Tissue-Specific Regulation of Cytochrome *c* Oxidase Efficiency by Nucleotides†

Felix Rohdich and Bernhard Kadenbach*

Fachbereich Chemie der Philipps-Universität, Hans-Meerwein-Strasse, D-3550 Marburg, FRG

Received October 14, 1992; Revised Manuscript Received May 19, 1993

ABSTRACT: Cytochrome *c* oxidase from bovine heart and liver was reconstituted in liposomes in the absence or presence of nucleotides. Intraliposomal ADP, and to a smaller extent intraliposomal ATP, increased the respiratory activity of the heart but not of the liver isozyme under uncoupled but not under coupled conditions, leading to increased respiratory control ratios. In a preceding publication [Anthony, G., Reimann, A., & Kadenbach, B. (1992) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1652–1656], the stimulatory effect of intraliposomal ADP could be related to interaction with the matrix domain of subunit VIa-h (heart type). The data suggest a regulatory effect of matrix nucleotides in heart and skeletal muscle mitochondria on the efficiency of energy transduction in COX.

The mammalian cytochrome *c* oxidase complex (COX) contains 13 different polypeptides including 10 "superstoichiometric" subunits which are absent in COX of bacteria (e.g., *Paracoccus denitrificans*), although both enzymes have almost identical electron-transfer and proton-pumping activities (Hendler et al., 1991; Pardhasaradhi et al., 1991). Also, other enzyme complexes of the respiratory chain from higher organisms contain additional subunits, not found in the bacterial analogs. However, only two superstoichiometric subunits of complex III could be ascribed a role: subunit I of cytochrome *c* reductase from *Neurospora crassa* (but not from yeast) was identified as a "processing enhancing protein" (PEP) (Schulte et al., 1989) and subunit III of cytochrome *c* reductase from potato as a "processing peptidase" (Braun et al., 1992). In yeast, the two COX isozymes, differing in the nuclear-coded subunit V isoform (Va or Vb), result in different turnover rates and rates of heme *a* reduction (Waterland et al., 1991). Until very recently (see below) the function of the superstoichiometric subunits of mammalian COX remained unknown.

The three large subunits of mammalian COX are encoded on mitochondrial DNA while the superstoichiometric subunits are nuclear-coded and partly expressed in tissue-specific forms (Kadenbach, 1983; Kadenbach et al., 1987). The heart-type isoforms of subunits VIa, VIIa, and VIII are expressed in heart and skeletal muscle, while the liver-type isoforms occur in most other tissues, e.g., liver, kidney, and brain (Schlerf et al., 1988; Anthony et al., 1990; Lightowlers et al., 1990; Seelan & Grossman, 1991). In smooth muscle, a different isoenzyme is expressed consisting of the liver types of subunits VIa and VIII but the heart type of subunit VIIa (Anthony et al., 1990). However, species-specific expression of isoforms has also been observed. In rat heart, both isoforms of subunit VIa (i.e., VIa-h and VIa-l) are expressed (Kadenbach et al., 1990) while in bovine heart only subunit VIa-h (heart type) has been found by protein sequencing (Yanamura et al., 1988). The heart type of subunit VIII is expressed in the heart of rat (Kadenbach et al., 1990) and bovine (Yanamura et al., 1988), whereas in human heart only the liver type could be identified (Van Kuilenburg et al., 1988).

The mitochondrial-coded subunits I and II are associated with the catalytic activity of the enzyme since they contain the prosthetic groups: two heme *a* and three copper atoms (Capaldi, 1990). For the nuclear-coded subunits a regulatory function was proposed (Kadenbach, 1986) on the basis of tissue-specific differences between the activity of COX from bovine heart and liver (Merle & Kadenbach, 1982; Büge & Kadenbach, 1986; Van Kuilenberg et al., 1991), and on effects of nucleotides (Hüther & Kadenbach, 1987), which were not found in COX from *Paracoccus* (Hüther & Kadenbach, 1988), lacking the nuclear-coded subunits (Ludwig, 1987). Recently the regulatory function of an individual nuclear-coded subunit could be elucidated. Subunit VIa-h from bovine heart (but not VIa-l from bovine liver) was shown to bind ADP at its N-terminal matrix-oriented domain, accompanied by an increase of the catalytic activity, as proven by competition with a monoclonal antibody (Anthony et al., 1992).

