Preliminary Notes

PN 10079

The complete amino acid sequence in baker's yeast cytochrome c

In a previous preliminary note, we reported the isolation and amino acid sequences of twenty-two peptides from the tryptic digest of baker's yeast cytochrome *c* (ref. 1). Some of them were the products of incomplete tryptic hydrolysis and they were able to overlap one another to constitute longer fragments. Thus the above twenty-two peptides could be combined into thirteen fragments. In order to find bridge peptides which overlap the above tryptic fragments, chymotryptic digestion of the cytochrome and fractionation of the peptides by a Dowex-50 X2 column were performed. The results of the structural analysis of the thirty-one chymotryptic peptides could combine the above tryptic fragments into six fragments (1–4*, 5–16, 17–32, 33–91, 92–104, and 105–108), but their mutual combination could not be determined, since all of the corresponding bridge peptides terminated at N-terminal lysine residue. Then thirty-three peptides from the peptic digest of the protein were analyzed on their amino acid compositions and N-terminal residues. The results provided an information concerning the mutual combination of the fragments 17–32 and 33–91 together with lots of confirmatory data on the amino acid sequence of already elucidated fragments.

From the reaction mixture of cyanogen bromide[2] with the cytochrome (0.1 N HCl, 36°, 24 h), three peptide fragments could be isolated by gel filtration on a Sephadex G-50 column and subsequent chromatography on an Amberlite CG-50 column. The one contained the heme group and its amino acid composition suggested that it corresponded to the peptide 1–69. Two-dimensional map by paper chromatography–electrophoresis of the tryptic digest of the above heme-peptide demonstrated the presence of the tryptic peptides which are expected to be derived from the peptide chain between the N-terminal and the 60th amino acid residues in the intact protein and the peptide 61–69 which contained homoserine instead of methionine residue. Consequently the above fragments 1–4, 5–16 and a part of 17–91 must be contained in the heme-containing fragment and the fragment 17–91 must be C-terminal part, since one of the other two fragments obtained by the cyanogen bromide treatment seemed to be the peptide 70–85. As was already reported, the peptide 1–4 was the N-terminal of the cytochrome[1]. Thus the amino acid sequence from the N-terminus to 91st residue is established. Constituent amino acids of the remaining fragment suggested that it corresponded to the peptide 86–108. The tryptic peptide 105–108 was shown to be C-terminal[1] and it can be concluded that the tryptic fragment 92–104 must be adjacent to the above C-terminal peptide. Thus the complete amino acid sequence in baker's yeast cytochrome *c* can be established as is shown in Fig. 1.

A remarkable difference in amino acid sequence between the yeast and vertebrate

* Figures denote the positions of amino acid residues from the N-terminus: e.g. 1–4 demonstrates tetrapeptide which contains amino acid residues from the N-terminal to the 4th.

cytochromes *c* is observed around the N-terminal group. Contrary to the N-terminal acetyl–Gly–Asp–Val–Glu– sequence in the cytochromes *c* of bovine[3], equine[3,4], human[5] and swine[6] hearts, the yeast protein terminated at an unsubstituted threonine residue, and N-terminal Thr–Glu–Phe–Lys–Ala– in the latter protein seems to correspond to the terminal acetyl group in the former. Such a relationship was also observed in α- and β-melanocyte-stimulating hormones[7,8]. Another difference can be seen in the C-terminal sequence. The above mammalian cytochromes terminated at –Thr–Asp (NH_2)–Glu, but –CySH–Glu in the yeast protein: namely the latter has one less amino acid residue near the C-terminus. Amino acid residues written in boldface type (total 62 residues) in Fig. 1 are common for the both groups of the cytochromes and a part

