Biochimica et Biophysica Acta, 636 (1981) 9–16
Elsevier/North-Holland Biomedical Press

BBA 48055

THE REACTION BETWEEN CYTOCHROME c_1 AND CYTOCHROME *c*

B.W. KÖNIG, J. WILMS and B.F. VAN GELDER

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam (The Netherlands)

(Received October 23rd, 1980)
(Revised manuscript received February 2nd, 1981)

Key words: Electron transfer; Cytochrome c_1; Cytochrome c; Ionic strength effect; (Kinetics)

The kinetics of electron transfer between the isolated enzymes of cytochrome c_1 and cytochrome *c* have been investigated using the stopped-flow technique. The reaction between ferrocytochrome c_1 and ferricytochrome *c* is fast; the second-order rate constant (k_1) is $3.0 \cdot 10^7$ $M^{-1} \cdot s^{-1}$ at low ionic strength (I = 223 mM, 10°C). The value of this rate constant decreases to $1.8 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ upon increasing the ionic strength to 1.13 M. The ionic strength dependence of the electron transfer between cytochrome c_1 and cytochrome *c* implies the involvement of electrostatic interactions in the reaction between both cytochromes. In addition to a general influence of ionic strength, specific anion effects are found for phosphate, chloride and morpholinosulphonate. These anions appear to inhibit the reaction between cytochrome c_1 and cytochrome *c* by binding of these anions to the cytochrome *c* molecule. Such a phenomenon is not observed for cacodylate. At an ionic strength of 1.02 M, the second-order rate constants for the reaction between ferrocytochrome c_1 and ferricytochrome *c* and the reverse reaction are $k_1 = 2.4 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ and $k_{-1} = 3.3 \cdot 10^5$ $M^{-1} \cdot s^{-1}$, respectively (450 mM potassium phosphate, pH 7.0, 1% Tween 20, 10°C). The 'equilibrium' constant calculated from the rate constants (0.73) is equal to the constant determined from equilibrium studies. Moreover, it is shown that at this ionic strength, the concentrations of intermediary complexes are very low and that the value of the equilibrium constant is independent of ionic strength. These data can be fitted into the following simple reaction scheme:

cytochrome c_1^{2+} + cytochrome c^{3+} ⇌ cytochrome c_1^{3+} + cytochrome c^{2+}.

Introduction

Cytochrome c_1 forms part of the mitochondrial ubiquinol-cytochrome *c* reductase complex (Complex III) and catalyses the electron transfer from cytochrome *b* to cytochrome *c*. Although the steady-state kinetics of the reaction between cytochrome *c* and cytochrome c_1 in mitochondrial membranes have been studied extensively [1–7], no exact kinetic data were available on the reaction between the isolated enzymes, since it was difficult to obtain a purified cytochrome c_1 preparation. In 1972, Yu et al. [8] succeeded in developing an isolation procedure for bovine cytochrome c_1 which yielded a highly purified enzyme. By studying the kinetics of electron transfer between this purified enzyme and cytochrome *c*, they were able to determine the rate constants for the electron transfer from cytochrome c_1 to cytochrome *c* and the reverse reaction ($k_1 = 3.3 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ and $k_{-1} = 1.0 \cdot 10^6$ $M^{-1} \cdot s^{-1}$, respectively; 50 mM potassium phosphate, pH 7.4, 10°C) [9]. Furthermore, it was shown that k_1 as well as k_{-1} is dependent on ionic strength, in contrast to the equilibrium constant (K_{eq}). From a K_{eq} value of 3.3, a difference in midpoint potential of 32 mV between the cytochromes has been calculated, cytochrome *c* having the higher midpoint potential. In contrast, Nicholls [2] reported

Abbreviation: Mops, 4-morpholinepropanesulphonic acid.

0005-2728/81/0000–0000/$02.50

a value of the midpoint potential for cytochrome *c* which is 5 mV lower than that of cytochrome c_1.

In a previous paper [10], it has been demonstrated that cytochrome c_1 prepared in our laboratory is in the monomeric state and contains only one polypeptide, in contrast to the cytochrome c_1 preparation of Yu et al. [8] that is isolated as a pentamer and contains two polypeptide chains. It remains questionable whether the second polypeptide found by Yu et al. [8] is an intrinsic part of the protein.