In the present study, we have investigated the kinetics and specificity of the stimulatory effect of intraliposomal nucleotides on the activity of reconstituted COX. Intraliposomal ADP increases the K_M for cytochrome *c*; it stimulates the uncoupled activity and improves the respiratory control ratio of the reconstituted enzyme from bovine heart but not from bovine liver. Other nucleotides, including ATP, have a smaller effect. It is proposed that in heart and skeletal muscle intramitochondrial ADP controls the efficiency of energy transduction.

MATERIALS AND METHODS

COX from bovine heart and liver was prepared from isolated mitochondria as described before (Kadenbach et al., 1986). Asolectin (L-α-phosphatidylcholine, type II-s from soybean) and cytochrome *c* (95% pure, from horse heart) were obtained from Sigma. Before use, asolectin was purified by the method of Kagawa and Racker (1971). Valinomycin and CCCP were bought from Boehringer, Mannheim.

Reconstitution of COX. COX was reconstituted in liposomes by the adsorption method. Asolectin (40 mg/mL) was first sonicated 4 × 2 min (Branson sonifier, microtip, 30% yield) in 1.5% sodium cholate, 10 mM K-Hepes, pH 7.4, and 40 mM KCl; then COX was added to a final concentration of 3 μM, together with nucleotides or salts, when indicated. The detergent was removed by adsorption to purified Amberlite XAD-2 (Serva, Heidelberg) (Schechter & Bloch, 1971) by incubation for 22 h at 4 °C under shaking with 50 mg/mL polymeric adsorbent. The orientation of COX in the vesicles was calculated from the reduced spectrum obtained by

† This paper was supported by the Deutsche Forschungsgemeinschaft (Ka 192/17-5) and the Fonds der Chemischen Industrie. Dedicated to Professor Britton Chance on the occasion of his 80th birthday.

0006-2960/93/0432-8499$04.00/0

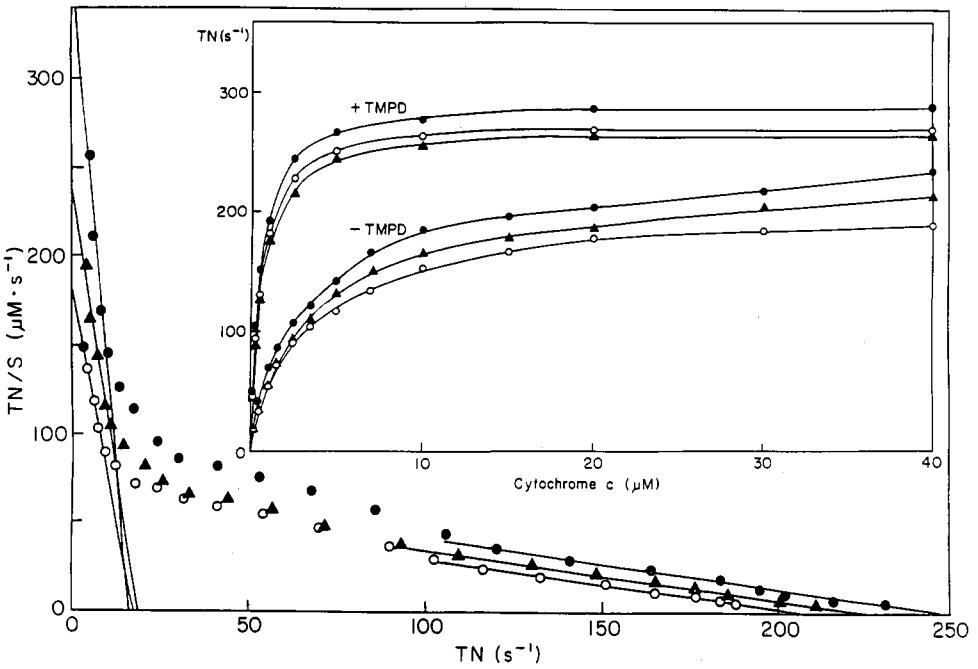

FIGURE 1: Influence of intraliposomal ADP and ATP on the polarographic activity of reconstituted COX from bovine heart. Proteoliposomes were prepared in the absence (open circles) or presence of 10 mM ADP (closed circles) or ATP (triangles), and the activity was measured under uncoupled conditions at increasing concentrations of cytochrome *c* in the absence or presence of 0.7 mM TMPD, as indicated. Main panel: Eadie–Hofstee plot of the kinetics of ferrocytochrome *c* oxidation in the absence of TMPD. Insert: Dependence of activity on the concentration of cytochrome *c*. The activity is presented as turnover number (TN = moles of cytochrome *c* for mole of cytochrome aa_3 per second). S = concentration of cytochrome *c*. For further details, see Materials and Methods.

impermeant (ascorbate and cytochrome *c*) and permeant (TMPD) reducing agents in the presence of cyanide as described by Casey et al. (1982).

