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Functional and Structural Comparisons between Prokaryotic and Eukaryotic *aa*$_3$-Type Cytochrome *c* Oxidases from an Evolutionary Point of View

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The specificities for cytochrome *c* of the *aa*$_3$-type cytochrome *c* oxidase were studied with enzymes derived from *Thiobacillus novellus*, *Nitrobacter agilis*, *Paracoccus denitrificans* and the cow in reaction with the cytochromes *c* from 5 prokaryotes and 7 eukaryotes. The *T. novellus* enzyme reacted most rapidly with the cytochromes *c* of *Candida krusei*, tuna and bonito as well as *T. novellus* cytochrome *c*; the specificity for cytochrome *c* of the *N. agilis* enzyme was similar to that of the *T. novellus* enzyme. The bovine enzyme reacted rapidly with all the eukaryotic cytochromes *c* tested. The *P. denitrificans* enzyme showed a specificity similar to that of the bovine enzyme, except that it reacted rapidly with *P. denitrificans* cytochrome *c*, while the bovine enzyme reacted with it very poorly. All four kinds of enzymes showed an extremely limited reaction with *Pseudomonas aeruginosa* cytochrome *c*.

The amino acid composition of subunit I of the *N. agilis* enzyme resembled that of the bovine enzyme, while the compositions of their subunits II were different. On the basis of these results, an evolutionary relationship between bacterial and eukaryotic enzymes was discussed.

Key words: Cyt *aa*$_3$ — Cyt *c* oxidase — Evolution — *Nitrobacter agilis* — *Paracoccus denitrificans* — *Thiobacillus novellus*.

The *aa*$_3$-type Cyt *c* oxidase [EC 1.9.3.1] is distributed among all eukaryotes and many aerobic prokaryotes (Keilin 1966, Lemberg and Barrett 1973). The enzyme has heme *a* and copper as the prosthetic groups and reduces molecular oxygen with reduced Cyt *c* as an electron donor (Okunuki 1966). We have shown that the eukaryotic oxidase reacts rapidly with almost all eukaryotic Cyts *c*, but little or not at all with many bacterial Cyts *c* (Yamanaka 1972, 1973, Yamanaka and Fukumori 1978). The finding that many bacterial Cyts *c* differ from eukaryotic Cyts *c* in their reactivity with the oxidase suggests that bacterial Cyt *c* oxidase may differ from eukaryotic oxidase in its specificity for Cyt *c*.

Recently, we have succeeded in purifying Cyt *c* oxidases derived from two bacteria and shown that although these enzymes resemble the eukaryotic enzymes in their spectral properties, their subunit structures differ greatly from those of the eukaryotic enzymes (Yamanaka et al. 1979, Yamanaka and Fujii 1980, Yamanaka et al. 1981).

In the present study, we determined the specificities for Cyt *c* of the Cyt *c* oxidases derived from *Thiobacillus novellus*, *Nitrobacter agilis* and *Paracoccus denitrificans*, and compared their specificities with that of the bovine enzyme from an evolutionary

point of view. Further, the amino acid compositions of the subunits obtained from the *N. agilis* enzyme were compared with those of the subunits of the bovine enzyme, and an evolutional relationship between the bacterial and eukaryotic enzymes is discussed on the basis of the structural features observed.

Materials and Methods

Preparation of cytochromes c *and oxidases*—Cyt *c* oxidases from *T. novellus*, *N. agilis* and *P. denitrificans* were purified according to Yamanaka and Fujii (1980), Yamanaka et al. (1981) and Fujii (1979), respectively. The *T. novellus* and *N. agilis* enzyme preparations were electrophoretically homogeneous. The *P. denitrificans* enzyme preparation contained Cyt c_1 of approximately 20 mol% or less, although the enzyme has been obtained in a pure state by Ludwig and Schatz (1980). The bovine enzyme was highly purified according to the method of Okunuki (1966). The purified preparation of this enzyme was kept frozen in liquid nitrogen until use.

