Phytochemistry, Vol. 21, No. 6, pp. 1317–1320, 1982.
Printed in Great Britain.

0031-9422/82/061317-04$03.00/0
Pergamon Press Ltd.

THE AMINO ACID SEQUENCE OF PLASTOCYANIN FROM *CUCUMIS SATIVUS*

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(*Revised received* 28 *September* 1981)

Key Word Index—*Cucumis sativus*; Cucurbitaceae; cucumber; plastocyanin; amino acid sequence.

Abstract—The amino acid sequence of plastocyanin from cucumber (*Cucumis sativus*) has been determined. Analysis was by the dansyl–phenylisothiocyanate method on tryptic and thermolytic peptides obtained from CNBr fragments of *S*-carboxymethylated protein. The protein consists of a single chain of 99 residues which is closely related to other plastocyanins. The evidence for residues 52 and 53 and for 75 and 76 was weak compared with that for the remainder of the sequence. Overlap between residues 54 and 55, 57 and 58, 77 and 78 and 92 and 93 was not demonstrated.

INTRODUCTION

Amino acid sequence data have now been determined for plastocyanin from a wide variety of sources [1] and the crystal structure of oxidized poplar plastocyanin has been reported [2]. Because of the unusual properties of the single copper site, plastocyanin has been examined in detail by spectroscopic methods [3]. NMR spectroscopy has been used extensively to study the nature of the copper environment [4–8] and the mechanism of electron transfer [9–12]. This paper reports the amino acid sequence of cucumber plastocyanin, which has been determined in order to allow more detailed interpretation of its NMR spectra [6, 10, 11].

RESULTS

The amino acid sequence of cucumber plastocyanin is shown in Fig. 1. The sequence is consistent with the amino acid analysis for the protein (see Table 2) except that the amino acid analysis indicates an extra Glx residue and one less Ser residue.

Cleavage of the *S*-carboxymethylated protein by CNBr gave three fragments which were readily separated by gel filtration. The largest of the fragments had N-terminal Ile, the same as the intact protein, and contained homoserine, so was therefore the N-terminal fragment of the protein. The smallest fragment had N-terminal Val and lacked homoserine, so was therefore the C-terminal of the protein. The remaining fragment had N-terminal Asx and contained homoserine, so represented the middle section of the sequence.

The two largest CNBr fragments were digested by trypsin and thermolysin and the resulting peptides purified by paper electrophoresis. The electophoretic mobilities and amino acid analyses of the peptides which were obtained pure for analysis are given in Table 1. These peptides were sequenced by the dansyl–phenylisothiocyanate method and the results of these analyses are shown in Fig. 1. The peptide sequence and amino acid analysis results are in agreement except for the analysis of peptide X1H6 where the analysis value for Ser was lower than expected.

For certain residues the sequence evidence was weak when compared with that for the remainder of the sequence. In peptide X1H6 both Ser(52) and Gly(53) were observed only as faint spots. Supporting evidence for Gly(53) was obtained from the amino acid analysis of the peptide, but these data did not provide extra support for Ser(52). If the evidence for this Ser is incorrect, then this would account for the low value observed for Ser in the total amino acid composition (Table 2). However, there was no evidence for any other residue being present in this position in the sequence. The tryptic peptide which would have included this region of the sequence was not isolated. The possibility of a deletion when compared to other plastocyanin sequences cannot be excluded. The evidence for residues Thr(75) and Glx(76) was also weak. In the analysis of the tryptic peptide X2T1 only faint spots were observed for these residues, and there was no evidence from the thermolytic digest as the corresponding peptide was not found. The amino acid analysis of peptide X2T1, however, provides supporting evidence for these residues. The evidence for some of the Lys residues was weak from the sequence analysis, but all were strongly supported by peptide amino acid analyses and by the observed tryptic specificities.

