). Finally, the isoalloxazine contribution is also large, due especially to its tight coplanar interaction with a conserved tyrosine (Tyr 94) and, to a lesser extent, to interaction with a conserved tryptophane (Trp 57).

The high affinity of apoflavodoxin for the FMN cofactor has been known for decades [3–4], and it has been naturally assumed that the holo form would be more stable than the apo one. However, only recently has the stability of the functional complex received attention. The initial reports on the stability of *Desulfovibrio desulfuricans* flavodoxin found little or no stabilization at all, and it was interpreted to mean that FMN remained bound to the unfolded polypeptide with an affinity similar to that of the native complex [46]. Subsequent work on the flavodoxins from *Desulfovibrio vulgaris* [26], *Azotobacter vinelandii*

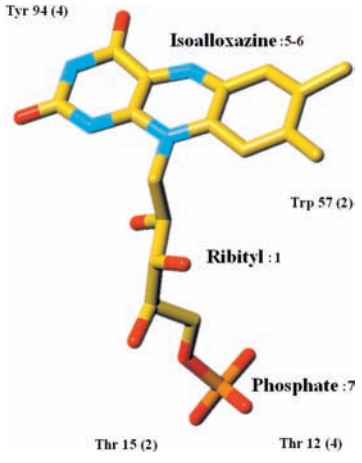

Figure 4. Structure of the FMN cofactor, and contributions (in kcal mol^{-1}) of the phosphate, the ribityl and the isoalloxazine triple ring to the binding energy of its complex with *Anabaena* apoflavodoxin. Decreases in affinity (in kcal mol^{-1}) associated with the replacement of several FMN binding residues by Ala (Trp57 and Tyr94) or Val (Thr 12 and Thr 15) are shown in parenthesis (adapted from [45]).

[18] and *Anabaena* PCC 7119 [47] have nevertheless shown that the functional complex is far more stable than the apoprotein, and have clarified that the extent of the observed stabilization corresponds exactly to that expected when the cofactor is fully released upon protein unfolding. The fact that small stabilizations have been observed in *Desulfovibrio desulfuricans* seems due to a significantly lower affinity of FMN for that particular apoprotein. In *Anabaena* flavodoxin, the bound FMN has been reported not only to increase the stability but also the cooperativity of the thermal unfolding. While the apo form unfolds in two steps via an equilibrium intermediate [21, 27], the complex unfolds at a much higher temperature following a simple two-state mechanism, the reason being that the FMN binding site overlaps with, and thus tightens, the weak region of the apoprotein that unfolds independently at lower temperatures giving rise to the intermediate [47]. In the presence of FMN, that region remains stable at moderate temperatures and unfolds with the rest of the protein. It is clear that, thanks to the tight apoprotein/cofactor interaction, flavodoxins are quite stable proteins, a fact that has probably contributed to stimulate their early and successful structural and functional characterization.

Tailoring of the FMN redox potentials by interaction with the apoprotein

At least in photosynthetic reactions, flavodoxins need to accept electrons one by one, an apparently simple task that, nevertheless, FMN cannot perform when it is free in solution, due to the very low stability of its one-electron reduced redox state [3–4]. Flavodoxin can do the job because the apoprotein modifies the redox potentials of the cofactor or, in other words, the relative stability of the oxidized, one-electron reduced (semiquinone) or two-electron reduced (hydroquinone) forms of FMN. The obvious mechanism to achieve this is forming complexes of different affinities with the three redox forms, and there has been much interest in identifying the residues of the polypeptide that are responsible for this differential binding. Here, valuable, extensive information has been provided by mutational work and NMR studies done mainly with flavodoxins from *Desulfovibrio vulgaris* [48–54] and *Clostridium Beijerinckii* [54–59], and also in those from *Anabaena* [60–61], *Anacystis nidulans* [62], *Megasphaera elsdenii* [63] and *Azotobacter vinelandii* [64]. The mutational analysis has highlighted the role of the conserved tyrosine residue that appears tightly packed onto the *si* face of the isoalloxazine ring [48, 60]. It seems that this residue establishes favourable interactions with the three redox forms of FMN, but those with the fully reduced state are much weaker than those with the oxidized and semireduced ones, which gives rise to substantial stabilization of the semiquinone relative to the hydroquinone, so contributing to shifting the semiquinone/hydroquinone redox potential to much more negative values than that of free FMN [60]. In addition, the negative electrostatic potential of the acidic flavodoxin, to which the FMN phosphate also contributes, destabilizes the negatively charged hydroquinone [49–51, 61–62], as it does the burial of its uncompensated negative charge in the reduced complex. On the other hand, the semiquinone is stabilized, relative to the oxidized form, because it can form a new hydrogen bond with a main chain group [52, 55–57, 63], which helps shift the oxidized/semireduced potential to moderately less negative values, relative to the potential of free FMN. The formation of this hydrogen bond seems assisted by local protein flexibility [58–59].