Former studies of the kinetics of the reactions of carboxydinitrophenyllysine-modified cytochromes *c* with our cytochrome c_1 preparation [11] and with the succinate-cytochrome *c* reductase complex [6,7] revealed that cytochrome c_1 in the isolated state and in the cytochrome bc_1 complex has the same reactivities towards various carboxydinitrophenyllysine-modified cytochromes *c*. Therefore, it is concluded that the ability of cytochrome c_1 to transfer electrons is not modified upon extraction of this component from the cytochrome bc_1 complex.

In this paper we investigate the kinetic properties of cytochrome c_1 by studying its reaction with cytochrome *c* at different ionic strengths.

Materials and Methods

Sephadex G-25 medium and G-75 superfine (Pharmacia) were swollen in 5 mM potassium phosphate (pH 7.0) at 90°C for 60 and 120 min, respectively. Ascorbic acid (BDH) was brought to pH 7.0 with KOH and was stored as 2 M stock solutions at −20°C. Mops, Tris, cacodylic acid and Tween 20 were obtained from Sigma. All other chemicals were purchased from British Drug Houses.

Reduced bovine cytochrome c_1 was isolated as described before [10]. Ferricytochrome c_1 was obtained by oxidation of ferrocytochrome c_1 with an excess of $K_3Fe(CN)_6$ which was removed by passing the mixture (0.2 ml of 800 μM ferricytochrome c_1) through a column of Sephadex G-75 superfine (25 × 0.5 cm) equilibrated at 4°C in 5 mM potassium phosphate (pH 7.0) and 1% Tween 20.

Cytochrome *c* from horse and bovine heart was isolated and purified according to the method of Margoliash and Walasek [12]. Ferrocytochrome *c* from horse heart was prepared by reducing ferricytochrome *c* (0.2 ml, 1500 μM) with an excess of potassium ascorbate (pH 7.0) which was removed by gel filtration chromatography on a column (40 × 0.5 cm) of Sephadex G-25 medium, equilibrated at 4°C in a deaerated potassium phosphate buffer (5 mM, pH 7.0).

Immediately before use, all cytochromes (0.1 ml, 300 μM) were passed through a column (20 × 0.5 cm) of Sephadex G-75 superfine, equilibrated in 5 mM potassium phosphate (pH 7.0) and 1% Tween 20. The concentration of cytochromes was determined spectrophotometrically using an extinction coefficient (reduced minus oxidized) of 19.2 $mM^{-1} \cdot cm^{-1}$ at 552.5 nm [13] for cytochrome c_1 and of 21.1 $mM^{-1} \cdot cm^{-1}$ for cytochrome *c* at 550 nm [14]. The difference extinction coefficient at 547.5 nm for the transfer of 1.0 mM reducing equivalent from cytochrome c_1 to cytochrome *c* can be calculated using extinction coefficients (reduced minus oxidized) at this wavelength of 4.3 and 17.5 $mM^{-1} \cdot cm^{-1}$ for cytochrome c_1 and cytochrome *c*, respectively. Spectra were recorded on a Cary-219 spectrophotometer equipped with a thermostatically controlled cuvette-house.

Reactions were followed at 10°C using a Durrum-Gibson stopped-flow apparatus, the stop system of which was changed into an adjustable block by a built-in microswitch, triggering a transient recorder (TR; Datalab 905). The optical pathlength reaction chamber is 2 cm. Storage and processing of the signals were carried out as described before [15]. The ionic strength (*I*) of the buffers (pH 7.0, 1% (v/v) Tween 20) was calculated using the Henderson-Hasselbach equation and a Debye-Hückel approximation [16] for the apparent pK_a values. The pK_a values (10°C) for phosphate, Tris, Mops and cacodylate at zero ionic strength [17] were 7.2, 8.3, 6.27 and 7.2, respectively.