The evidence for the two Met residues was indirect,

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as homoserine was present after the CNBr cleavage. This cleavage step also meant that there was no evidence for overlap after the Met residues. In addition, there was no evidence for overlap between residues 54 and 55, and 77 and 78. It has been assumed from homology with other higher plant plastocyanins [1] and from the amino acid composition of the protein that no additional residues are present.

The electrophoretic mobilities of the peptides at pH 6.5 were used to determine amide content [13] and this usually allowed for unambiguous assignment of amide residues. However, for the large and highly charged peptide X2T1 the amide content was uncertain. This peptide contains nine Asx/Glx residues of which three or four may be amides. Further information on which residues were amides was obtained from the thermolytic peptides. Electrophoretic analysis of aliquots from peptide X2H1 at successive degradation steps allowed unambiguous assignment of the single amide and three acidic residues of this peptide, and the mobility of peptide X2H2 showed that two of the four residues Asx(64), Glx(68), Glx(71) or Glx(73) were present as amides.

Additional support for the N-terminal 34 residues of the sequence was obtained by automatic sequence analysis using a Beckman 890c sequenator (D. Boulter and B. G. Haslett, personal communication).

DISCUSSION

The complete sequence of cucumber plastocyanin is closely related to other higher plant plastocyanin sequences [1]. However, when compared with the sequence of plastocyanin from vegetable marrow [14], which is also a member of the Cucurbitaceae, at least nine differences are found. This number may be