Flavodoxin reactions and protein partners

Flavodoxin was discovered as a photosynthetic protein that shuttled electrons from PS I to FNR [1]. The role of flavodoxin in this reaction has been mainly studied in *Anabaena* PCC 7119 [65–68], and also in *Synechococcus* sp. 2 PCC 700 [69] by a combination of laser-induced fast kinetics and mutagenesis that has clarified the contribution of several flavodoxin residues to the reaction and allowed to propose a kinetic scheme. A review of the initial work is available [70]. In addition, structural and functional details of the interaction between specific proteins in PS I and flavodoxin have been described [71–73] and a review is also available [74]. The interaction with FNR has also been specifically investigated, mainly in *Anabaena* PCC 7119 [75–77], and models have been proposed for the yet unsolved flavodoxin-FNR complex [78–79]. The stability of the oxidized flavodoxin-FNR complex and the rate of electron transfer between the two proteins seem modulated by a combination of electrostatic and hydrophobic interactions [68, 75].

On the other hand, the presence of flavodoxin in many non-photosynthetic organisms has fuelled the investigation of a variety of roles for the protein (Fig. 5). The flavodoxin and FNR of *Escherichia coli* [80–82] have raised much interest on the function of this well-known electron transfer couple [83], already investigated in the photosynthetic context and also known in their eukaryotic monogenic associations: P450 reductase, nitric oxide synthase or methionine synthase reductase. The electron acceptor specificity and structural details of the FNR-flavodoxin complex have been reported [84–85], and these proteins have been implicated in stress oxidative response [86]. Flavodoxin has also been shown to participate in nitrate reduction in *Azotobacter vinelandii* [87]. The role of flavodoxin in the synthesis of methionine through its interaction with *E. coli* cobalamin-dependent methionine synthase has also been investigated

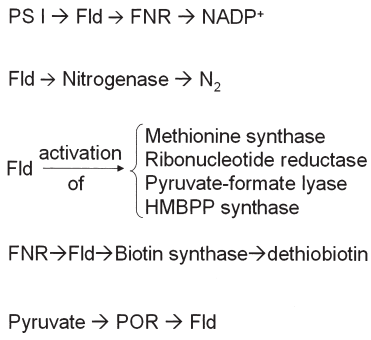

Figure 5. Some known physiological reactions involving flavodoxin. The essential role of flavodoxin in bacterial reactions, such as pyruvate oxidation by POR in *Helicobacter pylori*, makes flavodoxin an interesting drug target.

[88–91], and structural details of the complex have been provided [85]. Flavodoxin is also required for the synthesis of biotin in *Bacillus subtilis*, where it donates electrons to biotin synthase and cytochrome P450 BioI [92–93]. In addition, flavodoxin participates in the activation of pyruvate-formate lyase [94], ribonucleotide reductase [95] and HMBPP synthase (GcpE) [96], also playing an essential role in the oxidation of pyruvate in *Helicobacter pylori* [97–99]. The flavodoxin residues that are involved in the recognition of the different partners have started to be unveiled. A short loop in the flavodoxin from *Azotobacter chroococcum* has been proposed to participate in nitrogenase recognition [100], and a recent NMR study indicates that *E. coli* flavodoxin may use the long loop in the recognition of *E. coli* methionine synthase and FNR [84]. The role of flavodoxin in all these reactions and the details of its interactions with the several partner enzymes involved certainly deserve further investigation.

Future research

The past decade has witnessed a renewed interest in flavodoxin, both as an important redox protein on its own and as a model to understand the general physicochemical processes that drive the newly synthesized unfolded polypeptides into active proteins. In the last 10 years, the flavodoxin folding mechanism has been outlined [15–16], the first three-dimensional structures of apoflavodoxin have been determined [34, 38], the mechanism of apoflavodoxin/FMN recognition has been actively investigated [40–44], the stability of both the apo and holo forms are now largely characterized [20–27], the understanding of the role of the polypeptide in shaping redox potentials is much advanced [48–63] and new flavodoxin functions, beyond those of replacing ferredoxin, have been characterized. Indeed, flavodoxin is an essential protein for the survival of certain organisms, such as the pathogen *Helicobacter pylori* [39].