Cyts *c* derived from *Pseudomonas aeruginosa* (Horio et al. 1960), *Nitrosomonas europaea* (Yamanaka and Shinra 1974), *T. novellus* (Yamanaka et al. 1971), *P. denitrificans* (Scholes et al. 1971), *N. agilis* (Ketchum et al. 1969), *Candida krusei* (Yamanaka et al. 1964), *Saccharomyces oviformis* (Motonaga et al. 1965), tuna (Hagihara et al. 1958), bonito (Hagihara et al. 1958), cow (Hagihara et al. 1958) and man (Matsubara and Yasunobu 1961) were highly purified according to the methods described in the references cited.

Enzyme reactions—The reactivities of the oxidases with the Cyts *c* were determined spectrophotometrically (Yamanaka 1972); to 1.0 ml of about 20 μM ferrocytochrome *c* dissolved in 10 mM phosphate buffer was added 0.02 ml of 1–2 μM oxidase and the decrease in the absorbance at the α peak of each Cyt *c* was followed over time at 20°C. The reactions were performed at the pH optima for each enzyme: at pH 5.5 for the *T. novellus* and *P. denitrificans* enzymes and at pH 6.0 for the *N. agilis* enzyme. In the case of the bovine enzyme, the reactions were performed in 40 mM phosphate buffer at pH 6.5. The initial reaction rate was determined from the absorbance decrease at the α peak between 15 and 30 sec after the addition of oxidase; the molecular activity was calculated from the initial rate.

Enzyme subunit analysis—The subunits of the *N. agilis* oxidase were separated by polyacrylamide gel electrophoresis in the presence of SDS using a preparative gel electrophoresis apparatus (Canalco Prep-Disc, Miles Laboratories Inc., Elkhart, Indiana, U.S.A.). The gel was made from 12.5% acrylamide containing 0.1% SDS. The enzyme preparation was heated at 100°C for 1 min in the presence of 3% SDS before electrophoresis. The protein content in the eluate was monitored by the absorbance at 280 nm: two distinct peaks were detected in the elution curve. The proteins in each peak were confirmed to be subunits I and II by separate polyacrylamide gel electrophoresis in the presence of SDS. To analyze the amino acid compositions of subunits I and II, each was dialyzed for 48 hr against 0.001% SDS, lyophilized, and hydrolyzed in an evacuated sealed tube with 6 N HCl for 24 hr at 110°C, then analyzed with a high sensitivity amino acid analyzer (Iriac Instruments, Inc., model A-3300).

Results

Specificities for cytochrome c *of cytochrome* c *oxidases*

The reactivities with the various Cyts *c* of the four kinds of Cyt *c* oxidases are shown in Fig. 1.

T. novellus *cytochrome* c *oxidase*: The enzyme reacted very poorly with Cyts *c* derived from *P. aeruginosa*, *N. europaea* and *P. denitrificans*, while it reacted rapidly with Cyts *c* from *C. krusei*, tuna and bonito as well as with *T. novellus* Cyt *c*. However, it reacted relatively slowly with bovine, horse and human Cyts *c*. It is noteworthy that the enzyme reacted with human Cyt *c* more rapidly than with the bovine and horse Cyts *c*. The reactivity of the enzyme was greatly affected by ionic strength. The reaction rates with the Cyts *c* of *T. novellus*, tuna, horse and man decreased with an increase in the concentration of the phosphate buffer in the reaction mixture, while the reaction rates with yeast Cyts *c* showed maximal values in 30–40 mM buffer. Thus, the reactivity of *S. oviformis* Cyt *c* with the *T. novellus* oxidase in 40 mM phosphate buffer was comparable to that of tuna Cyt *c* in 40 mM phosphate buffer. However, the profile of specificity obtained from the reactions in 40 mM phosphate buffer did not differ much, as a whole, from that in 10 mM buffer except for the greater relative reactivity of *S. oviformis* Cyt *c*.

