Journal of Bioenergetics and Biomembranes, Vol. 23, No. 2, 1991

MINI-REVIEW

Evolutionary Aspects of Cytochrome *c* Oxidase

B. Kadenbach,[1] A. Stroh,[1] F.-J. Hüther,[1] A. Reimann,[1] and D. Steverding[1]

Received August 28, 1990

Abstract

The presence of additional subunits in cytochrome oxidase distinguish the multicellular eukaryotic enzyme from that of a simple unicellular bacterial enzyme. The number of these additional subunits increases with increasing evolutionary stage of the organism. Subunits I–III of the eukaryotic enzyme are related to the three bacterial subunits, and they are encoded on mitochondrial DNA. The additional subunits are nuclear encoded. Experimental evidences are presented here to indicate that the lower enzymatic activity of the mammalian enzyme is due to the presence of nuclear-coded subunits. Dissociation of some of the nuclear-coded subunits (e.g., VIa) by laurylmaltoside and anions increased the activity of the rat liver enzyme to a value similar to that of the bacterial enzyme. Further, it is shown that the intraliposomal nucleotides influence the kinetics of ferrocytochrome *c* oxidation by the reconstituted enzyme from bovine heart but not from *P. denitrificans*. The regulatory function attributed to the nuclear-coded subunits of mammalian cytochrome *c* oxidase is also demonstrated by the tissue-specific response of the reconstituted enzyme from bovine heart but not from bovine liver to intraliposomal ADP. These enzymes from bovine heart and liver differ in the amino acid sequences of subunits VIa, VIIa, and VIII. The results presented here are taken to indicate a regulation of cytochrome *c* oxidase activity by nuclear-coded subunits which act like receptors for allosteric effectors and influence the catalytic activity of the core enzyme via conformational changes.

Key Words: Cytochrome *c* oxidase evolution; subunit composition; regulation of activity; adenine nucleotides; allosteric effectors.

Introduction

After the advent of molecular oxygen in the atmosphere, due to water cleavage by photosystem II of photosynthetic bacteria, oxidases evolved

[1]Biochemie, Fachbereich Chemie der Philipps-Universität, Hans-Meerwein-Strasse, D-3350 Marburg, Germany.

0145-479X/91/0400-0321$06.50/0 © 1991 Plenum Publishing Corporation

which used dioxygen as electron acceptor for energy-yielding or other oxidative reactions. Among various oxidases which could transfer one, two, or four electrons to oxygen, respectively, those yielding two molecules of water, by translocating four electrons to dioxygen, were chosen as terminal electron transferases of the respiratory chain of aerobic organisms. These bacteria produce ATP by oxidative phosphorylation. The energy-conserving reaction is connected with the ability of the membrane-bound oxidases to translocate protons across the membrane. A large variety of such four-electron translocating oxidases have evolved, and present-day oxidases of bacteria differ in their electron donor (reviewed by Ludwig, 1980, 1987; Poole, 1983; Yamanaka *et al.*, 1985; Fee *et al.*, 1986). Thus the *E. coli* cytochrome b_0 complex accepts electrons from a quinol (Saraste *et al.*, 1988), cytochrome caa_3 from *Thermus thermophilus* and *Bacillus* PS3 (Fee *et al.*, 1986, 1988) accept electrons from cytochrome c_1, which is tightly associated with the enzyme complex, and cytochrome aa_3 from *Paracoccus denitrificans* accepts electrons from soluble cytochrome *c* (Ludwig and Schatz, 1980; Albracht *et al.*, 1980), although in the latter case the physiological electron donor is thought to be a membrane-bound cytochrome c_{552} (Bolgiano *et al.*, 1989). Recently a second oxidase, cytochrome ba_3, was isolated from *T. thermophilus* (Zimmermann *et al.*, 1988). In *P. denitrificans* up to five different oxidases have been identified (Ludwig, 1987). The energy-transducing oxidases appear to have evolved from a common precursor protein, because the amino acid sequences show homology (Steffens *et al.*, 1983), which amounts to 37% between the largest subunit of the far related quinol oxidase cytochrome b_0 of *E. coli* and the cytochrome *c* oxidase from *P. denitrificans* (Saraste *et al.*, 1988).

The terminal oxidase of the respiratory chain of mitochondria of all eukaryotic organisms is similar to cytochrome *c* oxidase of *P. denitrificans*. Mitochondria are suggested to originate from an endosymbiotic event between a urkaryote (an anaerobic bacterium) and an eubacterium (an aerobic bacterium capable of oxidative phosphorylation and/or photosynthesis) (Yang *et al.*, 1985; Darnell and Doolittle, 1986). While most of the genes of the aerobic eubacterium have been transferred to the nucleus during evolution of the eukaryotic cell, few remained in the mitochondrial DNA, including the genes for the subunits I, II, and III of cytochrome *c* oxidase (COX). Thus large amino acid sequence homology exists among the subunits I, II, and III of COX from mitochondria and *P. denitrificans* as well as *T. thermophilus* (Steffens *et al.*, 1983; Raitio *et al.*, 1987; Buse *et al.*, 1989). Correspondingly many similarities in the functional properties of the two enzymes are observed (Ludwig, 1987), including the possible occurrence of a third copper atom (Steffens *et al.*, 1987; Bombelka *et al.*, 1986). The function of subunit I is to bind the two heme *a* groups and Cu_B (Müller *et al.*, 1988), and possibly the third copper (Steffens *et al.*, 1987) and to react with dioxygen.

