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THE AMINO ACID SEQUENCE OF PLASTOCYANIN FROM *RUMEX OBTUSIFOLIUS*

BARRY G. HASLETT, CHRISTOPHER J. BAILEY*, JOHN A. M. RAMSHAW†, MICHAEL D. SCAWEN‡ and DONALD BOULTER

Department of Botany University of Durham, Durham DH1 3LE, England; * Department of Biochemistry, Trinity College, Dublin 2, Ireland

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Key Word Index—*Rumex obtusifolius*; Polygonaceae; dock; plastocyanin; amino acid sequence.

Abstract—The amino acid sequence of plastocyanin from dock has been completed. It is a single polypeptide chain of 99 residues which is closely related to other plant plastocyanins. Compared to a preliminary sequence presented earlier, the completed sequence now shows two changes, at positions 53 and 92.

INTRODUCTION

The amino acid sequence of dock plastocyanin has been determined as part of a continuing programme of using protein sequence data to examine plant affinities by computer generated phylogenetic trees [1, 2]. Further, comparative sequence data for plastocyanin is of use in interpreting the various spectroscopic studies on the nature of the copper binding site [see 3].

A preliminary communication on studies of the amino acid sequence of dock plastocyanin has been presented [1]. Completion of this study has confirmed the sequence originally presented except for two residues in the sequence, positions 53 and 92.

RESULTS AND DISCUSSION

The amino acid sequence of dock plastocyanin is given in Fig. 1 together with the evidence for it derived from those peptides which were obtained sufficiently pure for analysis. Dock plastocyanin consists of a single polypeptide chain of 99 residues.

A Beckman 890c automatic sequencer was used to determine 35 of the *N*-terminal residues unambiguously and allowed probable identification of a further 5 residues. These results were confirmed and the remainder of the sequence was established from a consideration of the results of analyses of thermolysin, chymotrypsin and papain peptides.

The majority of the sequence was established from the analysis of the thermolysin peptides, of which peptides H13 and H17a are indistinguishable. The fragment(s) representing residues 82–92 was not recovered in sufficient purity and quantity for accurate analysis. This region of the sequence however is represented by strong papain and chymotrypsin peptides. Most of the expected papain peptides were obtained in sufficient yield for analysis; Peptides from regions 31–48 and 91–93 were either not found or could not be obtained sufficiently pure. Chymotryptic peptides covering all of the sequence were obtained. The large peptide C4 was further digested with subtilisin and three fragments were obtained which were analysed. In case of peptides C4S1 and C4S2 the length of the peptide was not established. With peptide C7, methionine was clearly present from its amino acid composition, but after 8 steps of degradation insufficient material remained for identification of the residue. However further evidence for this methionine residue is available from CNBr cleavage results and from the amino acid analysis of the total protein (Table 1). Preliminary experiments on the CNBr cleavage of dock plastocyanin were unsuccessful and it was concluded that methionine 92 may be absent despite the very strong indication of 2 methionine residues being present in the amino acid analysis [1]. On reinvestigation, CNBr cleavage of a Met–Val bond was shown by the presence of a new Val *N*-terminal residue after cleavage. This further supports the Met 92 assignment The leucine residue suggested in [1] was based on the *N*-terminal of a misidentified peptide and was not confirmed on further analysis. The other

Table 1. Amino acid analysis of dock plastocyanin

	24 hr hydrolysate	Sequence
Aspartic acid	9.41	10
Threonine	4.83	5
Serine	4.93	6
Glutamic acid	10.04	10
Proline	5.63	5
Glycine	11.16	12
Alanine	10.10	11
Cysteine	n.d.	1
Valine	9.67	10
Methionine	1.75	2
Isoleucine	5.65	5
Leucine	5.42	5
Tyrosine	2.94	3
Phenylalanine	6.10	6
Histidine	1.69	2
Lysine	7.22	6
Tryptophan	n.d.	0
Arginine	0	0

n.d. not determined.

† Present Address: Museum of Comparative Zoology, Harvard University, Cambridge, MA02138, U.S.A.

‡ Present Address: Microbiological Research Establishment, Porton Down.