N. agilis *cytochrome* c *oxidase*: The enzyme reacted rapidly with Cyts *c* derived from *C. krusei*, *S. oviformis*, tuna and bonito as well as with *N. agilis* Cyt *c*. Further, it reacted with bovine and horse Cyts *c* rapidly as compared with the *T. novellus* enzyme. The *N. agilis* enzyme reacted very poorly with *P. aeruginosa* Cyt *c*, while it reacted rapidly with Cyts *c* derived from *N. europaea* and *T. novellus*. The reactivity of the enzyme with Cyts *c* was also affected greatly by ionic strength. The reaction rates with Cyts *c* of *N. agilis*, *C. krusei*, *S. oviformis*, tuna and horse decreased

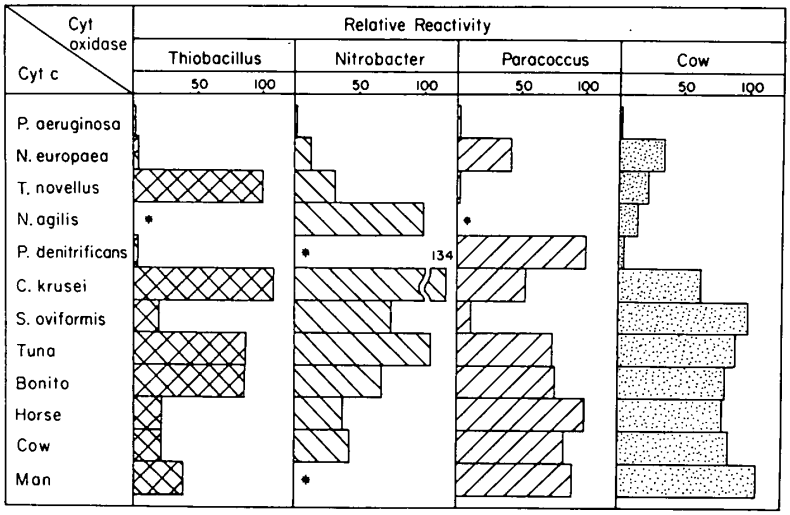

Fig. 1 Specificities for various Cyts *c* of Cyt *c* oxidases derived from *T. novellus*, *N. agilis*, *P. denitrificans* and cow. The reactivities were expressed as relative values; the molecular activity (mol of ferrocytochrome *c* oxidized/mol of enzyme) per min was taken as 100% in the reaction of each oxidase with Cyt *c* from its host organism, except for the bovine enzyme, where *S. oviformis* Cyt *c* was used in place of bovine Cyt *c*. Where indicated by *, the reactivity was not determined.

when the concentration of phosphate buffer increased in the reaction mixture. The reaction rates with *N. agilis* and *S. oviformis* Cyts *c* showed maximal values in 10 mM phosphate buffer, while the rate with *C. krusei* Cyt *c* showed a plateau at 5–10 mM.

P. denitrificans *cytochrome* c *oxidase*: The specificity for Cyt *c* of the enzyme resembled that of bovine Cyt *c* oxidase; it reacted rapidly with all the eukaryotic Cyts *c* tested in the present study with exception of *S. oviformis* Cyt *c*, with which it reacted considerably more slowly. Further, it reacted rapidly with Cyt *c* derived from *N. europaea*, while it reacted poorly with *P. aeruginosa* and *T. novellus* Cyts *c*. The reaction rates of the enzyme with horse and tuna Cyts *c* decreased with an increase in the concentration of phosphate buffer in the reaction mixture, while that with *S. oviformis* Cyt *c* showed a maximal value around 30 mM. Thus the reactivity of *S. oviformis* Cyt *c* in 30 mM buffer was 14% of that of horse Cyt *c*, while in 10 mM buffer it was 20%. However, the profile of specificity obtained from the reactions in 30 mM phosphate buffer seemed not to differ much, as a whole, from that in 10 mM phosphate buffer except that relative reactivity with *S. oviformis* Cyt *c* was higher.

Although the enzyme preparation used here contained Cyt c_1, it seemed unlikely that this contamination affected its specificity for Cyt *c*, because the preparation reacted with the Cyts *c* of, for example, *S. oviformis* and *C. krusei* at considerably different rates, and the profiles of specificities for Cyts *c* did not differ among the preparations which contained varying amounts of Cyt c_1. If Cyt c_1 in the enzyme preparation does mediate electrons between Cyt *c* and the enzyme, a difference in the reactivity of the enzyme with different Cyts *c* should not be observed (see e.g., Yamanaka 1968).