Measurement of COX Activity. The activity of reconstituted COX was measured polarographically at 25 °C with a Clark-type electrode according to Ferguson-Miller (1978) in 10 mM K-Hepes, pH 7.4, 40 mM KCl, 25 mM Tris–ascorbate, 20 nM reconstituted COX (oriented right-side-out), 0.02–40 μM cytochrome *c* with the uncoupler valinomycin (1 μg/mL), and CCCP (3 μM) when indicated. Where indicated, TMPD was added at 0.7 mM with 0.1 mM EDTA, final concentrations. The activity under coupled conditions (absence of valinomycin and CCCP) was measured with 170 nM reconstituted COX. COX activity is presented as turnover number [TN = nmol of cytochrome *c* s^{-1} (heme aa_3)$^{-1}$].

RESULTS

COX Activity Is Stimulated by Intraliposomal Nucleotides. COX from bovine heart was reconstituted in liposomes in the presence or absence of 10 mM ADP or ATP, and the activity was measured polarographically with or without *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) at increasing concentrations of cytochrome *c*. Under the applied steady-state conditions (25 mM ascorbate), cytochrome *c* is largely reduced (also without TMPD more than 95%, not shown). Intraliposomal ADP stimulates the activity, when measured in the presence of uncoupler but in the absence of TMPD, at all concentrations of cytochrome *c* by about 25% (see insert of Figure 1). Intraliposomal ATP has also a stimulatory effect, but to a smaller extent than ADP. Addition of TMPD stimulates the oxygen uptake of the enzyme in particular at lower concentrations of cytochrome *c*. In the presence of TMPD, the relative stimulation by ADP is lower, and a slight decrease of activity is observed by ATP. In the main panel of Figure 1 is shown the Eadie–Hofstee plot of one representative titration, measured in the absence of TMPD. The V_{max} (TN_{max}) and apparent K_M values for the high- and low-affinity phases of cytochrome *c* oxidation were graphically determined according to Ferguson-Miller et al. (1976, 1978) from curves with average data of seven independent titrations. The maximal variations of TN and TN/S values of individual data in the Eadie–Hofstee plot were below 10%. The apparent K_M value for the high-affinity phase of ferrocytochrome *c* oxidation is more decreased by intraliposomal ADP (0.037 μM) than by intraliposomal ATP (0.060 μM), as compared to control vesicles (0.076 μM). The TN_{max} values for the high-affinity phase are the same for control (16 s^{-1}), ATP (17 s^{-1}), or ADP-containing proteoliposomes (16 s^{-1}). The apparent K_M value for the low-affinity phase of cytochrome *c* oxidation is almost the same for control (4.38 μM), ATP (3.97 μM), and ADP-containing proteoliposomes (4.03 μM). The TN_{max} of the low-affinity phase of cytochrome *c* oxidation (control = 210 s^{-1}) is slightly increased by intraliposomal ATP (230 s^{-1}) and even more by intraliposomal ADP (248 s^{-1}).

The increase of activity by intraliposomal nucleotide is not due to different orientations of the COX vesicles. From seven independent reconstitutions in the absence and in the presence of 10 mM ADP, the following average turnover numbers and standard deviations were obtained with 40 μM cytochrome *c* in the presence of uncoupler: 194 ± 10.1 (without ADP) and 239 ± 13.5 (with ADP). The percentage stimulation by intraliposomal ADP is calculated to 23.5% ± 0.89%. The orientations of COX in these proteoliposomes were the same in the absence (65.4% ± 2.1%) and in the presence of 10 mM intraliposomal ADP (64.8% ± 3.0%).