The reaction between cytochrome c_1 and cytochrome *c* can be represented by the following reaction scheme:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C + D \qquad (1)$$

The differential equation of this bimolecular reaction can be written in the form:

$$\frac{dc}{dt} = k_1 [A_0] [B_0] - k_{-1}([A_0] + [B_0])C + (k_1 - k_{-1}) C^2 \qquad (2)$$

In this equation, $[A_0]$ and $[B_0]$ are the initial concentrations of cytochromes (ferrocytochrome c_1 and ferricytochrome c or ferricytochrome c_1 and ferrocytochrome c). If $[C_0] = [D_0] = 0$, $[A_0] \geqslant 3[B_0]$ and $0.6 < K_{eq} < 1.6$, as will be shown in the experiments referred to in Fig. 6, the solution of Eqn. 2 can be approximated by one exponential with a contribution from a second exponential of less than 10%. The decay constant of the first exponential (the pseudo first-order rate constant) k' can be written as:

$$k' = k_1([A_0] + [B_0]) \quad (3)$$

The pseudo first-order rate constants (k') calculated from the experimental traces (cf. Ref. 15) were the mean values of 8–12 reaction traces. The standard deviation for k_1 was found to be 4–8%. The second-order rate constant (k_1) was determined from a plot of k' vs. ($[A_0] + [B_0]$), using a weighted least-squares method.

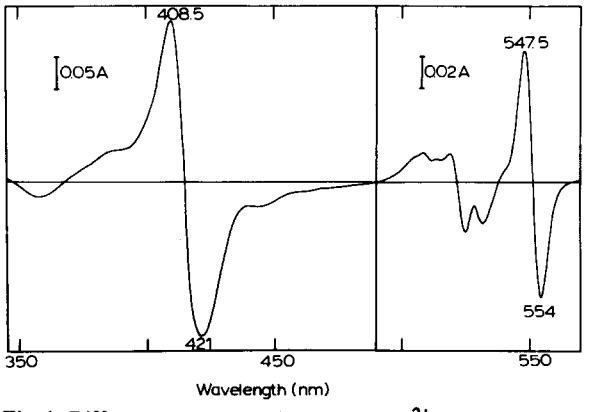
Results

The electron transfer between cytochrome c_1 and cytochrome c was monitored optimally at 547.5 nm (Fig. 1) because the background absorbance level at 408.5 nm is too high.

The absorbance changes at 547.5 nm at various concentrations of phosphate are shown in Fig. 2. It is obvious that an increase of the ionic strength (traces A–D) results in a decrease of the reaction rate. It is hardly possible to measure the reaction between both cytochromes at an ionic strength lower than 200 mM, since under these conditions, the reaction is completed within the mixing time of our stopped-flow apparatus ($k_1 > 1 \cdot 10^8\ M^{-1} \cdot s^{-1}$).

A plot of the pseudo first-order rate constant (k') of the reaction, calculated from traces such as those shown in Fig. 2, versus the total concentration of cytochromes is presented in Fig. 3. Note that the second-order rate constant of the reaction (k_1), determined from the slope of the line, decreases from $3.0 \cdot 10^7$ to $1.8 \cdot 10^5\ M^{-1} \cdot s^{-1}$, when the ionic strength is increased from 223 to 1130 mM. The use of bovine cytochrome c (Fig. 3, closed symbols), instead of horse cytochrome c, as electron acceptor did not change the values for the rate constants.

To investigate whether or not the inhibition by phosphate is due to a general ionic strength effect, the dependence of ionic strength on the reaction



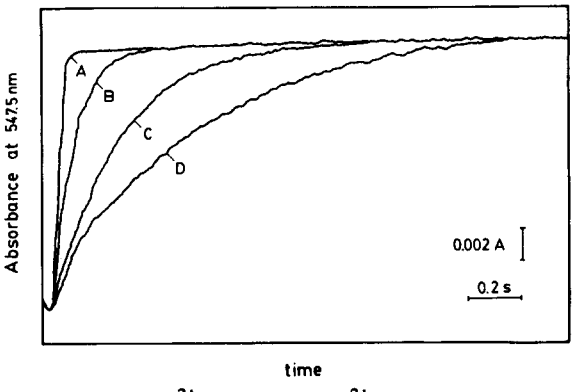
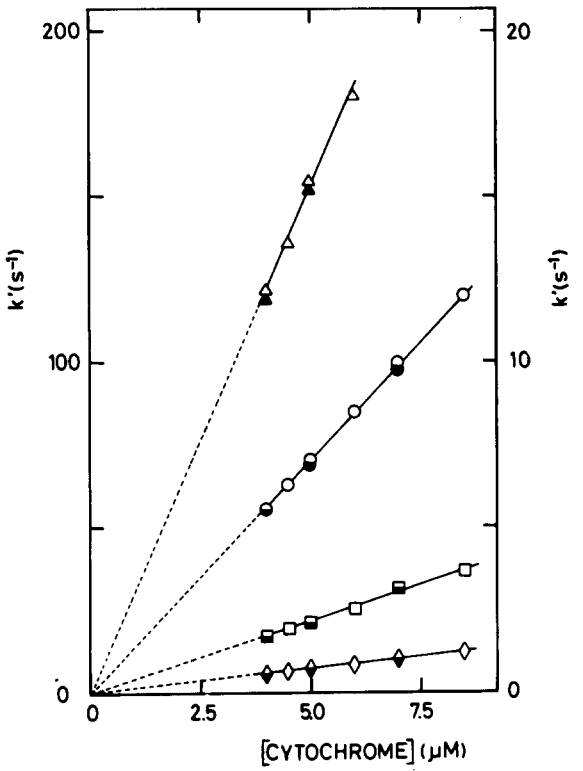