Bovine cytochrome c *oxidase*: The enzyme preparation used in the present studies differed from that used in the past (Yamanaka 1972, 1973); in the latter we used an enzyme preparation which was dissolved in 40 mM phosphate buffer pH 7.2, containing 0.25% Emasol 1130 (a non-ionic detergent), and was kept frozen at −20°C until use; while the enzyme preparation used in the present study was dissolved in 20 mM Tris-HCl buffer pH 8.0, containing 0.25% Emasol 1130, and was kept frozen at liquid nitrogen temperature until use. This new enzyme preparation showed a somewhat different specificity from that of the previous preparation, which did not react with either *P. aeruginosa* or *N. europaea* Cyt *c* at all. However, the present preparation reacted fairly rapidly with the latter Cyt *c* and reacted even with the former one, though very slowly. Further, its reactivity was almost the same with the Cyts *c* derived from tuna, bonito, horse and cow. This result is consistent with that reported by Smith et al. (1973).

Structural relationship between bacterial and eukaryotic enzymes

The molecule of *N. agilis* Cyt *c* oxidase is composed of two kinds of subunits (I and II) (Yamanaka et al. 1979, Chaudhry et al. 1980). The subunits of the enzyme obtained by the preparative polyacrylamide gel electrophoresis in the presence of SDS were not cross-contaminated, as shown in Fig. 2.

The amino acid compositions of the two subunits are compared in Fig. 3 with those of the bovine enzyme. The amino acid composition of subunit I of the *N. agilis* enzyme was very similar to that of the bovine enzyme, although the leucine content of the bovine enzyme was higher than that of the bacterial enzyme and the

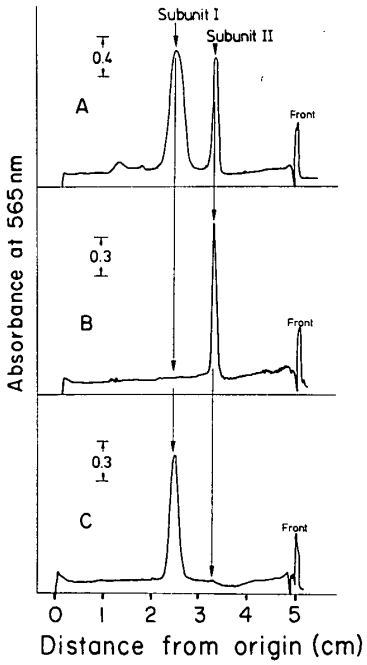

Fig. 2

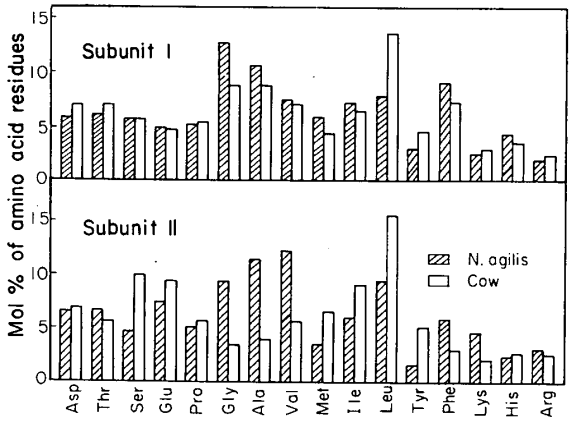

Fig. 3

Fig. 2 Electrophoretic profiles of *N. agilis* Cyt *c* oxidase and its subunits. The two protein fractions obtained by a preparative polyacrylamide gel electrophoresis in the presence of SDS (see text) were separately subjected to analytical polyacrylamide gel electrophoresis in the presence of SDS. As a control, the intact enzyme was also included. The gels were stained by Coomassie brilliant blue and scanned by a densitometer at 565 nm. A, intact enzyme; B, fast eluate at the preparative polyacrylamide gel electrophoresis; C, slow eluate.