Subunit II contains Cu_A and binds cytochrome *c* (Bisson *et al.*, 1982), whereas subunit III is suggested to participate in proton translocation (Prochaska and Fink, 1987; Püttner *et al.*, 1985) and was shown to be essential for assembly of COX from *P. denitrificans* (Haltia *et al.*, 1989).

The most intriguing difference between COX from *P. denitrificans* and mitochondria is the occurrence of additional subunits associated with the eukaryotic enzyme, which are coded by nuclear DNA. The number of nuclear-coded subunits increased with increasing evolutionary stage of the organism from 4 in *Dictyostelium discoideum* (Bisson and Schiavo, 1986, 1988), 5 in *Neurospora crassa* (Werner, 1977), 6 in yeast (Power *et al.*, 1984), 7–9 in birds and fishes (Montecucco *et al.*, 1987), to 10 in mammals (see Kadenbach *et al.*, 1983, 1987a, for review). Although the individual function of each nuclear-coded subunit is still unknown, some of the following results clearly demonstrate regulation of mammalian COX activity via nuclear-coded subunits.

Valinomycin Binds Equally to Bacterial and Mammalian COX

In a previous study we have shown a specific and stoichiometric binding of valinomycin to isolated as well as reconstituted COX from bovine heart. The enzyme-bound valinomycin was shown to induce proton translocation in proteoliposomes, because a maximal H^+/e^- stoichiometry was obtained when 1 mol of valinomycin was added per mole of reconstituted bovine heart COX (Steverding and Kadenbach, 1989). In Fig. 1 is shown the spectral change of the oxidized γ-band of COX from *P. denitrificans* induced by valinomycin. The spectral change is almost identical to that found with the bovine heart enzyme (Steverding and Kadenbach, 1989). Also a partial

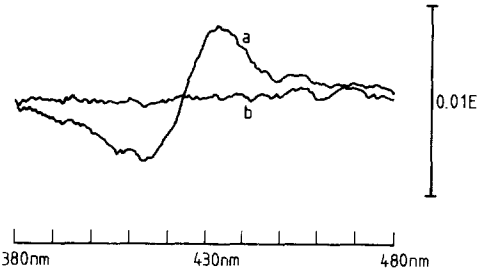

Fig. 1. Spectral changes of COX from *P. denitrificans* by valinomycin. Two cuvettes were filled with 1.24 μM isolated COX from *P. denitrificans* (two subunit enzyme) in 100 mM K-HEPES, 50 mM KCl, and 0.05% laurylmaltoside, pH 7.4, and 10 μM valinomycin in 1 μl ethanol (sample cuvette) or 1 μl ethanol (reference cuvette) were added. After 10 min the difference spectrum was recorded.

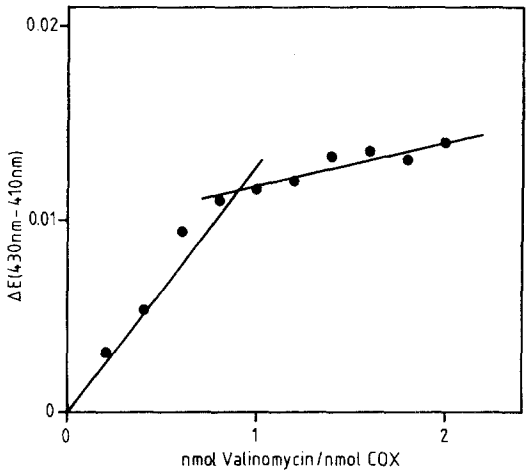

Fig. 2. Titration of the spectral change of isolated COX from *P. denitrificans* with valinomycin. For further details see legend to Fig. 1.

saturation of the spectral change is obtained at 1 mole of valinomycin added per mole of COX, similar to the bovine heart enzyme, as presented in Fig. 2. No spectral change was found in the absence of potassium ions (not shown). These data indicate that in subunit I or II of COX a highly conserved binding site for K^+-complexed valinomycin exists, which enables the bound ionophore to translocate K^+ ions through the membrane via the enzyme complex.

It was also shown that valinomycin increases the respiration of reconstituted COX from bovine heart not only via degradation of the membrane potential, but also at least in part, by direct interaction with the enzyme protein (Steverding and Kadenbach, 1990).

Nuclear-Coded Subunits of Mitochondrial COX Depress Its Catalytic Activity

In previous studies we have shown a biphasic effect of anions on the activity of laurylmaltoside-dissolved COX from pig heart (Kadenbach, 1986), bovine heart (Reimann *et al.*, 1988), and rat liver (Kadenbach *et al.*, 1987b, 1988). At low ionic strength the activity increased, and at high ionic strength it decreased, with increasing anion concentrations. With COX from *P. denitrificans* no increase but only a decrease of activity was found with increasing anion concentrations (Kadenbach *et al.*, 1987b, 1988). In contrast, only a slight stimulation of activity at low, but a large decrease of activity at high anion concentrations was found with rat liver mitochondria or mitoplasts

