The Amino Acid Sequence of Ferredoxin from *Brassica napus* (Rape)

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The amino acid sequence of the ferredoxin of *Brassica napus* was determined by using a Beckman 890C sequencer in combination with the characterization of peptides obtained by tryptic and chymotryptic digestion of the protein; some peptides were subdigested with thermolysin. The molecule consists of a single polypeptide chain of 96 amino acid residues and has an unblocked *N*-terminus. The primary structure shows considerable similarity with other plant-type ferredoxins.

Ferredoxins are a class of iron–sulphur proteins that act as electron carriers in a number of different biochemical processes, including photosynthetic electron transport and nitrogen fixation. Those from higher plants, eukaryotic algae and blue–green algae possess a 2Fe–2S active centre, and have a molecular weight of approx. 12000, whereas bacterial ferredoxins contain two 4Fe–4S active centres and have a molecular weight of approx. 6000. In the past few years the primary structures of ferredoxins from different sources of algae and higher plants have been determined (see Wakabayashi *et al.*, 1978; Takruri *et al.*, 1978), and the results suggest that these ferredoxins have evolved from a common ancestor.

The amino acid sequence of rape ferredoxin given in the present paper is very similar to those of other ferredoxins that have been sequenced from higher plants and algae.

Experimental

Materials

Seeds of *Brassica napus* were obtained from the Tyneside Seed Store, Gateshead, U.K., and grown in the University of Durham Botanical Gardens. Acrylamide and *NN'*-methylenebisacrylamide were obtained from BDH Chemicals, Poole, Dorset, U.K.; 40% ampholyte carrier (pH 3–10) was obtained from Serva, Heidelberg, Germany. Other chemicals and enzymes used were as described in Takruri *et al.* (1978).

Purification of ferredoxin

Ferredoxin was isolated and purified as described by Petering & Palmer (1970).

Isoelectric focusing

Isoelectric focusing was carried out with carboxymethylated ferredoxin as described by Wrigley (1968) in gels containing 5% (w/w) polyacrylamide, 2% (w/v) Ampholine (pH 3–10) and 6 M-urea. After the focusing the gels were fixed by immersion in 25% (w/v) trichloroacetic acid and the protein was located as a white precipitation band. The pI was determined by measuring the pH gradient in similar unfixed gels.

Carboxymethylation of ferredoxin

Ferredoxin was carboxymethylated as described by Milne & Wells (1970), and the carboxymethylated protein was desalted as described by Takruri *et al.* (1978).

Amino acid analysis, protein digestion, peptide purification and amino acid sequence determination

These methods were carried out as described by Takruri *et al.* (1978), except that a column of Bio-Gel P-4 was used instead of Sephadex G-50.

Results

Rape ferredoxin was isolated to a high degree of purity with an absorption index (A_{420}/A_{280}) 0.79. The homogeneity of the protein was shown by isoelectric focusing, which gave a single band corresponding in position to a pI value of 3.8.

The amino acid composition of rape ferredoxin is given in Table 1. The results are in good agreement with the sequence data except for arginine. The *N*-terminal 40 residues of the protein were determined by using an automated Beckman 890C protein sequencer.

Fig. 1. Amino acid sequence of *Brassica napus* ferredoxin

T, C and H represent peptides obtained by tryptic, chymotryptic and thermolytic digestion of carboxymethylated ferredoxin respectively. Arrows $\rightarrow$, $\dashrightarrow$ and $\dashleftarrow$ represent automatic sequencing, manual Edman degradation and carboxypeptidase digestion respectively. Values for electrophoretic mobility of peptide C6 after two and three cycles of Edman degradation are indicated.

Digestion of the protein with trypsin produced six peptides (see Fig. 1 and Table 2), of which the largest, peptides T3 and T5, were purified by gel chromatography of the mixture and the other peptides by paper electrophoresis. Digestion of peptide T3 with thermolysin produced five peptides, of

which peptide T3H3 was not isolated. Digestion of peptide T5 with thermolysin produced seven peptides, which were separated by paper electrophoresis. The sequences of the tryptic and thermolytic peptides were determined by Edman degradation, and their order in the parent protein was established

Table 1. *Amino acid composition of Brassica napus ferredoxin*
Cm-Cys (carboxymethylcysteine) was not determined after hydrolysis.

	Amino acid composition (mol of residue/mol)				
	Mean values, 24 h hydrolysis	Mean values, 48 h hydrolysis	Mean values, 72 h hydrolysis	Average values	Sequence values
Asx	11.4	10.6	11.4	11.2	11
Thr	5.6	5.6	5.4	5.8*	6
Ser	7.9	7.6	6.9	8.5*	8
Glx	16.1	15.8	15.0	15.7	16
Pro	3.6	3.2	3.1	3.3	3
Gly	6.5	6.2	6.3	6.3	6
Ala	10.0	9.0	9.5	9.5	9
Val	9.7	9.8	9.9	9.9†	10
Ile	3.2	3.5	4.0	4.0†	4
Leu	5.0	4.8	4.2	4.7	5
Tyr	3.6	3.7	3.2	3.5	4
Phe	4.0	3.1	3.7	3.6	4
His	1.2	1.2	1.2	1.2	1
Lys	3.4	3.7	3.7	3.6	4
Arg	1.8	1.7	2.0	1.8	1
Cm-Cys	—	—	—		4

* Calculated from 24 h and 72 h values, assuming first-order kinetics for destructions (Moore & Stein, 1963).