Fig. 3 Comparison of the amino acid compositions of subunits I and II of the *N. agilis* and bovine Cyt *c* oxidases. The compositions were expressed in mol%; those of subunits I and II for the cow enzymes were calculated from the data reported by Steffens and Buse (1979) and Yasunobu et al. (1979), respectively.

glycine content in the bacterial subunit a little higher than that in the bovine subunit. On the other hand, the composition of subunit II was clearly different in the prokaryotic and eukaryotic enzymes.

Discussion

These results concerning the specificity of the aa_3-type Cyt *c* oxidases for Cyt *c* suggest the various evolutionary stages of the bacterial enzymes. We infer that *T. novellus* and *N. agilis* enzymes, which react most rapidly with Cyts *c* of yeasts, tuna and bonito but slowly with the Cyts *c* of horse and cow, may be more primitive than the bovine enzyme which reacts very rapidly with all eukaryotic Cyts *c* tested. The specificity for Cyt *c* of the *P. denitrificans* enzyme resembles that of the bovine enzyme. In this respect, this bacterial enzyme may be assumed to be an intermediate in the evolutionary path from the *T. novellus* and *N. agilis* enzymes to the eukaryotic enzymes, or the enzyme could be evolutionarily closest to the eukaryotic enzymes among the bacterial enzymes tested here. Although only bovine Cyt *c*

oxidase was studied among the eukaryotic enzymes in the present investigation, the oxidases purified from many eukaryotes are known to be quite similar to the bovine enzyme in various properties (Malmström 1979, Darley-Usmar et al. 1981).

The proposal that the bacterial and eukaryotic aa_3-type Cyt *c* oxidases may be evolutionarily continuous is supported also by their structural features. A molecule of the eukaryotic Cyt *c* oxidase is composed of 7 (or 6) subunits (e.g. Malmström 1979), and 3 large subunits (I, II and III) among them are biosynthesized in the mitochondrion, while the 4 small subunits synthesized on the cytoplasmic ribosomes (Mason and Schatz 1973). The subunit structure of the bacterial enzymes is simpler; their molecules are composed of only two kinds of subunits (I and II) (Yamanaka et al. 1979, Sone et al. 1979, Ludwig and Schatz 1980, Hon-nami and Oshima 1980, Fee et al. 1980). Subunits I and II of the bacterial enzymes resemble, respectively, subunits I and II of the eukaryotic enzymes in respect to their molecular weight. In the present study, the amino acid composition of subunit I has been found to be very similar to that of the bovine enzyme. The similarity in the amino acid compositions between the two subunits I suggests that the subunits I of the bacterial and eukaryotic enzymes are closely related to each other in an evolutional sense. Recently, Ludwig (1980) has reported that subunit II of the *P. denitrificans* enzyme cross-reacts immunologically with subunit II of both the *Saccharomyces cerevisiae* and bovine enzymes.

Winter et al. (1980) have recently reported that all the heme *a* and copper of the bovine enzyme are located in its subunits I and II. Thus, these subunits are responsible for the ability of the eukaryotic enzyme to oxidize ferrocytochrome *c*. This conclusion appears consistent with the observations that the bacterial enzymes show spectral and enzymatic properties similar to eukaryotic enzymes and that the bacterial molecules are composed of two subunits which resemble in molecular weight those of subunits I and II of the eukaryotic enzymes. The function common to the bacterial and eukaryotic enzymes is the reduction of molecular oxygen, while their specificities for Cyt *c* are different. The similarity in the amino acid composition of the subunits I of the bacterial and eukaryotic enzymes suggests that the subunits I participate in the reduction of molecular oxygen. Indeed, Winter et al. (1980) have presented results which suggest that the oxygen-binding site in the bovine enzyme is located on subunit I. The dissimilar amino acid compositions of the bacterial and eukaryotic subunits II may indicate that the subunits II are responsible for the interaction with Cyt *c*. Some evidence has been obtained which shows that subunit II of the bovine enzyme may react directly with Cyt *c* (Bisson and Gutweniger 1978, Briggs and Capaldi 1978).

If the subunits of the eukaryotic enzyme synthesized in the mitochondrion have the functional ability to oxidize ferrocytochrome *c* as discussed above, then the eukaryotic enzyme may have been evolutionarily completed by the later addition of the "supplementary" subunits synthesized on the cytoplasmic ribosomes. It is difficult at present to postulate any advantage to the eukaryotic organism in adding these latter subunits to the bacterial enzyme-like molecule, if this is indeed what happened during the evolution of Cyt *c* oxidase.