Increasing concentrations of TMPD diminish or abolish the effects of ADP and ATP, respectively, as shown in Figure 2. It was assumed by Ferguson-Miller et al. (1978) that dissociation of ferricytochrome *c* represents the rate-limiting step of COX activity in the absence of TMPD. In the presence of TMPD, electron transfer from ascorbate via cytochrome

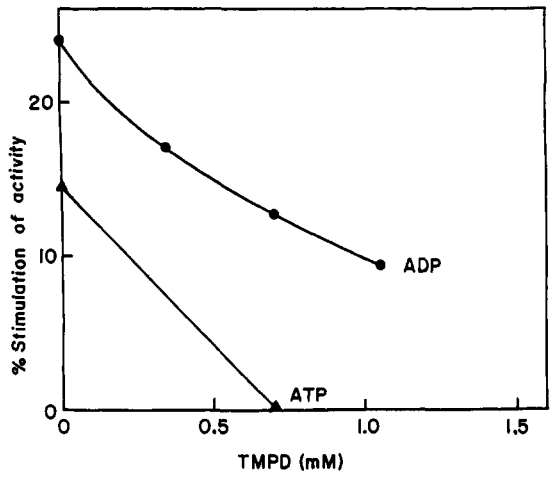

FIGURE 2: Stimulatory effect of intraliposomal nucleotides on the activity of reconstituted COX from bovine heart is abolished by TMPD. The activity of proteoliposomes, prepared in the presence or absence of 10 mM ADP or ATP, was measured with 40 μM cytochrome *c* in the presence of uncoupler at the indicated concentration of TMPD. Presented in the figure is the percentage stimulation of activity by intraliposomal ADP (circles) or ATP (triangles).

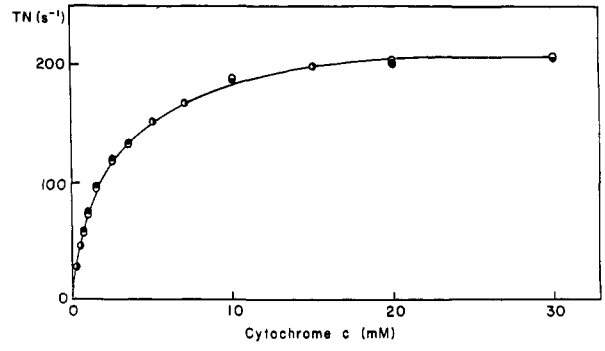

FIGURE 3: Intraliposomal ADP and ATP do not stimulate the activity of reconstituted COX from bovine liver. The activity of COX from bovine liver, reconstituted in the absence (open circles) or presence of 10 mM ADP (closed circles), was measured in the presence of uncoupler without TMPD at increasing concentrations of cytochrome *c*.

c to COX was suggested to occur without dissociation of ferricytochrome *c* from COX (Ferguson-Miller et al., 1978). The effect of ADP on the low-affinity phase of cytochrome *c* oxidation, shown in Figure 1 (increased TN_{max} and unaltered K_M), supports the previous assumption that ADP increases the dissociation of ferricytochrome *c* from the enzyme (Hüther & Kadenbach, 1987). In contrast, the effect of intraliposomal ADP on the high-affinity phase of cytochrome *c* oxidation (unaltered TN_{max} and decreased K_M) suggests increased substrate binding at low ferrocytochrome *c* concentrations. Thus, the molecular mechanism of the ADP effect is complex and not fully understood.

Intraliposomal Nucleotides Interact Tissue-Specific. In contrast to COX from bovine heart, the activity of COX from bovine liver is not stimulated by intraliposomal ADP. This is demonstrated in Figure 3, where the activity was measured polarographically in the presence of uncoupler but in the absence of TMPD. Also, intraliposomal ATP does not influence the activity of COX from bovine liver (not shown). This result, which corroborates the data presented in the preceding publication (Anthony et al., 1992), demonstrates that the ADP effect is tissue-specific.

Concentration Dependence of Nucleotide Effects. The concentration dependences of the effects of intraliposomal ADP and ATP are shown in Figure 4. COX from bovine

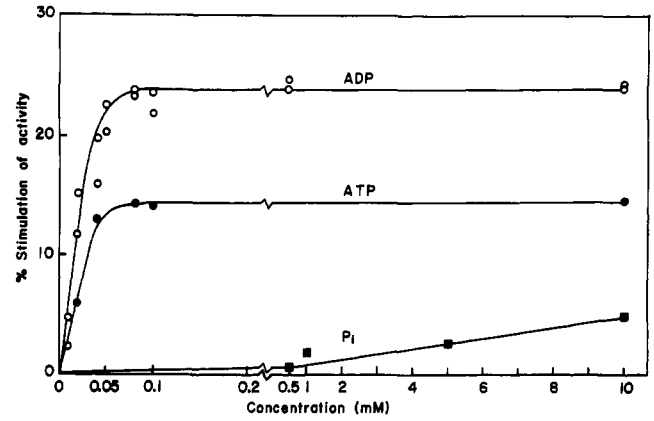

FIGURE 4: Concentration dependence of the effect of intraliposomal nucleotides and phosphate on the activity of reconstituted COX from bovine heart. Proteoliposomes were prepared at the indicated concentrations of ADP (open circles, two independent experiments), ATP (closed circles), or phosphate (squares). The activity was measured with 40 μM cytochrome *c* under uncoupled conditions without TMPD as described under Materials and Methods. Presented is the percentage stimulation of activity compared to that of proteoliposomes prepared without additions.