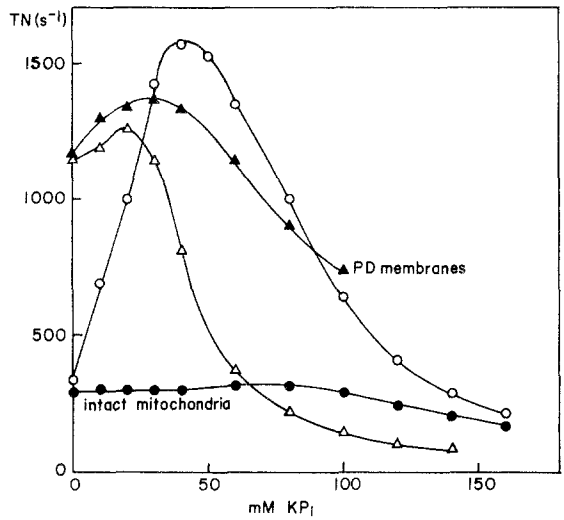

Fig. 3. Comparison of the anion sensitivity of COX in rat liver mitochondria and membranes from *P. denitrificans* and of the laurylmaltoside-dissolved enzymes. The activity of COX was measured polarographically with 7 mM ascorbate, 0.014 mM EDTA, 0.7 mM TMPD, 40 μM cytochrome *c*, 1 μg/ml valinomycin, and 3 μM CCCP in 250 mM sucrose, 10 mM HEPES, pH 7.6, and 4.5 nM COX either in the presence (○, △) or absence (●, ▲) of 0.042% laurylmaltoside at the indicated concentration of potassium phosphate, pH 7.6. Rat liver mitochondria: ●, ○; *P. denitrificans* membranes: ▲, △.

(Kadenbach *et al.* 1987b) and with the reconstituted enzyme from bovine heart (Kadenbach *et al.*, 1988; Kossekova *et al.*, 1989; Steverding *et al.*, 1990) in the absence of detergents. The inhibition of soluble as well as membrane-bound COX at high ionic strength is apparently due to weakening of the electrostatic interactions between ferrocytochrome *c* and COX (Koppenol and Margoliash, 1982).

The strong stimulation of the laurylmaltoside-dissolved mammalian COX, but not of the bacterial COX, by low phosphate concentrations is shown in Fig. 3. In the absence of anions the basal activity of the bacterial enzyme is 4-fold higher than the activity of the mammalian enzyme. A turnover number of only $65 s^{-1}$ was measured with fresh isolated rat liver mitochondria in the presence of uncoupler (CCCP) using ascorbate/TMPD as substrates (Yamazaki, 1975). Solubilization in laurylmaltoside does not change the activity of both, the mitochondrial and the bacterial COX, in the absence of anions.

The strong stimulation of activity of the laurylmaltoside-dissolved mammalian COX at low phosphate concentrations is suggested to be caused by dissociation of some of the nuclear-coded subunits from the catalytic core of the enzyme. This suggestion is based on the following results: (a) Affinity

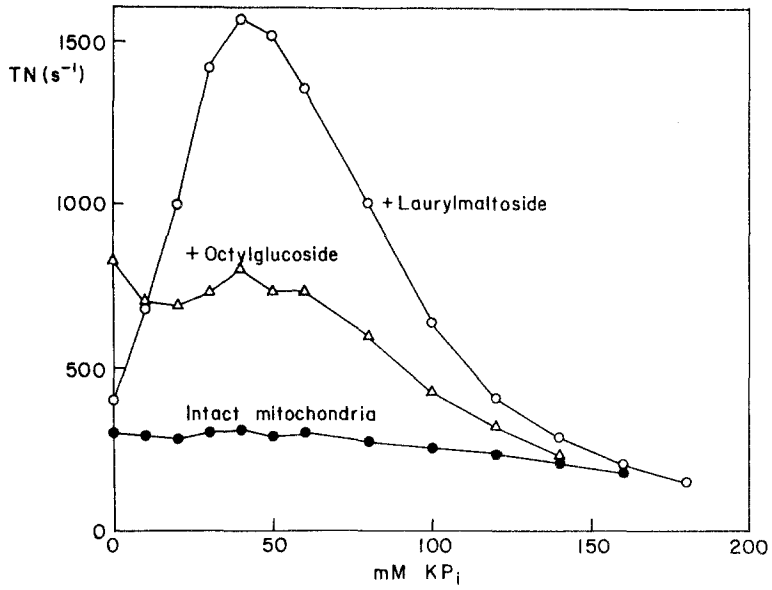

Fig. 4. Influence of increasing phosphate concentrations on COX activity of rat liver mitochondria in the presence and absence of laurylmaltoside or octylglycoside. The polarographic assay of COX activity was performed as described in the legend to Fig. 3 in the absence or presence of either 0.04% laurylmaltoside or 0.4% octylglycoside as indicated.

chromatography of laurylmaltoside-dissolved COX from rat liver results in removal of subunit III and of nuclear-coded subunits (mainly subunits VI) (Thompson and Ferguson-Miller, 1983; Thompson *et al.*, 1985; Hill and Robinson, 1986; Gregory and Ferguson-Miller, 1988). (b) Treatment of COX from bovine heart with 0.1% Triton X-100 in 0.375 M Tris/glycinate at pH 9.5 leads to removal of subunits III, VIa, VIb, and VIIa (see Fig. 3.4 in Wikström *et al.*, 1981; see also Capaldi *et al.*, 1983). (c) The laurylmaltoside-dissolved rat liver enzyme (Thompson and Ferguson-Miller, 1983) and the Triton X-100 treated enzyme from bovine heart (Penttilä *et al.*, 1979) reveal an elevated enzymatic activity. (d) The increased activity cannot be due to the removal of subunit III, because the membrane-bound bacterial enzyme, containing subunits I, II and III (Raitio *et al.*, 1987; Haltia *et al.*, 1989), exhibits approximately the same high activity as the laurylmaltoside-dissolved and phosphate-stimulated mammalian enzyme, and this activity cannot be stimulated by phosphate after solubilization in laurylmaltoside (Fig. 3).