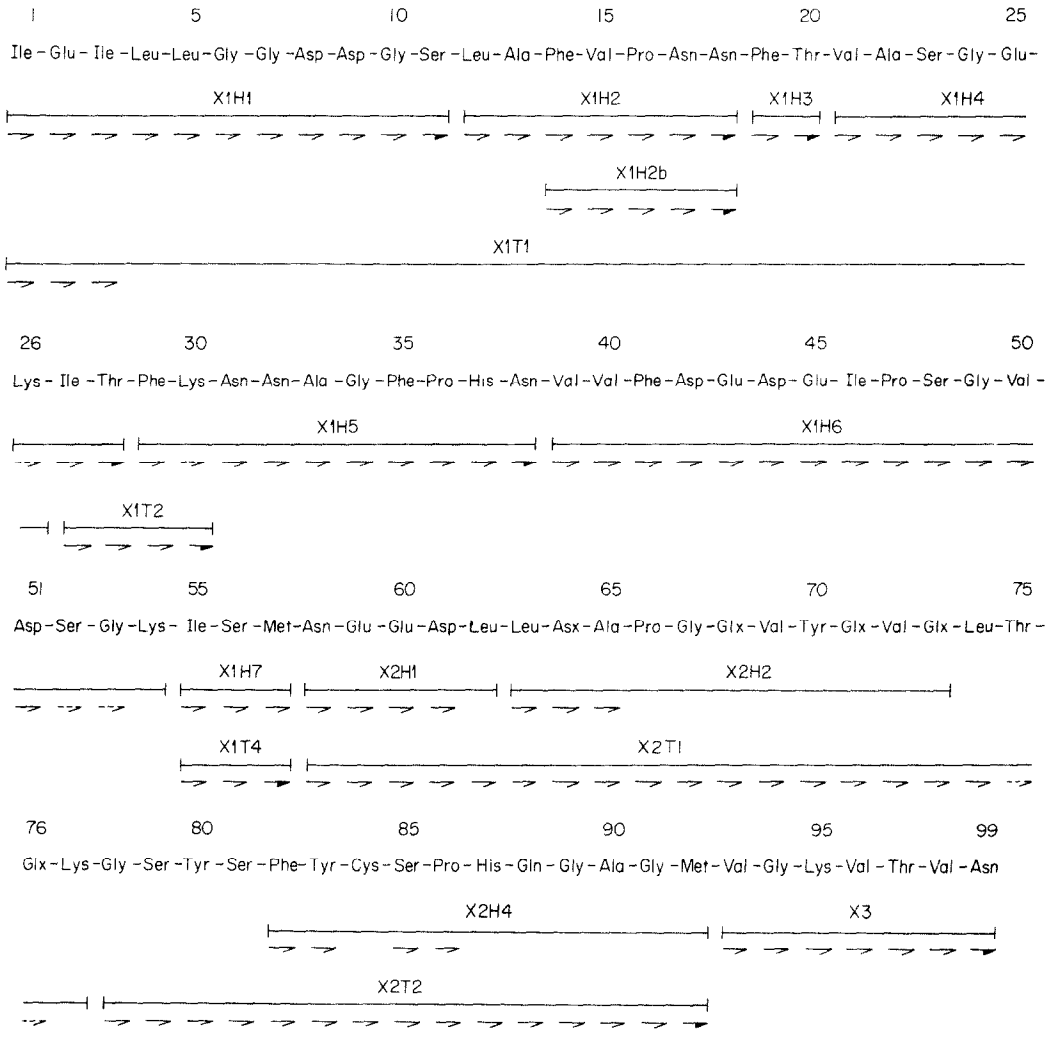

Fig. 1. The amino acid sequence of plastocyanin from cucumber (*Cucumis sativus*). Peptides which were purified for analysis are shown by a solid line. Cyanogen bromide fragments are labelled X; tryptic peptides T; thermolysin peptides H. Arrows → indicate residues which were identified by manual sequencing; where any ambiguity existed in identification, e.g. faint spots, an arrow ⇢ is used. An arrow ↛ indicates that the free amino acid was also observed. The evidence for residues 52, 53, 75 and 76 was weaker than that for the remainder of the sequence.

Table 1. Electrophoretic mobilities and amino acid analyses of purified peptides

Peptide	*m*(6.5)*	*m*(1.9)*	Analyses (20 hr hydrolysates)‡
X1H1	−1.70	0.21	Asp(2.1), Ser (0.9), Glu(1.3), Gly(2.7), Ile(1.7), Leu(2.2)
X1H2	0	0.30	Asp(1.9), Ser(0.3), Glu(0.3), Pro(1.0), Ala(1.2), Val(0.8), Leu(1.2), Phe(0.9)
X1H2a			Not isolated
X1H2b	0	0.51	Asp(2.1), Pro(0.9), Val(0.9), Phe(1.0)
X1H3	0	0.84	Thr(1.1), Phe(0.9)
X1H4	0	0.91	Thr(0.9), Ser(1.0), Glu(1.1), Gly(1.1), Ala(1.0), Val(0.9), Ile(0.8), Lys(1.1)
X1H5	1.15	0.85	Asp(3.1), Pro(1.0), Gly(1.1), Ala(0.9), Phe(2.0), His(1.0), Lys(1.0)
X1H6	−1.58	0.49	Asp(2.9), Ser(1.2), Glu(2.0), Pro(1.0), Gly(2.1), Val(3.3), Ile(1.1), Phe(0.9), Lys(1.0)
X1H7	0 1.35	0.73	Not analysed (see XIT4)
X1T1	−0.96	0.26	Asp(4.3), Thr(0.8), Ser(2.2), Glu(2.3), Pro(1.0), Gly/Ala†, Val(2.2), Ile(1.8), Leu(3.0), Phe(2.0), Lys(1.0)
X1T2	1.02		Thr(1.0), Ile(1.0), Phe(1.0), Lys(1.0)
X1T3			Not isolated
X1T4	0 1.35	0.73	Ser(1.0), Ile(1.1), +ve for homoserine
X2T1	−1.73		Asp(3.0), Thr(1.1), Glu(6.4), Pro(1.1), Gly(1.4)†, Ala(0.6)†, Val(2.1), Leu(3.1), Tyr(0.8), Lys(0.9)
X2T2	0.23 0	0.52	Ser(2.8), Glu(1.3), Pro(1.1), Gly(3.3)†, Ala(0.7)†, Tyr(1.9), Phe(1.0), His(1.0) + ve for homoserine, + ve for CM-Cys
X2H1	−2.50		Asp(1.8), Glu(2.1), Leu(1.0)
X2H2	−1.70	0.20	Not analysed
X2H3			Not isolated
X2H4	0.26	0.52	Ser(1.0), Glu(1.2), Pro(1.0), Gly(1.8), Ala(0.8), Tyr(0.8), Phe(0.9), His(0.9), +ve for homoserine, +ve for CM-Cys
X3	0.92		Asp(1.0), Thr(1.0), Gly(1.0), Val(3.1), Lys(0.9)