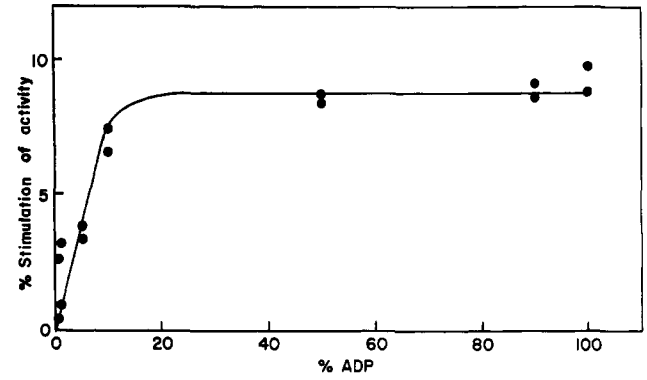

FIGURE 5: Concentration dependence of the effect of intraliposomal ADP in the presence of ATP on the activity of COX from bovine heart. Proteoliposomes were prepared in the presence of 10 mM total nucleotides (ADP + ATP) but at the indicated percentage of ADP. The activity was measured with 40 μM cytochrome *c* under uncoupled conditions without TMPD as described under Materials and Methods. The data of two independent experiments are presented.

heart was reconstituted in the presence of the indicated concentrations of ADP, ATP, or phosphate, and the activity was measured in the presence of uncoupler and the absence of TMPD. Half-maximal stimulation of activity is obtained at 20 mM intraliposomal ADP as well as ATP. Above 0.1 mM nucleotide concentrations, no further stimulation of COX activity is obtained. While the maximal stimulation by ADP is 24%, that obtained by ATP—which has a higher ionic strength than ADP—is only 14.5%. Intraliposomal phosphate has no effect on the activity at low concentrations, but stimulates the activity at 10 mM to a small extent (5%). No stimulation of COX activity was obtained with 48 mM intraliposomal NaCl (not shown). These results demonstrate that the stimulation of COX activity by intraliposomal nucleotides is not due to unspecific ionic effects.

When the concentration dependence of the ADP effect is measured in the presence of ATP at 10 mM total intraliposomal nucleotides, half-maximal stimulation of activity is obtained at about 5% intraliposomal ADP (Figure 5). If both nucleotides bind at the same site and have the same affinity (see Figure 4), half-maximal stimulation should be expected at 50% ADP. The maximal stimulation of activity by ADP in the presence of ATP is only 8%, indicating no additive

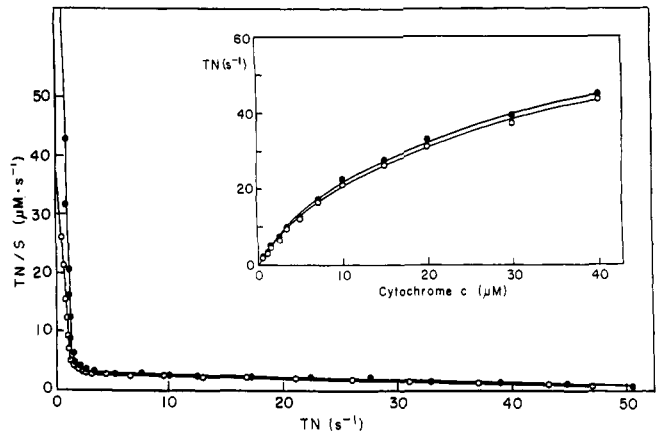

FIGURE 6: Effect of intraliposomal ADP on the kinetics of reconstituted COX from bovine heart under coupled conditions. COX was reconstituted in the absence (open circles) or presence of 10 mM ADP (closed circles). The activity was measured in the absence of uncouplers and TMPD at increasing concentrations of cytochrome *c* as described under Materials and Methods. Main panel: Eadie–Hofstee plot. Insert: Dependence of activity on cytochrome *c* concentration.