Most of the nuclear-coded subunits contain a hydrophobic membrane spanning domain [IV, VIa, VIc, VIIa, VIIc, VIII (Kadenbach *et al.*, 1987a) and VIIb (Lightowlers *et al.*, 1989)] and are assumed to be tightly associated

to the catalytic subunits I and II via hydrophobic binding and electrostatic interactions of charged amino acids (Kadenbach *et al.*, 1987b, 1988). Dissociation of nuclear-coded subunits from the active core may thus require both, separation of membrane domains as well as abolition of charge interactions. The highest activity of mammalian COX, obtained in the presence of laurylmaltoside (Rosevear *et al.*, 1980) and anions (Fig. 3), could thus be due to optimal dissociation of membrane-spanning domains, similar to the dissociation by SDS, which also contains a hydrophobic dodecyl-rest and has a negative charge, but less drastic than dissociation by SDS. This conclusion is further supported by the different effects of anions on the laurylmaltoside- and octylglucoside-dissolved rat liver COX, shown in Fig. 4. The octylglucoside-dissolved enzyme cannot be stimulated by anions. Its hydrocarbon-rest may be too short for dissociation of nuclear-coded subunits from the active core of the enzyme.

Regulation of eukaryotic COX via Nuclear-Coded Subunits

Nucleotides Influence the Mammalian but not the Bacterial Enzyme

Many catalytic properties of COX from *P. denitrificans* and bovine heart are the same (reviewed in Ludwig, 1987). Thus, it was previously assumed that at least some of the 10 nuclear-coded subunits of the mammalian enzyme may not be essential for its catalytic function. In yeast it could be shown, however, that mutation of any of the six nuclear-coded subunits of the COX is accompanied by complete or partial loss of respiratory activity (McEwen *et al.*, 1986; Poyton *et al.*, 1988). We have suggested that the additional subunits, not found in the bacterial enzyme, have a regulatory function by binding allosteric effectors like hormones, nucleotides, and anions and changing the rate of respiration and efficiency of energy transduction via conformational change of the catalytic center (Kadenbach and Merle, 1981; Kadenbach 1986; Kadenbach *et al.*, 1987a). A modulation of COX activity by ATP (Montecucco *et al.*, 1986; Rigoulet *et al.*, 1987; Bisson *et al.*, 1987; Malatesta *et al.*, 1987; Hüther and Kadenbach, 1986, 1988; Antonini *et al.*, 1988), ADP (Hüther and Kadenbach, 1987, 1988), phosphate (Malatesta *et al.*, 1987; Büge and Kadenbach, 1986) and free fatty acids (Labonia *et al.*, 1988; Thiel and Kadenbach, 1989) has in fact been demonstrated, but could not be related to a specific interaction of the effector with one subunit. The specific labelling of subunits IV and VIII of soluble bovine heart COX by 8-azido-γ-^{32}P-ATP, described by Montecucco *et al.* (1986), could not be demonstrated in other investigations (Hüther *et al.*, 1988; Reimann *et al.*, 1988).

The regulatory function of nuclear-coded subunits became evident from the influence of intraliposomal nucleotides on the kinetics of ferrocytochrome *c* oxidation of the reconstituted bovine heart enzyme but not on the reconstituted COX from *P. denitrificans* (Hüther and Kadenbach, 1988). Figure 5 shows that 10 mM intraliposomal ATP increases, and 10 mM intraliposomal ADP decreases, the K_m for cytochrome *c* of the reconstituted bovine heart enzyme. In contrast, even 15 mM intraliposomal ATP or ADP had no influence on the reconstituted two-subunit enzyme from *P. denitrificans*. It should be pointed out that the medium of the spectrophotometric assay was free of nucleotides. Since cytochrome *c* interacts with COX from the extraliposomal side, the nucleotides must have changed the binding domain for cytochrome *c* via conformational change through the lipid bilayer.

Environmental-Specific COX Isozymes Occur in Unicellular Eukaryotes

In COX of *Dictyostelium discoideum* two isozymes have been found which differ in the smallest subunit VII. One type is expressed at the exponential (VIIe), the other at the stationary growth phase (VIIs) (Bisson and Schiavo, 1986, 1988). It could be demonstrated that the different expression is triggered by the oxygen concentration.

A similar oxygen-dependent expression of two genes for COX subunit V in yeast, which corresponds to subunit IV of the mammalian enzyme, was described by Poyton and coworker (Cumsky *et al.*, 1987; Trueblood and Poyton, 1987; Poyton *et al.*, 1988). It was concluded that the nuclear-coded subunits are modulators of COX activity in eukaryotes and that this modulation is effected by the differential expression of their genes in response to environmental and/or developmental signals (Poyton *et al.*, 1988).

