THE INHIBITION OF CYTOCHROME OXIDASE ACTIVITY BY CYTOCHROME C PEPTIDE FRAGMENTS

Yoshihiko Baba, Hiroshi Mizushima,
Akira Ito and Hiroshi Watanabe
Central Research Laboratories, Sankyo Co. Ltd.
Shinagawa, Tokyo, Japan

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There are several indications that a specific protein-protein interaction, such as found in an antigen-antibody reaction, might be responsible for the cytochrome c-cytochrome oxidase interaction. We attempted to find the positions in cytochrome c responsible for the interaction by investigating the inhibitory effect of cytochrome c peptide fragments on cytochrome c-cytochrome oxidase binding.

Cytochrome c was split into seven fragments by chemical and enzymatic cleavage, and the inhibitory effect of every fragment was examined. The fragments containing residues -2~9, 39~54 and 81~103 showed considerable inhibition. Other fragments, however, did not, in spite of the fact that among them was present the fragment including the longest amino acid sequence which is invariable in all cytochromes c so far examined.

Materials and Methods ; Chromatographically pure cytochrome c was prepared from Saccharomyces oviformis M_2. Its amino acid sequence had been elucidated by Narita et al. (1963) (Fig. 1). Cytochrome oxidase was isolated and highly purified from the above described strain following Sekuzu's method (1964). Seven fragments of cytochrome c were obtained as described in Table I and purified by paper chromatography and gel-filtration on Sephadex G 10. Amino acid ratios of these peptides were determined by amino acid analysis (Hitachi KLA-2 amino acid automatic analyzer). Assays

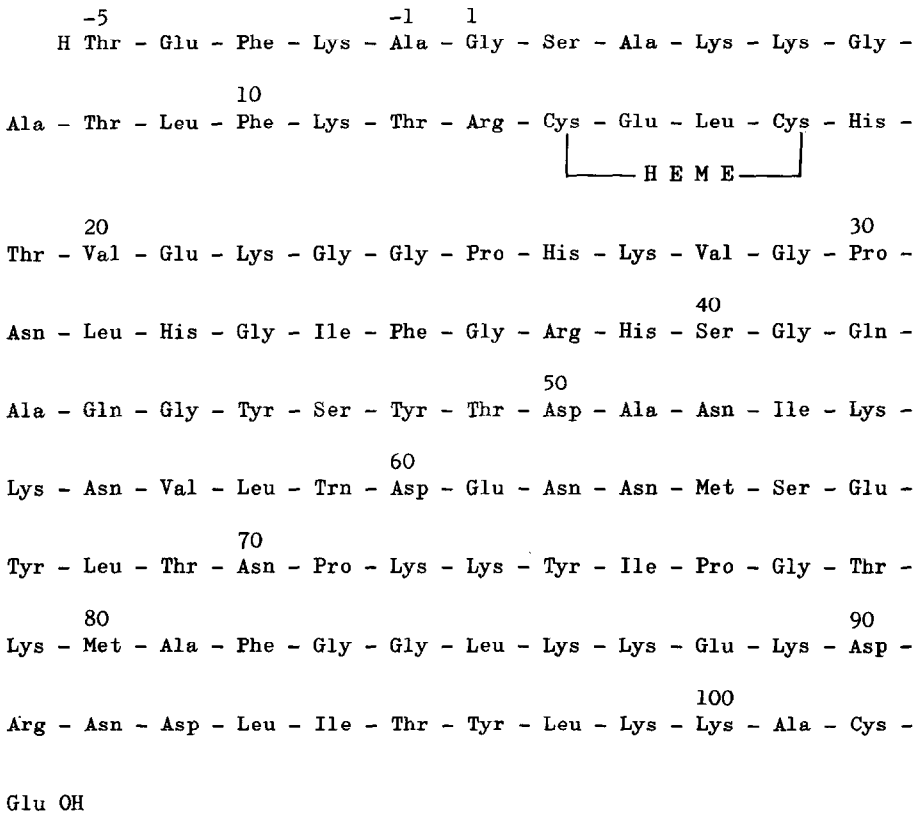

Fig. 1 Amino acid sequence of cytochrome c (Saccharomyces oviformis M_2) (Narita et al., 1963)

for cytochrome oxidase activity were performed spectrophotometrically by determining the decrease in absorbancy of ferrocytochrome c at 550 mμ at 20°C in 0.15 M phosphate buffer (K^+) pH 6.0. The concentrations of ferrocytochrome c, cytochrome oxidase and peptide fragments as inhibitors were 2×10^{-5} M, 10^{-8} M and 2×10^{-4} M each. Cytochrome oxidase was pre-incubated with the peptide fragments for two minutes at 20°C before the addition of ferrocytochrome c.