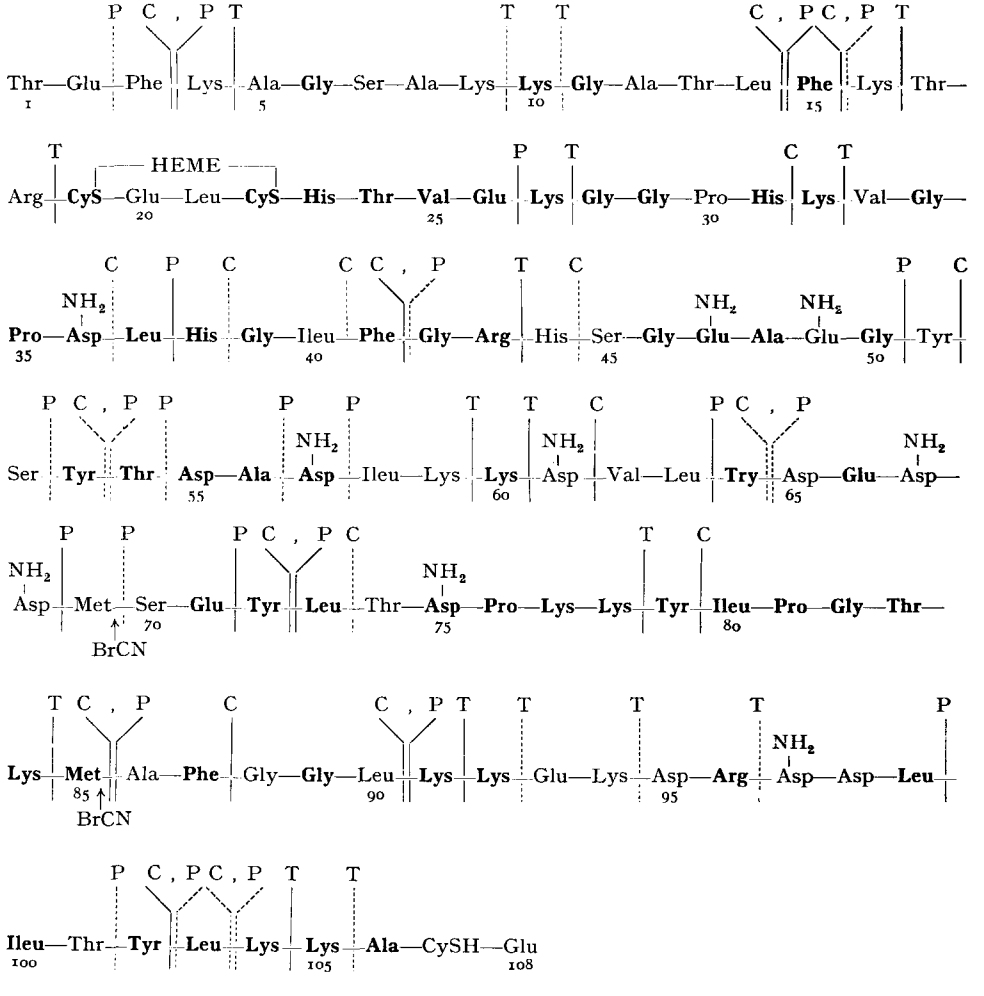

Fig. 1. The amino acid sequence of cytochrome *c* from baker's yeast. Amino acid residues written in boldface type denote that they are identical to those found in the equine-heart protein. The solid and dashed lines indicate the major and minor points of hydrolysis by trypsin (T), chymotrypsin (C) and pepsin (P), respectively.

of them probably contributes, at least, to maintain secondary and tertiary structures for active conformation of the protein.

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Soluble cytrochromes in *Escherichia coli*

It is well known that four cytochrome pigments occur in *Escherichia coli*, *i.e.* membrane-bound cytochromes b_1, a_1 and a_2 (ref. 1) and a CO-binding pigment or cytochrome *o* of unknown intracellular localization[2,3]. Recently a fifth pigment has been added to this list by GRAY *et al.*[4,5] who showed that a soluble cytochrome of the *c* type ("cytochrome *c*-551") is synthesized in *E. coli* and related facultative anaerobes when they are grown anaerobically. While studying the enzyme system involved in nitrate respiration[6], we have also noticed the presence of a closely similar cytochrome ("cytochrome *c*-552") in a soluble fraction obtained from *E. coli* (Yamagutchi) grown anaerobically in the presence of nitrate. Furthermore, it has been disclosed that cytochrome *c*-552 was always accompanied by smaller amounts of a second cytochrome of the *c* type ("cytochrome *c*-550") and a pigment of the *b* type ("cytochrome *b*-562"). This paper describes briefly the purification and properties of these soluble cytochromes.

E. coli (Yamagutchi) was cultivated anaerobically in a medium containing glucose, inorganic salts (including 0.1% $NaNO_3$) and 0.05% yeast-extract powder, and the cells harvested at the log phase were disrupted in 0.1 M phosphate (pH 7.0) by sonic oscillation (9 kcycles) for 10 min. When the sonicate was centrifuged at 105 000 × *g* for 3 h, practically all of the cytochrome b_1 as well as the cytochromes of the *a* type were recovered in the particulate sediment; the cytochrome b_1 content in the intact cells was determined by the previously reported method[7] to be 0.34 mμmole/mg of