Expression of Tissue-Specific Isoforms of Nuclear-Coded Subunits in Mammals

Further indication for the regulatory role of nuclear-coded subunits comes from the occurrence of tissue-specific isoforms for subunits VIa, VIIa, and VIII of the mammalian enzyme, based on N-terminal amino acid sequences (Kadenbach *et al.*, 1982; Yanamura *et al.*, 1988) and two different *c*DNAs for COX subunit VIa of the rat (Schlerf *et al.*, 1988) and subunit VIII of bovine (Lightowlers *et al.*, 1990). Recently two isoforms of subunit VIIa have also been identified in COX from human skeletal muscle by HPLC separation and N-terminal amino acid sequencing (Van Beeumen *et al.*, 1990). The tissue-specific expression of the isoforms appears to be species-specific. In bovine only the liver-type (l) or the heart-type (h) of subunits VIa, VIIa, and VIII are expressed in liver and heart, respectively (Yanamura *et al.*,

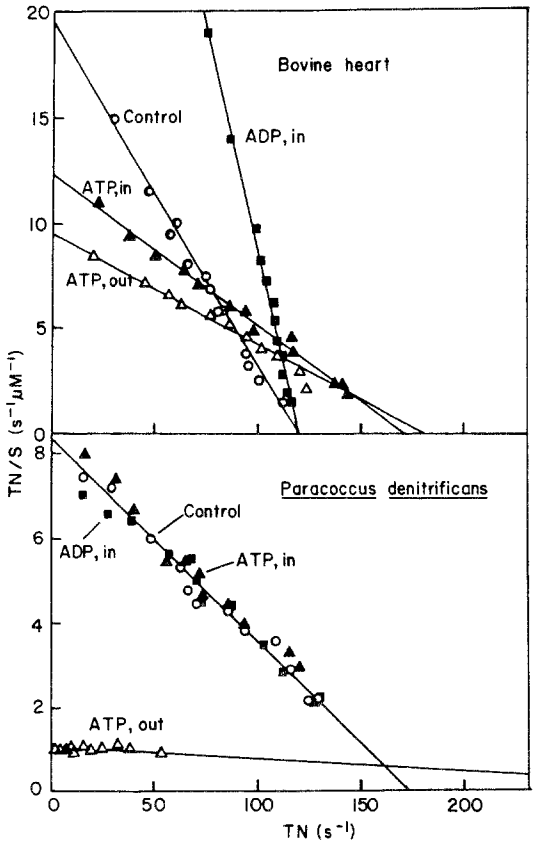

Fig. 5. Effect of intra- and extraliposomal ATP and ADP on the kinetics of ferrocytochrome *c* oxidation of reconstituted COX from bovine heart and *P. denitrificans* (Hüther and Kadenbach, 1988). Isolated COX from bovine heart was reconstituted in the presence of 10 mM (bacterial COX, 15 mM) ATP or ADP (in) or absence of ATP (out). The activity was measured spectrophotometrically at various concentrations of ferrocytochrome *c* (1–80 μM) in the absence (in) or presence of 10 mM (bacterial COX, 15 mM) ATP (out). The molecular turnover (TN) is presented in a reversed Eadie–Hofstee plot.

1988). In the rat both subunits, VIa-l and VIa-h, are expressed in the heart, whereas in liver only VIa-l is expressed (Schlerf *et al.*, 1988; Kadenbach *et al.*, 1990). Thus in rat heart at least two different isozymes of COX are expressed (VIa–l/VIII-h and VIa–h/VIII-h), which differ from the liver isozyme (VIa–l/VIIIl). One isozyme of the heart (VIa–l/VIII-h) which is not found in skeletal muscle (Schlerf *et al.*, 1988) was also found in COX of brown fat tissue (Kadenbach *et al.*, 1990). In human heart only the liver type of subunit VIII is expressed (Van Kuilenberg *et al.*, 1988; Kadenbach *et al.*, 1990), which contrasts the expression of subunit VIII-h in the heart of rat, pig, bovine and