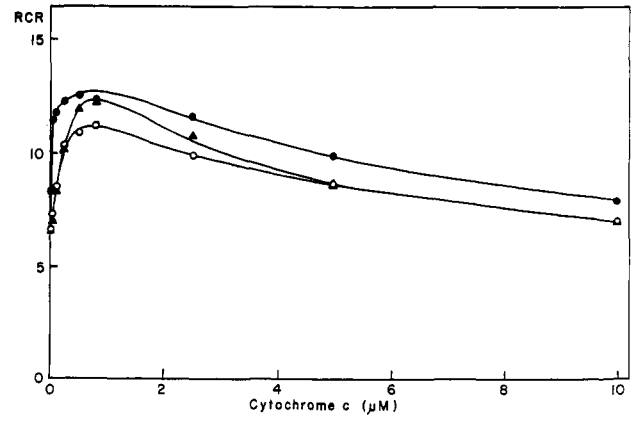

FIGURE 7: Intraliposomal ADP stimulates the respiratory control ratio of reconstituted COX from bovine heart. Proteoliposomes were prepared in the absence (open circles) or presence of 10 mM ADP (closed circles), and the activity was measured without TMPD at increasing concentrations of cytochrome *c* in the presence and absence of uncoupler as described under Materials and Methods. Presented is the respiratory control ratio (RCR = ratio of the rate of oxygen uptake in the presence to that in the absence of uncoupler).

effects by ADP and ATP. These results cannot be simply explained. Neither a single binding site for both nucleotides nor two independent binding sites would correspond to the data. Instead, complex conformational changes may be induced by the nucleotides.

Effect of Nucleotides on Respiratory Control Ratios. The effect of intraliposomal nucleotides on the activity of reconstituted COX from bovine heart was also measured in the absence of uncoupler. The coupled respiration of COX from bovine heart is hardly stimulated by intraliposomal ADP, as shown in the insert of Figure 6. The Eadie–Hofstee plot of the data (main panel of Figure 6) indicates very strong biphasic behavior of COX kinetics under coupled conditions. The high- and low-affinity phases of ferrocytochrome *c* oxidation, which represent almost straight lines, are strongly separated by a narrow intermediate phase. Intraliposomal ADP decreases the K_M for the high-affinity phase of ferrocytochrome *c* oxidation (0.012 μM as compared to 0.033 μM of the control), but has no influence on the low-affinity phase. Thus, the affinity of COX for its substrate is stimulated by intraliposomal ADP under coupled as well as uncoupled conditions.

The lower stimulation of COX activity by intraliposomal ADP under coupled as compared to uncoupled conditions results in an increased respiratory control ratio (RCR). Figure 7 presents the RCR values measured at increasing concentrations of cytochrome *c*. Apparently, the RCR value is not constant, but shows a biphasic dependence on the concentration of cytochrome *c*, i.e., the rate of respiration. The RCR value has a maximum at about 0.7 μM cytochrome *c*. Under all concentrations of cytochrome *c*, intraliposomal ADP increases the RCR value, but the highest stimulation is found at low respiratory rates. The increase of the RCR indicates a more efficient energy transduction in the presence of intraliposomal ADP. ATP does not stimulate the RCR at low and high concentrations of cytochrome *c*, but shows some stimulation around the maximal RCR value of the control. Under all concentrations of cytochrome *c*, the stimulation of RCR by intraliposomal ADP is higher than by ATP.

Effect of Other Nucleotides. Finally we investigated the specificity of the nucleotide effect. COX from bovine heart was reconstituted in the presence of 10 mM ADP, GDP, IDP, ATP, or CTP. Intraliposomal GDP and IDP stimulated the activity, measured with 40 μM cytochrome *c* in the presence of uncoupler, almost to the same degree as intraliposomal ADP (93 and 90% from the stimulation by ADP, respectively), while ATP and CTP stimulated to a lesser extent (61 and 42% from the stimulation by ADP, respectively). Thus, it may be concluded that both the base of the nucleotide (purine or pyrimidine) and the number of phosphate groups influence the degree of stimulation of COX activity by intraliposomal nucleotides.