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References

Bisson, R. and H. Gutweniger (1978) Photoaffinity labeling of yeast cytochrome oxidase with arylazido cytochrome *c* derivatives. *FEBS Lett.* 92: 219–226.

Briggs, M. M. and R. A. Capaldi (1978) Cross-linking studies on a cytochrome *c*-cytochrome *c* oxidase complex. *Biochem. Biophys. Res. Commun.* 80: 553–559.

Chaudhry, G. R., I. Suzuki and H. Lees (1980) Cytochrome oxidase of *Nitrobacter agilis*: Isolation by hydrophobic interaction chromatography. *Can. J. Microbiol.* 26: 1270–1274.

Darley-Usmar, V. M., N. Alizai, A. I. Al-Ayash, G. D. Jones, A. Sharpe and M. T. Wilson (1981) A comparison of the structural and functional properties of cytochrome *c* oxidase isolated from beef (*Bos tauros*), camel (*Camelus dromedarius*), chicken (*Gallus domesticus*) and rat (*Rattus norvegicus*). *Comp. Biochem. Physiol.* 68B: 445–456.

Fee, J. A., M. E. Choc, K. L. Findling, R. Lorence and T. Yoshida (1980) Properties of a copper containing cytochrome c_1aa_3 complex: A terminal oxidase of the extreme thermophile *Thermus thermophilus* HB 8. *Proc. Natl. Acad. Sci. USA* 77: 147–151.

Fujii, K. (1979) Cytochrome *a*-type terminal oxidases purified from *Thiobacillus novellus* and *Paracoccus denitrificans*. Master's Thesis, Graduate School of Science, Osaka University.

Hagihara, B., I. Morikawa, K. Tagawa and K. Okunuki (1958) Crystalline animal cytochrome *c*. *In* Biochemical Preparations. Edited by C. S. Vestling. vol. 6, p. 1–7. Wiley, New York.

Hon-nami, K. and T. Oshima (1980) Cytochrome oxidase from an extremely thermophile *Thermus thermophilus* HB 8. *Biochem. Biophys. Res. Commun.* 92: 1023–1029.

Horio, T., T. Higashi, M. Sasagawa, K. Kusai, M. Nakai and K. Okunuki (1960) Preparation of crystalline *Pseudomonas* cytochrome *c*-551 and its general properties. *Biochem. J.* 77: 194–201.

Keilin, D. (1966) *The History of the Cell Respiration and Cytochrome.* Cambridge University Press, Cambridge.

Ketchum, P. A., H. K. Sanders, J. W. Gryder and A. Nason (1969) Characterization of cytochrome *c* from *Nitrobacter agilis*. *Biochim. Biophys. Acta* 189: 360–365.

Lemberg, R. and J. Barrett (1973) *Cytochromes.* Academic Press, New York.

Ludwig, B. (1980) Heme aa_3-type cytochrome *c* oxidase from bacteria. *Biochim. Biophys. Acta* 594: 177–189.

Ludwig, B. and G. Schatz (1980) A two-subunit cytochrome *c* oxidase (cytochrome aa_3) from *Paracoccus denitrificans*. *Proc. Natl. Acad. Sci. USA* 77: 196–200.

Malmström, B. G. (1979) Cytochrome *c* oxidase. Structure and catalytic activity. *Biochim. Biophys. Acta* 549: 281–303.

Mason, T. L. and G. Schatz (1973) Cytochrome *c* oxidase from baker's yeast II. Site of translation of the protein. *J. Biol. Chem.* 248: 1355–1360.

Matsubara, H. and K. T. Yasunobu (1961) Comparative biochemical studies I. Purification and crystallization of human heart cytochrome *c*. *J. Biol. Chem.* 236: 1701–1705.

Motonaga, K., E. Misaka, E. Nakajima, S. Ueda and K. Nakanishi (1965) Structure of yeast cytochrome *c*. *J. Biochem.* 57: 22–28.