Fig. 1. Difference spectrum ((cytochrome c_1^{2+} + cytochrome c^{3+}) minus (cytochrome c_1^{3+} + cytochrome c^{2+})) for the reduction of ferricytochrome c by ferrocytochrome c_1. Before the reaction, both the sample and the reference tender cell (d = 0.876 cm) contained 15.4 μM ferrocytochrome c_1 and 15.0 μM ferricytochrome c in their respective compartments. After scanning the baseline, the contents of the two chambers of the sample cell were mixed. The difference spectrum was recorded at 10°C in 100 mM potassium phosphate (pH 7.0), 1% Tween 20 with a Cary-219 spectrophotometer.

Fig. 2. Single traces of the time course of the reaction of ferrocytochrome c_1 with ferricytochrome c at various phosphate concentrations. The experiments were carried out at 10°C in a potassium phosphate buffer (pH 7.0) containing 1% Tween 20. Absorbance changes were followed at 547.5 nm. Concentrations of ferrocytochrome c_1 and of ferricytochrome c were 5.0 and 1.0 μM, respectively. Concentrations of potassium phosphate were for traces A–D 100, 250, 375 and 500 mM, respectively.



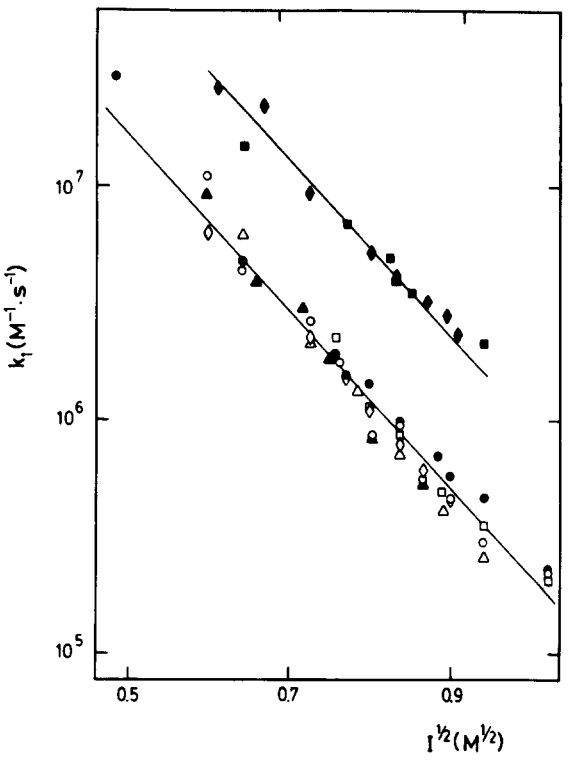


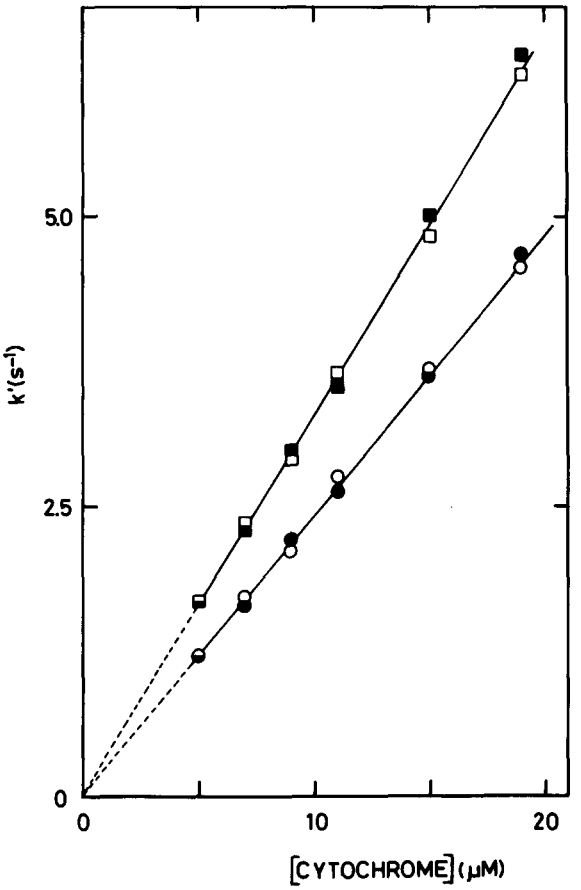
Fig. 3. Relationship between the observed pseudo first-order rate (k') and the total concentration of cytochromes at various phosphate concentrations. Conditions as in Fig. 2: ferricytochrome c, 1.0 μM; ferrocytochrome c_1, 3.0–7.5 μM. The left-hand ordinate refers to the reaction at 100 mM potassium phosphate (△———△), whereas the right-hand ordinate refers to the reactions at the other potassium phosphate concentrations (○———○, 250 mM; □———□, 375 mM; ◇———◇, 500 mM). For the reaction between bovine ferrocytochrome c_1 and horse ferricytochrome c, the values for k' are represented by open symbols, whereas those for the reaction between bovine ferrocytochrome c_1 and bovine ferricytochrome c are given by closed symbols.

Fig. 4. Relationship between the second-order rate constant (k_1) and the ionic strength. Experiments were carried out at 10°C in buffers (pH 7.0, 1% Tween 20) of different ionic strength. Absorbance changes were monitored at 547.5 nm and k_1 values were determined as described in Materials and Methods. The ionic strength was provided either by increasing the concentrations of the buffer: ●———●, potassium phosphate; ◆———◆, Tris-cacodylate; ■———■, sodium cacodylate; ▲———▲, sodium/Mops (closed symbols) or by the addition of KCl to an appropriate buffer with a concentration of 20 mM (open symbols).