DISCUSSION

ADP and ATP Influence the Activity of COX. The effects of nucleotides on the activity of COX have been described in the literature repeatedly (Ferguson-Miller et al., 1976; Robert & Hess, 1977; Hüther & Kadenbach, 1986, 1987, 1988; Rigoulet et al., 1987; Malatesta et al., 1988; Hüther et al., 1988). It was difficult, however, to distinguish between the interaction of nucleotides with the COX enzyme or with its substrate cytochrome *c*. In addition, nucleotides could interact with the cytosolic as well as with the matrix domain of the enzyme. From kinetic studies with reconstituted COX vesicles where the nucleotides could be added either from the outside (cytosolic) or during reconstitution from the intraliposomal (matrix) space, we could demonstrate an effect of ATP from the cytosolic side (Hüther & Kadenbach, 1986; Hüther et al., 1988), and of ADP and ATP from the matrix side (Hüther et al., 1987, 1988). The effect of ATP on the redox state of COX in yeast mitochondria was due to interaction from the cytosolic side (Rigoulet et al., 1987).

ADP and ATP Bind to the Same Site at Subunit VIa-h. Recently the binding site for intraliposomal ADP at the N-terminal matrix domain of subunit VIa-h (heart type) was identified with the help of a specific monoclonal antibody (Anthony et al., 1992). This interaction site is tissue-specific, as corroborated in the present study, where no effect of intraliposomal nucleotides could be found with COX from bovine liver. In a recent publication, we have titrated COX from bovine heart with trinitrophenyl-ATP and identified two binding sites for ATP with a K_D value of 1.6 μM (Reimann & Kadenbach, 1992). It was proposed that the two binding sites at COX from bovine heart are located on both sides of the transmembranous enzyme complex. The present results describe the effects of "matrix nucleotides" on COX from heart. Intraliposomal ADP and ATP (and to a lesser extent

other nucleotides) appear to bind at the same site (located at subunit VIa-h), because the stimulatory effects on respiration are not additive. Although both nucleotides stimulates respiration and decrease the K_M for cytochrome *c*, the magnitude of the effect is higher with ADP, indicating high specificity and excluding unspecific ionic effects.

ADP Decreases the K_M for Cytochrome c (High-Affinity Phase). In a previous publication, the effect of intraliposomal nucleotides could only be measured with the spectrophotometric, not with the polarographic, method of assay in the presence of TMPD (Hüther & Kadenbach, 1987). Here we show that the effects of intraliposomal nucleotides can also be measured polarographically if TMPD is omitted. In the previous study, opposite effects of ADP and ATP on the K_M for cytochrome *c* were obtained by the spectrophotometric assay, in which only a single phase of cytochrome *c* oxidation was obtained. The present results show a decrease of the K_M for cytochrome *c* by ADP as well as ATP, but only for the high-affinity phase. The lack of biphasic kinetics in the previous study is due to accumulation of product between the start of the reaction and the recording of the rates by the manual spectrophotometric method, since ferricytochrome *c* was shown to change the multiphasic into monophasic COX kinetics (Reimann et al., 1992).

ADP Increases the Efficiency of COX from Heart. ADP stimulates more strongly the uncoupled than the coupled respiration of reconstituted COX from bovine heart, resulting in an improved respiratory control ratio (RCR). The RCR shows a strong dependence on the concentration of cytochrome *c* (i.e., rate of respiration). This result is in accordance with the variable proton-pumping activity of COX in mitochondria (Papa et al., 1991) and in proteoliposomes (Capitanio et al., 1991). Both the RCR and the H^+/e^- ratio reveal a maximum at intermediate rates of respiration. Therefore, we suggest that the RCR is a measure of the proton-pumping efficiency of COX. A variation of proton-pumping efficiency (or "slippage" of proton pumping) has been shown recently by chemical modification of isolated COX from bovine heart (Steverding & Kadenbach, 1991), and after removal of subunit III from COX of *Paracoccus* (Steverding et al., 1993). Under all rates of respiration, intraliposomal ADP stimulates the RCR of reconstituted COX (Figure 7). Therefore, "matrix ADP" could act as a regulator of the efficiency of COX from heart and skeletal muscle.

The increased efficiency of COX by ADP could have a physiological meaning. It was suggested that the heart participates in nonshivering thermogenesis of vertebrates (Puchalski et al., 1987). At high work load, the heart produces severalfold more heat than under resting conditions, if the efficiency of energy metabolism is constant. The decrease of efficiency of oxidative phosphorylation at low work load (high concentrations of ATP) would improve heat production under resting conditions (maintenance of body temperature), while the increase of efficiency at high work load (increased concentrations of ADP) would diminish the excess amount of heat generated under high beating rates.

