Primary Structure of Rice Cytochrome c

Eigo MORI and Yuhei MORITA

The Research Institute for Food Science, Kyoto University, Uji 611, Japan

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The primary structure of cytochrome c from many plants has been determined to construct a phylogenetic tree for higher plants,¹⁾ although little work has been done on monocotyledonous species. As the primary structure of plant cytochrome c differs in certain characteristic features from that of vertebrates,²⁾ the determination of the three-dimensional structure of plant cytochrome c will provide valuable information on the structure-function relationship of the molecule. Morita and Ida³⁾ have reported preliminary crystallographic data for the hexagonal crystals of cytochrome c from rice embryos, and further structural investigation is under way. In this communication we describe the primary structure of rice cytochrome c.

Rice cytochrome c with an A_{550} (red)/ A_{280} (ox) ratio of 1.03 was used in the sequence study,⁴⁾ and was denatured with ethanol according to the method of Margoliash *et al.*⁵⁾ Cleavage of the denatured protein (5.9 μ moles) at two methionine sites with cyanogen bromide (CNBr) by the method of Steers *et al.*⁶⁾ yielded three major fragments (I, II and III) with overlapping fragments and the unreacted protein in minor quantities. These fragments were separated by gel filtration on Sephadex G-75 and -50 columns, followed by Aminex 50W-X2 column chromatography. The CNBr peptides I and II each contained one homoserine or homoserine lactone, whereas III contained neither. Peptide I was also found to contain the N-terminus of the protein. Therefore, the CNBr peptides must be aligned: I-II-III. The final yields of the purified peptides were: I (residues -8 to 55) 15%, II (56 to 80) 8.1%, III (81 to 103) 28%. Pepti-

des I and III were further fragmented by trypsin and II by chymotrypsin at pH 8.0 and at 37°C. The tryptic and chymotryptic peptides were fractionated on an Aminex 50W-X2 column (0.8 $\times$ 65 cm) and purified on Sephadex G-50, -25 and -15 columns (1.5 $\times$ 240 cm) or by high-voltage paper electrophoresis at pH 6.5 or pH 1.9. The yields and charge at pH 6.5 of these purified peptides were: IT1, 43%, 0; IT2, 23%, 0; IT3, 66%, +1; IT4, 15%, N.D.; IT5, 23%, 0; IT6, 56%, N.D.; IT7, 18%, 0; IT8, 9.5%, -1; IT9, 12%, 0; IIC1, 50%, N.D.; IIC2, 32%, N.D.; IIC2', 48%, N.D.; IIC3, 67%, N.D.; IIIT1, 65%, +2; IIIT2, 70%, -1; IIIT3, 81%, +1; IIIT4, 75%, -1, where N.D. means "not determined." To align these peptides derived from CNBr fragments the overlapping peptides were obtained from the chymotryptic digest of the denatured protein (2.0 μ moles). Fourteen peptides were purified by similar procedures. Their yields and charge at pH 6.5 were: C1, 54%, N.D.; C2, 56%, 0; C3, 9.3%, N.D.; C4, 30%, 0; C5, 32%, 0; C6, 24%, 0; C7, 8.6%, N.D.; C8, 43%, -2; C9, 26%, +1; C9', 8.7%, +2; C9'', 25%, N.D.; C10, 41%, +1; C11, 47%, N.D.; C12, 59%, 0, where N.D. means "not determined." Peptide numbering is based on the position of the peptide in the complete amino acid sequence of the protein starting at the N-terminus, and the residue number is based on vertebrate cytochrome c numeration.

Amino acid analyses were performed with a Hitachi KLA-5 amino acid analyzer or a micro-amino acid analyzer (1~10 nmoles) constructed by Hayashi. Lysine and ϵ -N-trimethyllysine (TML) were separated in the amino acid analysis, as described by Hempel and Lange.⁷⁾ IT1, IT2, IT3, IT4, IT5, IT6, IIC3, IIIT1, IIIT2, IIIT3, IIIT4, C4, C5, C6, C7, C8 and C9 were sequenced by the subtractive Edman method.⁸⁾ The others were sequenced by the Edman-Dansyl method⁹⁾ directly or on subfragments after thermolytic digestion (C2 and C11) or chymotryptic digestion (IT1). The C-terminal sequence of peptides was determined by carboxypeptidase

FIG. 1. The Complete Amino Acid Sequence of Rice Cytochrome c.

The residue numbers used in this figure are based on vertebrate cytochrome c numeration. The Roman numbers refer to CNBr peptides. The prefix T refers to purified tryptic peptides, and the prefix C to purified chymotryptic peptides. TML = ϵ -N-trimethyllysine.

A, B or Y digestion methods.^{10,11)}

The complete amino acid sequence of rice cytochrome c is shown in Fig. 1. The amides were assigned by the method of Offord¹²⁾ but Gln²⁸, Asn³¹, Asn³³, Gln³⁹, Asn⁶², Gln⁸⁹ and Glu⁹⁰ were assigned by homology with the sequences of other plant cytochromes c already reported. The primary structure of rice cytochrome c has general features common to other plant cytochromes c, *i. e.*, an N-acetylated 'tail' of eight residues and two TML residues in positions 72 and 86. However, the residue in position 96 in rice cytochrome c is serine, the same as in Ginkgo, whereas other plant cytochromes c so far examined have alanine in this position. The experimental details of sequence determination will be described elsewhere along with a discussion of taxonomical comparison and structural homology.¹³⁾

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