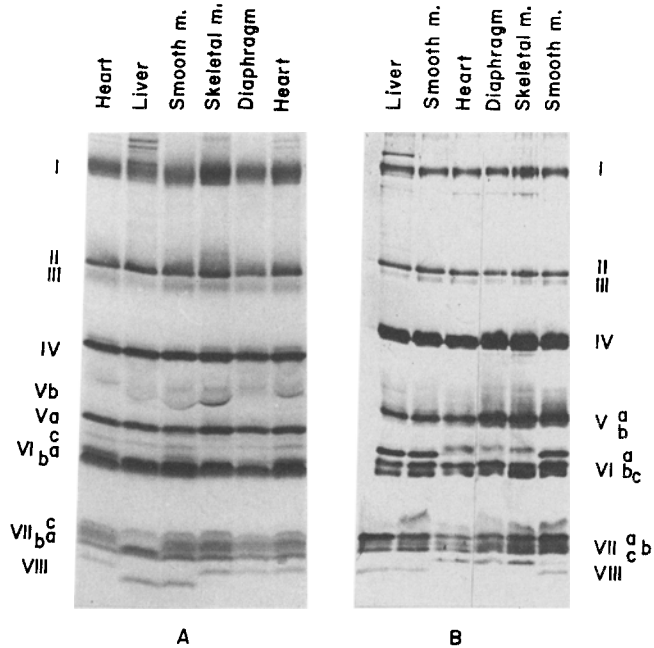

Fig. 6. Subunit pattern of isolated cytochrome *c* oxidase from bovine heart, liver, stomach smooth muscle, skeletal muscle, and diaphragm. The enzymes were separated by the system of Schägger and von Jagow (1987) using 16.5% acrylamide and 0.5% bisacrylamide (A) or Kadenbach *et al.* (1983) (B). The proteins were silver-stained (B) or stained with Coomassie blue (A). The double band of subunit VIIa of the muscle enzymes and the variable appearance of subunit Vb in A are due to the occurrence of SH groups in these subunits (Stroh and Kadenbach, 1986).

chicken (Kadenbach *et al.*, 1981, 1990). This indicates that tissue-specific expression of COX isozymes varies in different species.

The variation of COX isozyme expression in different bovine tissues is presented in Fig. 6. The gel-electrophoretic pattern includes isolated enzymes from liver, heart, diaphragm, skeletal muscle, and smooth muscle (stomach). In liver, in heart or skeletal muscle, and in smooth muscle three different COX isozymes are found, based on different apparent molecular weights of subunits VIa, VIIa, and VIII.

Tissue-Specific Regulation of Mammalian COX Activity

The kinetics of ferrocytochrome *c* oxidation were found to be different with COX from bovine heart and liver (Merle and Kadenbach, 1982). Also the phospholipid composition of liposomes had a different influence on the activity of reconstituted COX from bovine heart and liver, and intraliposomal

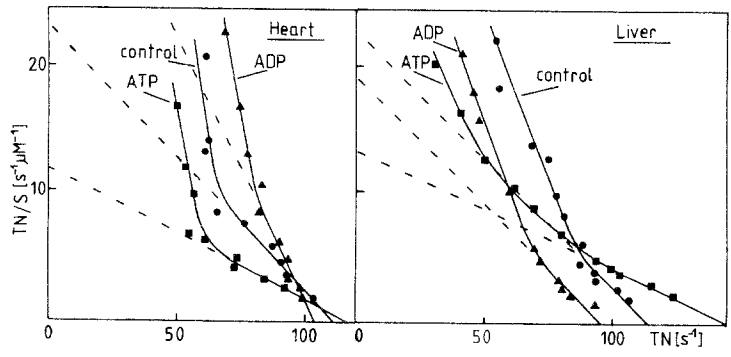

Fig. 7. Influence of intraliposomal ADP and ATP on the kinetics of ferrocytochrome *c* oxidation by reconstituted COX from bovine heart and bovine liver under uncoupled conditions. Proteoliposomes were prepared in the absence (●) or in the presence of 10 mM ADP (▲) or 10 mM ATP (■), respectively, by the cholate-dialysis method. The spectrophotometric assay was performed with 4 nM COX from bovine heart or bovine liver and 2–60 µM ferrocytochrome *c* in a buffer containing 10 mM K-Hepes, pH 7.4, 40 mM KCl, 3 µM CCCP, and 1 µg/ml valinomycin by computer-driven data collection. The data were evaluated using a mixed-exponential fit-function.

phosphate modified the K_m for cytochrome *c* and changed the V_{max} of reconstituted COX from the two tissues into opposite directions (Büge and Kadenbach, 1986). A partial decrease of the respiratory control ratio of reconstituted COX by nonesterified fatty acids was found with the enzyme from bovine heart (Labonia *et al.*, 1988) but not from bovine liver (Thiel and Kadenbach, 1989), suggesting the involvement of the heart but not of the liver in nonshivering thermogenesis.

A further difference between COX from bovine heart and liver is shown in Fig. 7, which presents the influence of intraliposomal ATP and ADP on the V_{max} and K_m for cytochrome *c* of the reconstituted enzymes from bovine heart and liver. In contrast to Fig. 5, where only the low-affinity phase of ferrocytochrome *c* oxidation is presented, both phases are seen on this figure, because the spectrophotometric recordings were stored on-line in a computer and thus allowed to measure the kinetics at low ferrocytochrome *c* concentrations (high-affinity phase). While the intraliposomal ATP changed the K_m for cytochrome *c* at the low-affinity phase of both enzymes, the intraliposomal ADP decreased the K_m for cytochrome *c* of the heart but not of the liver enzyme, at the low-affinity phase of ferrocytochrome *c* oxidation.

Taken together, these data clearly indicate a different modulation of the catalytic activity of COX from bovine heart and liver by allosteric effectors. Since the two enzymes only differ in the structure of nuclear-coded subunits VIa, VIIa, and VIII, the allosteric effectors must interact via these tissue-specific subunits. The specific interaction of an allosteric effector with one subunit, however, remains yet to be established.

Acknowledgments

We thank Prof. B. Ludwig for supplying us with samples of membranes and isolated cytochrome *c* oxidase from *P. denitrificans*. For careful typing of the manuscript, Roswitha Roller-Müller is gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft (Ka 192/21-1) and Fonds der Chemischen Industrie.

References

Albracht, S. P. J., Van Verseveld, H. W., and Kalkman, M. L. (1980). *Biochim. Biophys. Acta* **593**, 173–186.