REFERENCES

Anthony, G., Stroh, A., Lottspeich, F., & Kadenbach, B. (1990) *FEBS Lett. 277*, 97–100.

Anthony, G., Reimann, A., & Kadenbach, B. (1992) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1652–1656.

Braun, H.-P., Emmermann, M., Kruft, V., & Schmitz, U. K. (1992) *EMBO J. 11*, 3219–3227.

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

Capaldi, R. A. (1990) *Annu. Rev. Biochem. 59*, 569–596.

Capitanio, N., Capitanio, G., De Nitto, E., Villani, G., & Papa, S. (1991) *FEBS Lett. 288*, 179–182.

Casey, R. P., Ariano, B. H., & Azzi, A. (1982) *Eur. J. Biochem. 122*, 313–318.

Ferguson-Miller, S., Brautigan, D. L., & Margoliash, E. (1976) *J. Biol. Chem. 251*, 1104–1115.

Ferguson-Miller, S., Brautigan, D. L., & Margoliash, E. (1978) *J. Biol. Chem. 253*, 149–159.

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

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

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

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

Kadenbach, B. (1983) *Angew. Chem., Int. Ed. Engl. 22*, 275–282.

Kadenbach, B., Stroh, A., Ungibauer, M., Kuhn-Nentwig, L., Büge, U., & Jarausch, J. (1986) *Methods Enzymol. 126*, 32–45.

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

Kadenbach, B., Stroh, A., Becker, A., Eckerskorn, C., & Lottspeich, F. (1990) *Biochim. Biophys. Acta 1015*, 368–372.

Kagawa, Y., & Racker, E. (1971) *J. Biol. Chem. 246*, 5477–5487.

Lightowlers, L., Ewart, G., Aggeler, R., Zhang, Y. Z., Calavetta, L., & Capaldi, R. A. (1990) *J. Biol. Chem. 265*, 2677–2681.

Ludwig, B. (1987) *FEMS Microbiol. Rev. 46*, 41–56.

Malatesta, F., Antonini, G., Sarti, P., & Brunori, M. (1988) *Biochem. J. 248*, 161–165.

Merle, P., & Kadenbach, B. (1982) *Eur. J. Biochem. 125*, 239–244.

Papa, S., Capitanio, N., Capitanio, G., De Nitto, E., & Minuto, M. (1991) *FEBS Lett. 288*, 183–186.

Puchalski, W., Böckler, H., Heldmaier, G., & Langefeld, M. (1987) *J. Exp. Zool. 242*, 263–271.

Reimann, A., & Kadenbach, B. (1992) *FEBS Lett. 307*, 294–296.

Reimann, A., Röhm, K.-H., & Kadenbach, B. (1993) *J. Bioenerg. Biomembr.* (in press).

Robert, H., & Hess, B. (1977) *Biochim. Biophys. Acta 462*, 215–234.

Schlerf, A., Droste, M., Winter, M., & Kadenbach, B. (1988) *EMBO J. 7*, 2387–2391.

Schulte, U., Arretz, M., Schneider, H., Tropschug, M., Wachter, E., Neupert, W., & Weiss, H. (1989) *Nature 339*, 147–149.

Seelan, R. S., & Grossman, L. I. (1991) *J. Biol. Chem. 266*, 19752–19757.

Shechter, J., & Bloch, K. (1971) *J. Biol. Chem. 246*, 7690–7696.

Steverding, D., & Kadenbach, B. (1991) *J. Biol. Chem. 266*, 8097–8101.

Steverding, D., Köhnke, D., Ludwig, B., & Kadenbach, B. (1993) *Eur. J. Biochem. 212*, 827–831.

Van Kuilenberg, A. B. P., Muijers, A. O., Demol, H., Dekker, H. L., & Van Beeumen, J. J. (1988) *FEBS Lett. 240*, 127–132.

Van Kuilenberg, A. B. P., Dekker, H. L., Van den Bogert, C., Nieboer, P., Van Gelder, B. F., & Muijsers, A. O. (1991) *Eur. J. Biochem. 199*, 615–622.

Waterland, R. A., Basu, A., Chance, B., & Poyton, R. A. (1991) *J. Biol. Chem. 266*, 4180–4186.

Yanamura, W., Zhang, Y.-Z., Takamiya, S., & Capaldi, R. A. (1988) *Biochemistry 27*, 4909–4914.