† For valine and isoleucine maximal values (72 h hydrolysis) were taken.

Table 2. *Electrophoretic mobilities and amino acid compositions of peptides from Brassica napus ferredoxin*

The mobilities of peptides during electrophoresis at pH 6.5 were measured from dansyl-arginine relative to aspartic acid and at pH 1.9 from 5-dimethylaminonaphthalene-1-sulphonic acid relative to dansyl-arginine. Amino acids present in less than 0.3 residue/molecule were disregarded. This indicates that some peptides were not completely pure. Carboxymethylated cysteine was not determined after hydrolysis. —, Not determined.

Peptide	Electrophoretic mobility		Amino acid composition of peptide (mol of residue/mol)														
	pH	pH															
	1.9	6.5	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
C1	0.6	0		0.81					1.20				0.99				
C2	1.38	0.91								0.92				0.77		2.15	
C3	0.19	−1.03	2.90	1.02		5.46	0.71	1.06	0.29	2.24	0.95		0.92				
C4	0.19	−0.81	2.17			2.31	0.83	0.71	2.66	1.00	0.68	1.88	0.93				
C5	0.21	—			5.54			2.75	1.84	2.30				0.54		1.00	1.05
C6	—	−0.74	2.19		1.55	2.01				0.87				1.00			
C7	0.81	−0.98	2.75			2.11		0.77	0.85		0.77	0.89		0.89			
C8	0.79	0								0.93		0.89					
C9	0.21	−0.30	0.83	4.08	0.50	4.15	0.65		2.23	1.88	1.11	0.98	0.45		1.28	0.39	
T1	0.98	0.42		1.01					1.15				1.01			0.79	
T2	1.24	0.70								0.99						1.05	
T3	—	—	5.41	1.27	1.49	6.51	2.00	2.16	3.17	3.39	1.82	1.93		1.15			0.76
T4	—	−0.25			2.59			1.77	1.76							0.93	
T6	0.29	−0.75				3.13				0.92		0.78					

Table 3. Matrix of amino acid sequence differences for representative plant-type ferredoxins
I and II refer to two different pokeweed ferredoxin types.

	No. of differences in sequence							
	1	2	3	4	5	6	7	8
1. <i>Brassica napus</i> (rape)	0							
2. <i>Leucaena glauca</i> (koa)	24	0						
3. <i>Colocasia esculenta</i> (taro)	22	20	0					
4. <i>Spinacia oleracea</i> (spinach)	24	20	18	0				
5. <i>Medicago sativa</i> (alfalfa)	24	23	16	19	0			
5. <i>Triticum aestivum</i> (wheat)	20	20	20	21	19	0		
7. <i>Phytolacca americana</i> I (pokeweed)	24	35	28	30	27	32	0	
8. <i>Phytolacca americana</i> II (pokeweed)	31	37	29	31	27	34	23	0

from overlap with chymotryptic peptides and from the *N*-terminal sequence of the protein. Thus the thermolytic peptides obtained from peptide T3 were overlapped by the automatic-sequencer data, and those obtained from peptide T5 by chymotryptic peptides. Peptides T1, T2 and T3 were ordered by overlaps with chymotryptic and sequencer data, and peptides T4, T5 and T6 were ordered by overlap with chymotryptic peptides C5, C6, C7, C8 and C9; that peptide T6 follows peptide T5 without an insertion between follows only from the amino acid composition of peptide C9. The acidic and amide residues in the *N*-terminal 40-residue region were determined by direct identification of their phenylthiohydantoin derivatives. These assignments agree with the electrophoretic mobilities of the constituent peptides. The amide residue within peptide C6 was established from changes in the electrophoretic mobility after two and three cycles of Edman degradation (see Fig. 1). The *C*-terminal amide position in peptide T5H3 was established by carboxypeptidase-A digestion and subsequent identification of the released amide after dansylation, coupled with the fact that the mobility (-0.91 at pH 6.5) of this peptide indicated the presence of three acidic residues and only one amide residue.

The amino acid compositions of tryptic and chymotryptic peptides are given in Table 2 and the sequence data for these peptides and the thermolytic peptides are shown in Fig. 1.

Discussion

The ferredoxin of *Brassica napus* contains 96 amino acid residues, as compared with other plant/algal ferredoxins, which contain 96–98. Its *pI* is similar to those of barley (*Hordeum vulgare*), 3.75, wheat (*Triticum aestivum*) (Takruri & Boulter, 1979), 3.9, *Anabaena flosaquae*, 3.7 (Andrew *et al.*,

1975), *Porphyra umbilicalis*, 3.8 (Andrew *et al.*, 1976), and *Clostridium pasteurianum*, 3.7 (Lovenberg *et al.*, 1963). The molecular weight deduced from the sequence is 10774, including the active centre. Rape ferredoxin contains four cysteine residues which bind the two iron atoms of the catalytic site, although no quantitative evidence of cysteine content is presented. Neither tryptophan, as suggested by the absorption index, nor methionine residues were detected in the sequence, in contrast with other plant ferredoxins. The *C*-terminal sequence of rape ferredoxin shows considerable difference to this region to other plant/algal sequences.

Table 3 gives the number of amino acid differences that exist between this sequence and that of other selected plant-type ferredoxins. *Triticum aestivum* ferredoxin shows the greatest sequence similarity to it, with 20 amino acid differences.

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