*Electrophoretic mobilities at pH 6.5 (*m* 6.5) were measured from DNS-Arg relative to DNS-Arg-Arg and at pH 1.9 (*m* 1.9) were measured from DNS-OH relative to DNS-Arg as previously described [21].

†Gly and Ala were poorly resolved.

‡Ratios of <0.3 are not included.

more, depending on the location of the unassigned amide residues and on the position of a Lys to Ser substitution which has been reported between two different cultivars of marrow [15]. The large differences which are found between plastocyanins from closely related species such as cucumber and marrow, French bean and broad bean [16, 17] contrast greatly with the sequence data for cytochrome *c* where proteins from closely related families, including the Cucurbitaceae and Leguminosae, generally differ by only 1–3 residues [18]. It has been suggested that plastocyanin has evolved about twice as quickly as cytochrome *c* [19], but this would seem to be an under-estimate.

The amino acid sequence of plastocyanin from cucumber has been determined in order to allow a more detailed interpretation of the NMR spectra which have been obtained for this protein [6, 10, 11]. Assignments for some of the aromatic residue resonances have been made for the NMR spectra of French bean plastocyanin [6] and these aromatic residues are conserved in the cucumber plastocyanin sequence.

EXPERIMENTAL

Plastocyanin was purified from either fresh or frozen material as previously described [20] except that NaCl was omitted from the extraction buffer, and all chromatographic steps were in 0.1 M NaOAc buffer, pH 6. In addition, final purification of plastocyanin was by gradient elution from DE(50) Sephadex with 100–300 mM NaCl in 0.1 M NaOAc buffer, pH 6. This step was repeated with the protein alternately in the oxidized and then reduced forms until a constant ratio for A_{278}/A_{597} of 1.1 was obtained.

Reduction and carboxymethylation of cucumber plastocyanin, cleavage with CNBr, gel filtration of CNBr fragments and enzyme digestions with trypsin and thermolysin were all as previously described [21]. 80% of the largest CNBr fragment was digested with thermolysin and the remainder with trypsin; 80% of the middle CNBr fragment was digested with trypsin and the remainder with thermolysin. The resulting peptides and the smallest CNBr fragment were purified by paper electrophoresis at pH 6.5 and 1.9 and had their sequences determined as previously described [21]. For amino acid analysis, samples were hydrolysed in evacuated tubes by 6 M HCl at 108° and analysed on a Beckman 121b amino acid analyser.

Table 2. Amino acid analysis of cucumber plastocyanin

	Analysis*	Sequence
Asx	13.94	14
Thr†	3.94	4
Ser†	7.09	8
Glx	11.70	11
Pro	4.82	5
Gly	12.39	12
Ala	5.09	5
Cys	n.d.	1
Val	10.08	10
Met	1.62	2
Ile	4.62	5
Leu	6.07	6
Tyr	2.61	3
Phe	5.67	6
His	2.09	2
Lys	4.94	5
Arg	0	0
Trp‡	0	0

*From hydrolysates of duplicate samples at both 20 hr and 70 hr.

†Corrected to zero time of hydrolysis. n.d., Not determined.

‡Determined spectrophotometrically relative to Tyr [22].

Acknowledgements—We wish to thank Professor H. C. Freeman, V. A. Norris, Dr. M. Murata and Dr. P. E. Wright for a sample of cucumber plastocyanin from the same source as used for NMR studies; Professor D. Boulter and Dr. B. G. Haslett for their unpublished data for the N-terminal of cucumber plastocyanin; and M. L. Coe for assistance with amino acid analyses.

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