Antonini, G., Malatesta, F., Sarti, P., Vallone, B., and Brunori, M. (1988). *Biochem. J.* **256**, 835–840.

Bisson, R., and Schiavo, G. (1986). *J. Biol. Chem.* **261**, 4373–4376.

Bisson, R., and Schiavo, G. (1988). *Ann. N.Y. Acad. Sci.* **550**, 325–336.

Bisson, R., Steffens, G. C. M., Capaldi, R. A., and Buse, G. (1982). *FEBS Lett.* **144**, 359–363.

Bisson, R., Schiavo, G., and Montecucco, C. (1987). *J. Biol. Chem.* **262**, 5992–5998.

Bolgiano, B., Smith, L., and Davies, H. C. (1989). *Biochim. Biophys. Acta* **973**, 227–234.

Bombelka, E., Richter, F.-W., Stroh, A., and Kadenbach, B. (1986). *Biochem. Biophys. Res. Commun.* **140**, 1007–1014.

Büge, U., and Kadenbach, B. (1986). *Eur. J. Biochem.* **161**, 383–390.

Buse, G., Hensel, S., and Fee, J. A. (1989). *Eur. J. Biochem.* **181**, 261–268.

Capaldi, R. A., Malatesta, F., and Darley-Usmar, V. M. (1983). *Biochim. Biophys. Acta* **726**, 135–148.

Cumsky, M. G., Trueblood, C. E., Ko, C., and Poyton, R. O. (1987). *Mol. Cell. Biol.* **7**, 3511–3519.

Darnell, J. E., and Doolittle, W. F. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 1271–1274.

Fee, J. A., Kuila, D., Mather, M. W., and Yoshida, T. (1986). *Biochim. Biophys. Acta* **853**, 153–185.

Fee, J. A., Mather, M. W., Springer, P., Hensel, S., and Buse, G. (1988). *Ann. N.Y. Acad. Sci.* **550**, 33–38.

Gregory, L. C., and Ferguson-Miller, S. (1988). *Biochemistry* **27**, 6307–6314.

Haltia, T., Finel, M., Harms, N., Nakari, T., Raitio, M., Wikström, M., and Saraste, M. (1989). *EMBO J.* **8**, 3571–3579.

Hill, B. C., and Robinson, N. C. (1986). *J. Biol. Chem.* **261**, 15356–15359.

Hüther, F.-J., and Kadenbach, B. (1986). *FEBS Lett.* **207**, 89–94.

Hüther, F.-J., and Kadenbach, B. (1987). *Biochem. Biophys. Res. Commun.* **147**, 1268–1275.

Hüther, F.-J., and Kadenbach, B. (1988). *Biochem. Biophys. Res. Commun.* **153**, 525–534.

Hüther, F.-J., Berden, J., and Kadenbach, B. (1988). *J. Bioenerg. Biomembr.* **20**, 503–516.

Kadenbach, B. (1986). *J. Bioenerg. Biomembr.* **18**, 39–54.

Kadenbach, B., and Merle, P. (1981). *FEBS Lett.* **135**, 1–11.

Kadenbach, B., Büge, U., Jarausch, J., and Merle, P. (1981). In *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F., *et al.*, eds.), Elsevier North-Holland Biomedical Press, pp. 11–23.

Kadenbach, B., Hartmann, R., Glanville, R., and Buse, G. (1982). *FEBS Lett.* **138**, 236–238 (1982).

Kadenbach, B., Jarausch, J., Hartmann, R., and Merle, P. (1983). *Anal. Biochem.* **129**, 517–521.

Kadenbach, B., Kuhn-Nentwig, L., and Büge, U. (1987a). *Curr. Top. Bioenerg.* **15**, 113–161.

Kadenbach, B., Stroh, A., Hüther, F.-J., and Berden, J. (1987b). In *Cytochrome Systems: Molecular Biology and Bioenergetics* (Papa, S., Chance, B., and Ernster, L., eds.), Plenum Press, New York, pp. 399–406.