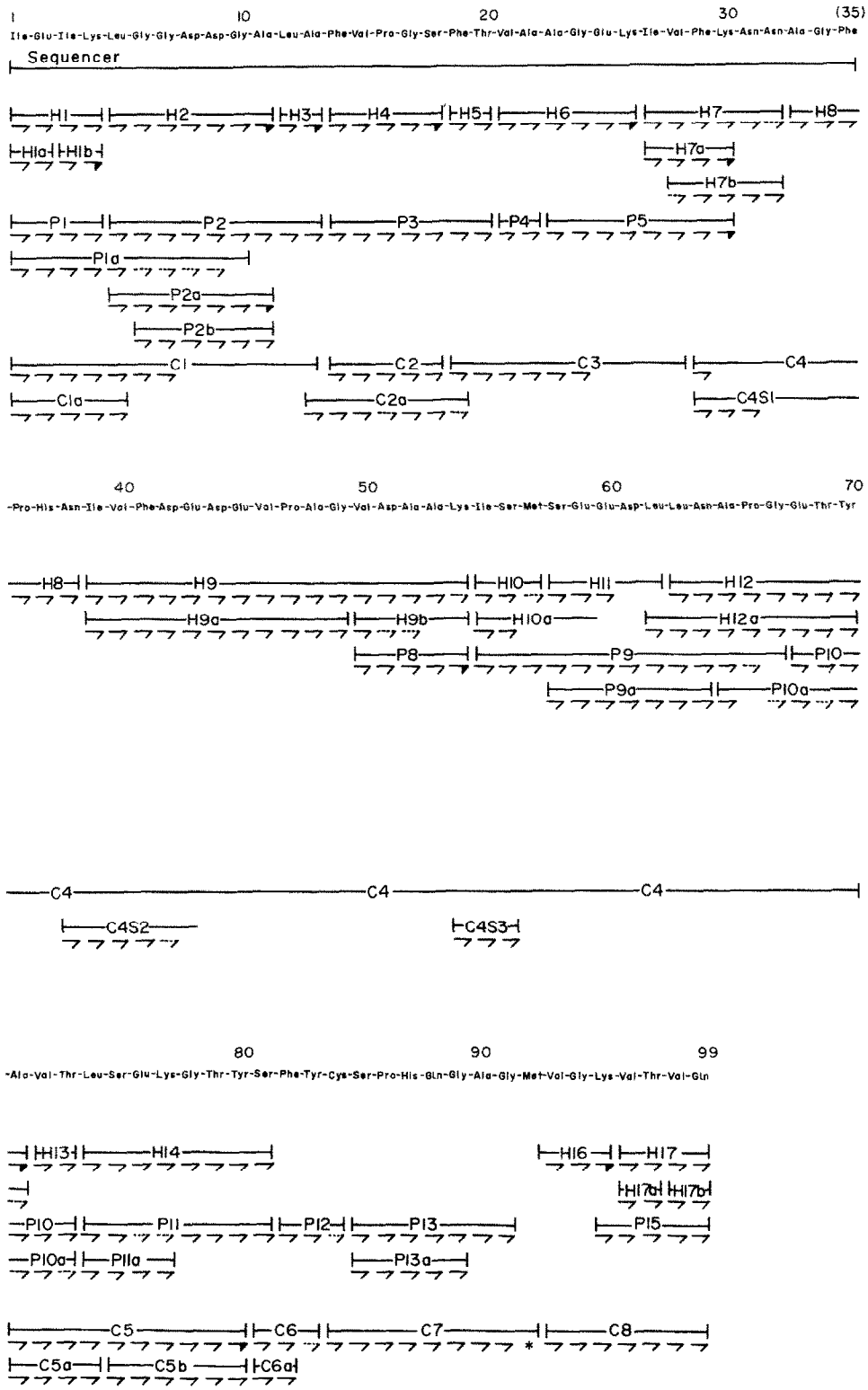

Fig. 1. The amino acid sequence of plastocyanin from dock. Peptides purified for analysis are shown by solid lines. They are labelled: H for thermolysin, P for papain, C for chymotrypsin and S for subtilisin. Arrows ——→ indicate residues postively identified by automatic or manual sequencing. Where some ambiguity existed an arrow - - - → is used An arrow ——→ indicates that the free amino acid was also identified. An * indicates presence in the peptide composition [15] (see text also).

change between the sequence now presented and the preliminary results is that residue 53 is alanine. The serine originally proposed was based on the weak evidence of peptide H9b. Completion of the analysis of peptide H9 and the identification of peptide P8 from the papain digest have shown unambiguously that residue 53 is alanine.

Assignment of amide residues was either from the automatic sequencer data or from peptide mobilities at pH 6.5 [4]. For peptides H9 and H9a, the mobilities (−1.71 and −2.14 with respect to dansyl–arg–arg) were in close agreement with those found for the equivalent peptides in potato plastocyanin (−1.50 and −2.15) for which the presence of the 4 successive acidic residues has been clearly established [5]. The various peptides isolated from the *C*-terminal of the protein suggest that the *C*-terminal glutamine residue is either particularly liable to deamidation or else glutamic acid is present as heterogeneity which is not resolved during the protein preparation. Apparent deamidation of the *C*-terminal amide residue has been observed in other plastocyanins [5–7].

The peptides examined provide evidence for the complete sequence of dock plastocyanin. The evidence for residues 33–38 is weaker than that for the rest of the sequence in that only one peptide is given. Similarly, the evidence for position 92 relies on amino acid composition and cleavage specificity. In certain places in the sequence there is no overlap evidence or it is not extensive. However the homology with French bean and *Chlorella* plastocyanins for which complete overlap was shown [8, 9] is extensive. The sequence is in reasonable agreement with the determined amino acid analysis (Table 1) except for the value for lysine. The peptides examined show no evidence for an additional lysine residue in the sequence.

The evidence showing that residue 92 is methionine rather than leucine, as incorrectly suggested in [1], is important as this means that this residue is still invariant among plastocyanins. Spectroscopic evidence suggests the proximity of a methionine to the copper atom [10, 11] and this methionine has been suggested as a copper ligand [12].

EXPERIMENTAL

Preparation and *S*-carboxymethylation of plastocyanin from dock leaves was as previously described [5,13]. Enzyme digestions and separation of peptides by high voltage paper electrophoresis and PC were as previously described [5]. Peptide amino acid sequences were determined by the dansyl–phenyl isothiocyanate method [14] as previously described [15]. Automatic sequence determinations of native protein (400 nmol) were carried out on a Beckman 890c automatic sequencer using a fast protein program [16]. Identification of phenylthiodydantoin amino acids by TLC, GLC and HI regeneration methods was as previously described [17]. Amino acid analyses were performed on a Locarte amino acid analyser.

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