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N-TERMINAL AMINO ACID SEQUENCES OF FLAVODOXINS FROM *CHONDRUS CRISPUS* AND *NOSTOC* STRAIN MAC

I. TAKRURI, D. BOULTER, M. P. FITZGERALD*, G. N. HUTBER* and L. J. ROGERS*

Department of Botany, University of Durham, Durham, DH1 3LE, U.K.; *Department of Biochemistry, University College of Wales, Aberystwyth, SY23 3DD, U.K.

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Key Word Index—*Nostoc* strain MAC; Cyanophyceae; *Chondrus crispus*; Rhodophyceae; flavodoxin.

Abstract—The amino terminal sequences of the flavodoxins from a cyanobacterium *Nostoc* strain MAC and a red alga *Chondrus crispus* have been determined. A comparison of these data with those for other flavodoxins within the larger molecular weight group (M_r 21 000) of these electron transport proteins suggests that the cyanobacterial flavodoxins and that from *Azotobacter vinelandii* are most related and may be distinct from the flavodoxins from *Chrondrus crispus* and *Rhodospirillum rubrum*.

INTRODUCTION

Flavodoxins are low molecular weight proteins which contain a single molecule of non-covalently bound FMN as their redox active component and which transfer electrons at low potential. The number of organisms from which flavodoxins have been isolated and characterized are limited (see Table 2 later), but amongst these those from a few non-photosynthetic bacteria (M_r *ca* 15 000) have been extensively investigated since they are the smallest flavoproteins and therefore represent the simplest models for studying the chemistry and biochemistry of this group of proteins [1]. Amongst photosynthetic organisms, the flavodoxins from a photosynthetic bacterium *Rhodospirillum rubrum* [2], the cyanobacteria *Nostoc* strain MAC [3, 4], *Synechococcus* 6301 (*Anacystis nidulans*) [5] and *Synechococcus lividus* [6], and the eukaryotic algae *Chlorella fusca* [7] and *Chondrus crispus* [8] have been characterized to varying extent. In all but the latter, the flavodoxin was only produced to significant extent during growth in media low in iron. With the exception of *Synechococcus lividus* flavodoxin, these have M_r values close to 22 000, as does the protein from *Azotobacter vinelandii* [9]. The properties of these flavodoxins have been reviewed recently [10].

RESULTS AND DISCUSSION

Amino acid sequence information on the flavodoxins from photosynthetic organisms is very limited, being confined to the amino terminal sequence of that from *Rhodospirillum rubrum* [2] and some two-thirds of the sequence of the flavodoxin from *Synechococcus* 6301 [11]. The *N*-terminal sequences are of interest both from a phylogenetic view and because part of the binding site for FMN is located in these regions. The comparable sequences for the flavodoxins from another cyanobacterium *Nostoc* MAC and a red alga, *Chondrus crispus*, are now reported.

The amino acid sequences of the flavodoxins were determined by using the dansyl/phenyl isothiocyanate method [12]. Dansyl-amino acids were identified by chromatography on polyamide thin layers using the solvent systems described elsewhere [13]. Automated sequence analysis of reduced and carboxymethylated protein was carried out on a Beckman 890C protein sequencer by the 'fast protein' programme (Beckman prog. No. 072172C). Phenylthiohydantoin derivatives were identified as described in ref. [14].

The *N*-terminal amino acid sequences for the two flavodoxins are shown in Table 1, and are compared with those for *Synechococcus* 6301 [11, 15] and *Rhodospirillum rubrum* [2]. The conservation of certain residues in the *N*-terminal sequence in other flavodoxins holds for these proteins also. In this limited group from photosynthetic organisms five positions have invariant residues; for the three organisms exhibiting oxygenic photosynthesis there is greater similarity with 11 invariant positions. When all the flavodoxins for which sequence information is available are compared only three residues in these *N*-terminal sequences are invariant; Gly_{13}, Thr_{15} and Ala_{19}.