Kadenbach, B., Reimann, A., Stroh, A., and Hüther, F.-J. (1988). In *Oxidases and Related Redox Systems* (King, T. E., Mason, H. S., and Morrison, M., eds.), A. R. Liss Inc., New York, pp. 653–668.
Kadenbach, B., Stroh, A., Becker, A., Eckerskorn, C., and Lottspeich, F. (1990). *Biochim. Biophys. Acta* **1015**, 368–372.
Koppenol, W. H., and Margoliash, E. (1982). *J. Biol. Chem.* **257**, 4426–4437.
Kossekova, G., Atanasov, B., Bolli, R., and Azzi, A. (1989). *Biochem. J.* **262**, 591–596.
Labonia, N., Müller, M., and Azzi, A. (1988). *Biochem. J.* **254**, 139–145.
Lightowlers, R., Takamiya, S., Wessling, R., Lindorfer, M., and Capaldi, R. A. (1989). *J. Biol. Chem.* **264**, 16858–16860.
Lightowlers, R., Ewart, G., Aggeler, R., Zhang, Y.-Z., Calavetta, L., and Capaldi, R. A. (1990). *J. Biol. Chem.* **265**, 2677–2681.
Ludwig, B. (1980). *Biochim. Biophys. Acta* **594**, 177–189.
Ludwig, B. (1987). *FEMS Microbiol. Rev.* **46**, 41–56.
Ludwig, B., and Schatz, G. (1980). *Proc. Natl. Acad. Sci. USA* **77**, 196–200.
Malatesta, F., Antonini, G., Sarti, P., and Brunori, M. (1987). *Biochem. J.* **248**, 161–165.
McEwen, J. E., Ko, C., Kloeckner-Gruissem, B., and Poyton, R. O. (1986). *J. Biol. Chem.* **261**, 11872–11879.
Merle, P., and Kadenbach, B. (1982). *Eur. J. Biochem.* **125**, 239–244.
Montecucco, C., Schiavo, G., and Bisson, R. (1986). *Biochem. J.* **234**, 241–243.
Montecucco, C., Schiavo, G., Bacci, B., and Bisson, R. (1987). *Comp. Biochem. Physiol.* **87B**, 851–856.
Müller, M., Schläpfer, B., and Azzi, A. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 6647–6651.
Penttilä, T., Saraste, M., and Wikström, M. (1979). *FEBS Lett*, **101**, 295–300.
Poole, R. K. (1983). *Biochim. Biophys. Acta* **726**, 205–243.
Power, S. D., Lochrie, M. A., Sevarino, K. A., Patterson, T. E., and Poyton, R. O. (1984). *J. Biol. Chem.* **259**, 6564–6570.
Poyton, R. O., Trueblood, C. E., Wright, R. M., and Farrell, L. E. (1988). *Ann. N.Y. Acad. Sci.* **550**, 289–307.
Prochaska, L. J., and Fink, P. S. (1987). *J. Bioenerg. Biomembr.* **19**, 143–166.
Püttner, J., Carafoli, E., and Malatesta, F. (1985). *J. Biol. Chem.* **260**, 3719–3723.
Raitio, M., Jalli, T., and Saraste, M. (1987). *EMBO J.* **6**, 2825–2833.
Reimann, A., Hüther, F.-J., Berden, J. A., and Kadenbach, B. (1988). *Biochem. J.* **254**, 723–730.
Rigoulet, M., Guerin, B., and Denis, M. (1987). *Eur. J. Biochem.* **168**, 275–279.
Rosevear, P., Van Aken, T., Baxter, J., and Ferguson-Miller, S. (1980). *Biochemistry* **19**, 4108–4115.
Saraste, M., Raitio, M., Jalli, T., Chepuri, V., Lemieux, L., and Gennis, R. (1988). *Ann. N.Y. Acad. Sci.* **550**, 314–324.
Schägger, H., and von Jagow, G. (1987). *Anal. Biochem.* **166**, 368–379.
Schlerf, A., Droste, M., Winter, M., and Kadenbach, B. (1988). *EMBO. J.* **7**, 2387–2391.
Steffens, G. C. M., Buse, G., Oppliger, W., and Ludwig, B. (1983). *Biochem. Biophys. Res. Commun.* **116**, 335–340.
Steffens, G. C. M., Biewald, R., and Buse, G. (1987). *Eur. J. Biochem.* **164**, 295–300.
Steverding, D., and Kadenbach, B. (1989). *Biochem. Biophys. Res. Commun.* **160**, 1132–1139.
Steverding, D., and Kadenbach, B. (1990). *J. Bioenerg. Biomembr.* **22**, 197–205.
Stroh, A., and Kadenbach, B. (1986). *Eur. J. Biochem.* **156**, 199–204.
Thiel, C., and Kadenbach, B. (1989). *FEBS Lett.* **251**, 270–274.
Thompson, D. A., and Ferguson-Miller, S. (1983). *Biochemistry* **22**, 3178–3187.
Thompson, D. A., Gregory, L., and Ferguson-Miller, S. (1985). *J. Inorg. Biochem.* **23**, 357–364.
Trueblood, C. E., and Poyton, R. O. (1987). *Mol. Cell. Biol.* **7**, 3520–3526.
Van Beeumen, J. J., Van Kuilenberg, A. B. P., Van Bun, S., Van den Boert, C., Tager, J. M., and Muijsers, A. O. (1990). *FEBS Lett.* **263**, 213–216.
Van Kuilenberg, A. B. P., Muijsers, A. O., Demol, H., Dekker, H. L., and Van Beeumen, J. J. (1988). *FEBS Lett.* **240**, 127–132.
Werner, S. (1977). *Eur. J. Biochem.* **79**, 103–110.

Wikström, M., Krab, K., and Saraste, M., eds. (1981). *Cytochrome Oxidase. A Synthesis*, Academic Press, New York.
Yamanaka, T., Fukumori, Y., Yamazaki, T., Kato, H., and Nakayama, K. (1985). *J. Inorg. Biochem.* **23**, 273–277.
Yamazaki, R. K. (1975). *J. Biol. Chem.* **250**, 7924–7930.
Yanamura, W., Zhang, Y.-Z., Takamiya, S., and Capaldi, R. A. (1988). *Biochemistry* **27**, 4909–4914.
Yang, D. Y., Oyaizu, Y., Oyaizu, H., Olsen, G. J., and Woese, C. R. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 4443–4447.
Zimmermann, B. H., Nitsche, C. I., Fee, J. A., Rusnak, F., and Münck, E. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 5779–5783.