Okunuki, K. (1966) Cytochromes and cytochrome oxidase. *In* Comprehensive Biochemistry. Edited by M. Florkin and E. H. Stotz. vol. 14, p. 232–308. Elsevier, Amsterdam.

Scholes, P. B., G. McLain and L. Smith (1971) Purification and properties of a *c*-type cytochrome from *Micrococcus denitrificans*. *Biochemistry* 10: 2072–2076.

Smith, L., M. E. Nava and E. Margoliash (1973) The relationship of the amino acid sequence and of substances bound to cytochrome *c* to its reaction with cytochrome *c* oxidase. *In* Oxidases and Related Redox Systems. Edited by T. E. King, H. S. Mason and M. Morrison. vol. 2, p. 629–638. University Park Press, Baltimore.

Sone, N., T. Ohyama and Y. Kagawa (1979) Thermostable single band cytochrome oxidase. *FEBS Lett.* 106: 39–42.

Steffens, G. J. and G. Buse (1979) Chemical constitution and subunit function of polypeptide II from cytochrome *c* oxidase. *In* Cytochrome Oxidase. Edited by T. E. King, Y. Orii, B. Chance and K. Okunuki. p. 79–90. Elsevier, Amsterdam.

Winter, D. B., W. J. Bruyninckx, F. G. Foulke, N. P. Grinich and H. S. Mason (1980) Location of heme *a* on subunits I and II and copper on subunit II of cytochrome oxidase. *J. Biol. Chem.* 255: 11408–11414.

Yamanaka, T. (1968) Discussion. *In* Structure and Function of Cytochromes. Edited by K. Okunuki, M. D. Kamen and I. Sekuzu. p. 439–440. University of Tokyo Press, Tokyo.

Yamanaka, T. (1972) Evolution of cytochrome *c* molecule. *Adv. Biophys.* 3: 227–276.

Yamanaka, T. (1973) Cytochrome *c* and evolution of the energy acquiring system. *Space Life Sci.* 4: 490–504.

Yamanaka, T. and K. Fujii (1980) Cytochrome *a*-type terminal oxidase derived from *Thiobacillus novellus*. Molecular and enzymatic properties. *Biochim. Biophys. Acta* 591: 53–62.

Yamanaka, T., K. Fujii and Y. Kamita (1979) Subunits of cytochrome *a*-type terminal oxidases from *Thiobacillus novellus* and *Nitrobacter agilis*. *J. Biochem.* 86: 821–824.

Yamanaka, T. and Y. Fukumori (1978) An evolutionary aspect of the reactivity of cytochrome *c* with cytochrome oxidase. *In* Evolution of Protein Molecules. Edited by H. Matsubara and T. Yamanaka. p. 387–399. Japan Scientific Societies Press, Tokyo.

Yamanaka, T., Y. Kamita and Y. Fukumori (1981) Molecular and enzymatic properties of "cytochrome aa_3"-type terminal oxidase derived from *Nitrobacter agilis*. *J. Biochem.* 89: 265–273.

Yamanaka, T., H. Mizushima, H. Katano and K. Okunuki (1964) Comparison of three yeast cytochrome *c*'s purified from *Saccharomyces cerevisiae*, *Kloeckera* sp. and *Candida krusei*. *Biochim. Biophys. Acta* 85: 11–17.

Yamanaka, T. and M. Shinra (1974) Cytochrome *c*-552 and cytochrome *c*-554 derived from *Nitrosomonas europaea*: Purification, properties and their function in hydroxylamine oxidation. *J. Biochem.* 75: 1265–1273.

Yamanaka, T., S. Takenami, N. Akiyama and K. Okunuki (1971) Purification and properties of cytochrome *c*-550 and cytochrome *c*-551 derived from facultative chemoautotroph, *Thiobacillus novellus*. *J. Biochem.* 70: 349–358.

Yasunobu, K. T., M. Tanaka, M. Haniu, M. Sameshima, N. Reimer and T. Eto (1979) Sequence studies of bovine heart cytochrome oxidase subunits. *In* Cytochrome Oxidase. Edited by T. E. King, Y. Orii, B. Chance and K. Okunuki. p. 91–101. Elsevier, Amsterdam.

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