An expected outcome of new knowledge is to define better what we still do not understand and to shape future research. Although flavodoxin is recognized as the archetype of an entire class of proteins (the three-layered $\alpha\beta\alpha$ sandwiches) comprising many different folds [101], the evolution of the flavodoxin molecule is still unclear. No flavodoxins have been found in higher eukaryotes, but the ability of the flavodoxin gene product to bind FMN and participate in electron transfer reactions seems to have been very useful to the higher organisms that, through gene fusion events, have incorporated it into multidomain proteins such as P450 reductase and sulfite reductase, where the original flavodoxin sequence and fold can be clearly traced [13–14]. In contrast, flavodoxin is widely distributed within the prokaryote realm, and its distribution among the different groups is suggestive of a complex process involving many horizontal transfer events [M. Bueno and J. Sancho, unpublished observations]. Moreover, two types of flavodoxins exist that, although deriving from the same gene, have evolved to either contain or lack a surface-exposed 20-residue loop [3–4] that might be used in the recognition of protein partner enzymes involved in a variety of reactions [12, 85]. The overall history of the flavodoxin molecule, its origin, distribution and progressive involvement in a variety of metabolic pathways, ultimately leading to the appearance of more complex multidomain proteins, is still to be disclosed.

The apoflavodoxin/FMN recognition process will also deserve continuous attention. Although the available mutational evidence points to the isoalloxazine pocket, rather than to the phosphate binding region, as the recognition site [40], the details of the binding process are still unclear. The two X-ray apoflavodoxin structures solved [34, 38] indicate that, even in solutions of moderate ionic strength, both the isoalloxazine site (which is closed) and the phosphate site (which bears a bound anion) are not ready for FMN binding. Whether the closed isoalloxazine site becomes available as a consequence of a fast equilibrium with a more open conformation [36–37, 40], or in a process modulated by the presence of bound ions at the phosphate site [34, 43–44], or concomitantly with the observed hydrophobic encounter with the isoalloxazine [40] needs to be clarified. Both experimental and computational studies may help to understand the binding mechanism, which may shed light on the general field of molecular recognition.

In spite of the remarkable advance in the comprehension of the several strategies by which apoflavodoxin tailors the redox potential of its inevitable FMN redox partner to make them suit the needs of specific electron transfer reactions [48–64], we still lack a quantitative explanation of the observed changes in redox potentials brought about

by the complex. Since the changes in redox potentials experienced by free FMN upon incorporation into the apoflavodoxin fold are precisely known, and they are straightforwardly related to differences in free energies of binding of the oxidized, semireduced and fully reduced complexes, the goal here is to dissect the contribution of the different effects so that they quantitatively match the observed changes in redox potentials. One potential difficulty here is that the different contributions might not be additive [59]. Achieving the goal would represent a major advance towards the rational engineering of redox potentials, which could be of practical interest.

Another field where much progress is expected over the next 10 years is in the structural characterization of the complexes of flavodoxin with its growing number of partner enzymes. The availability of three-dimensional structures for the complexes would greatly help to guide the mutational investigation of the different reactions where flavodoxin plays a role and might help to understand how a single protein can recognize a variety of binding sites, which is related to the emerging realization of the existence of complex protein networks in the cell.

Perhaps the simplest and one of the more interesting goals for the next years is to advance in our understanding of the different reactions in which flavodoxin plays an important role. In some cases, flavodoxin just replaces ferredoxin in low iron media, where it is induced in a tightly regulated manner involving Fur proteins and possibly additional transcription regulators [102–103]. Particularly appealing, however, seem those reactions that flavodoxin does not share with other proteins. One such reaction is the oxidative cleavage of pyruvate by pyruvate oxydoreductase (POR) in certain organisms. In this reaction, pyruvate is decarboxylated into acetyl-coenzyme A, with transfer of electrons from POR to flavodoxin. It has been demonstrated that, probably due to the importance of the pathway, certain organisms cannot grow without flavodoxin. This is of great interest since they include human pathogens such as *Helicobacter pylori* [39]. Flavodoxin is thus a potential target for drug design [39, 104–106]. Indeed, the flavodoxin from *Helicobacter pylori* displays a peculiar structural feature that can be exploited to bind inhibitory molecules next to the active site [9]. As new or poorly defined roles of flavodoxin in bacterial metabolic pathways are discovered or clarified, the possibility of flavodoxin becoming a useful drug target will increase. In achieving this goal, the vast structural and functional data already gathered over years of careful flavodoxin investigation will be very valuable.

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