The structure of the oxidized flavodoxin of *Synechococcus* 6301 flavodoxin to 0.165 nm resolution has been elucidated [15]. Despite uncertainties in detailed interpretation because the full sequence of the flavodoxin has not yet been determined it is clear that the polypeptide backbone follows the β–α–β conformation seen in the low M_r flavodoxins from non-photosynthetic bacteria, and which characterizes the nucleotide-binding domains of the pyridine nucleotide dehydrogenases. In the sequence of the *Clostridium* MP flavodoxin [16] residues 9–15, 55–61 and 88–99 contribute to the FMN-binding site. Here Ser_{10}, Thr_{12} and Thr_{15} (with Ser_{58}) hydrogen-bond to the FMN phosphate and Asn_{14} (with Thr_{59}, Asp_{129}) to the 4′-hydroxyl of the ribityl side-chain. In the cyanobacterial and algal flavodoxins there is a Thr at position 10 and Asn_{14} in both cyanobacterial flavodoxins is replaced by Lys or Val. Differences in binding of the flavin which are a consequence of these substitutions give subtle differences in flavin environments and might explain the range of redox potentials found in these flavodoxins [17].

A matrix of amino acid differences in the *N*-terminal

Table 1. Comparison of the *N*-terminal amino acid sequences of flavodoxins from photosynthetic organisms

	10										20										30									
Chondrus crispus	—	—	K	I	G	I	F	F	S	T	S	T	G	N	T	(T)	E	V	A	D	F	I	G	K	T	L	G	A	K	A	S	A	P	I	(S)	V
Nostoc MAC	S	K	K	I	G	L	F	Y	G	T	Q/E	T	G	K	T	E	S	V	A	E	I	I	—	D	E	F	G	D	E	V	V	T	L	D	I	D
Synechococcus 6301	—	A	K	I	G	L	F	Y	G	T	Q	T	G	V	T	Q	T	I	A	E	S	I	Q	Q	E	F	G	G	E	S	I	V	D	L	N	D
Rhodospirillum rubrum	—	—	G	T	T	V	I	Y	G	S	D	G	G	T	T	E	G	V	A	K	R	I	A	A/S	P	L	E/Q	A	K	V	V	D/N	I	K	(V)	A

Comparison is based on the sequences aligned for maximum homology. Data for *Synechococcus* 6301 and *R. rubrum* flavodoxins are from refs [11] and [2], respectively.

Table 2. Amino acid difference matrix of flavodoxins

	1	2	3	4	5	6	7	8	9
(1) *Chondrus crispus*		36	35	40	33	36	39	42	36
(2) *Nostoc* strain MAC	24		26	37	32	39	38	36	39
(3) *Synechococcus* 6301	25	15		45	28	41	44	39	43
(4) *Rhodospirillum rubrum*	29	26	29		44	40	30	43	37
(5) *Azotobacter vinelandii*	24	19	20	28		40	44	41	44
(6) *Clostridium* MP	27	27	28	27	27		22	30	26
(7) *Clostridium pasteurianum*	28	28	29	23	27	14		33	20
(8) *Desulfovibrio vulgaris*	26	24	26	26	27	19	20		32
(9) *Megasphaera elsdenii*	26	29	30	26	30	18	16	20	

The matrix is based on the *N*-terminal sequences (first 36 positions) given in Fig. 1 and on sequences listed in ref. [18]. Figures above the diagonal indicate the minimum base changes between pairs of sequences with gaps counted as 2. Differences in residues with gaps counted as 1, are shown below the diagonal. Sequences were aligned for maximum homology.

sequence for all flavodoxins for which such data are known is shown in Table 2. This comparison and the consideration of minimum base changes for these pairs of partial sequences suggests there are considerable differences between the flavodoxins, despite the fact that this portion of the peptide chain possesses the highest homology [18]. The flavodoxins are thought to be ancient proteins and may predate ferredoxins in evolution. However, consideration of even this limited sequence information suggests that the cyanobacterial flavodoxins are more related to that from *Azotobacter vinelandii* than to the flavodoxins from *Chondrus crispus* and *Rhodospirillum rubrum* which are the other members of the larger M_r group. The smaller flavodoxins form a separate group; amongst these the complete sequences which are known show a homology of about 25–35% [19]. The differences between the cyanobacterial and red algal proteins are unexpected, although the flavodoxin from the latter organism is known to possess a number of unusual properties [17].

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