Results and Discussion ; Cytochrome c is a basic heme-protein which has an isoelectric point at pH 10-11, while cytochrome oxidase is an acidic heme-protein, resulting in an electrostatic coupling. This type of reaction had been suggested, and then also proved by chemical modifications

Table I Fractionations of cytochrome c peptides

method for cleavage of peptide bonds	tryptic digestion	Chymotryptic digestion	Cyanogen bromide treatment
chromatographic separation			
column	Dowex 50 x 2 200-400 mesh	Dowex 50 x 2 200-400 mesh	IRC 50
temperature	45°C	30°C	room temperature
buffer system	linear gradient elution from 0.1 N pyridine-formate pH 3.1 to 2.0 N pyridine acetate pH 5.0	linear gradient elution from 0.1 N pyridine-formate pH 3.1 to 2.5 N pyridine acetate pH 5.0 (According to Yaoi 1966)	linear gradient elution from 10% pyridine-5% acetic acid to 20% pyridine-10% acetic acid
fragments selected as inhibitors	Fragment IV Fragment V	Fragment I Fragment II Fragment III	Fragment VI Fragment VII

of lysine residues in cytochrome c (Takemori et al. 1962). Lysine residues, which are the predominant amino acid residues in cytochrome c, were shown to be of great importance in the interaction. Fragment I and Fragment VII, which contain several lysine residues, showed considerable inhibition as expected (Table II).

Fragment IV exhibits the strongest inhibition in spite of having only one lysine residue. We wondered which other residue is responsible for the inhibitory effect of the fragment. Some of the positions remaining invariant in cytochromes c of known primary structure are supposed to be responsible, because cytochromes c from various species, in spite of their differences in amino acid sequences, react readily with the same cytochrome oxidase*. A good candidate is the tyrosine residues in positions 46 and 48 on account of the distinct constancy in the distribution of

Table II Inhibition of cytochrome oxidase activity by cytochrome c peptide fragments

peptide fragment	location in cytochrome c	inhibition (%)
Fragment I	-2 ~ 9	15.0
Fragment II	11 ~ 26	a little
Fragment III	27 ~ 36	6.7
Fragment IV	39 ~ 54	25.3
Fragment V	56 ~ 73	0
Fragment VI	65 ~ 79	0
Fragment VII	81 ~ 103	17.8

aromatic amino acids in cytochromes c. Further studies on the tyrosine residues of cytochrome c are now in progress.

The mode of inhibition by Fragment I and Fragment IV was found to be competitive as shown in Fig. 2.

* Y. Baba, unpublished data ; I. Sekuzu, private communication

It was impossible to determine the inhibitory activity of Fragment II because of its oxidative effect on ferrocytochrome c. (On this interesting effect we will report in another place)

No inhibition was obtained by Fragment V. Also, Fragment VI containing almost the longest constant segment of cytochrome c (70~80) did not have any inhibitory activity. This result suggest that this part (70~80) does not play a role in the cytochrome c-cytochrome oxidase coupling mechanism. It may, however, be important for other properties of cytochrome c.

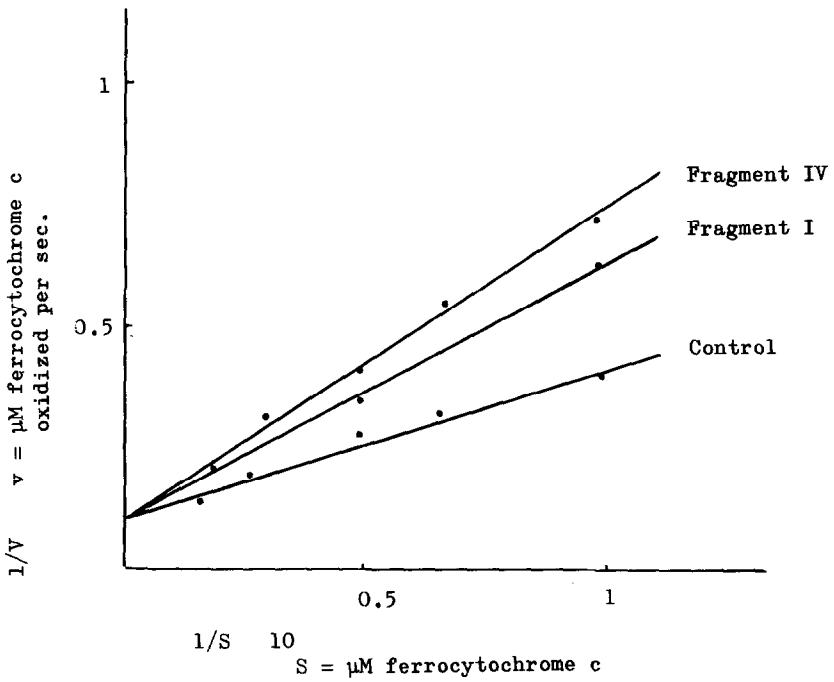

Fig. 2 Competitive inhibitions of cytochrome oxidase activity by Fragment I and Fragment IV

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