between cytochrome c_1 and cytochrome c was studied in more detail. As cytochrome c is able to bind several anions with variable affinity [18–24], the rate of the reaction between ferrocytochrome c_1 and ferricytochrome c was measured in media containing variable amounts of anions which bind to cytochrome c (phosphate, chloride and morpholinosulphonate) and a non-binding anion (cacodylate) [18–23]. The results are presented in Fig. 4 as a Brønsted plot (log k_1 vs. $\sqrt{I}$). This figure shows that an increase in the ionic strength upon addition of KCl or by increasing the concentration of buffers results in a decrease of the reaction rate. In addition to this general ionic strength effect there is also a specific anion effect. In the presence of cacodylate a higher value for k_1 is found at the same ionic strength, compared to the rate constants determined in media containing phosphate, chloride or morpholinosulphonate. The slope of the lines drawn through the experimental data is the same for both types of anion.

As the difference in redox potential between cytochrome c_1 and cytochrome c is small (5–30 mV) [2,9], electron transfer in both directions may be of physiological importance. The reversibility of the

reaction was therefore investigated by examination of the effect of the redox state of the cytochromes on the reaction rate by measuring the reaction between oxidized cytochrome c_1 and reduced cytochrome c, as well as the reaction between reduced cytochrome c_1 and oxidized cytochrome c. All forms of cytochrome being successively present in excess. The second-order rate constants calculated from Fig. 5 are of the same order of magnitude; k_1 for the reaction between ferrocytochrome c_1 and ferricytochrome c is $2.4 \cdot 10^5$ $M^{-1} \cdot s^{-1}$; the reduction of cytochrome c by cytochrome c_1 yields a rate constant of $3.3 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ (450 mM potassium phosphate, pH 7.0, 1% Tween 20, 10°C). Interchanging the concentrations of reactants did not affect the value of the rate constants (k_1 and k_{-1}). Using the values determined for k_1 and k_{-1}, a 'kinetic equilibrium constant' (k_1/k_{-1}) of 0.73 can be calculated. This value, close to unity, reflects a small difference in midpoint potential between cytochrome c_1 and cytochrome c (8 mV). Dependence on



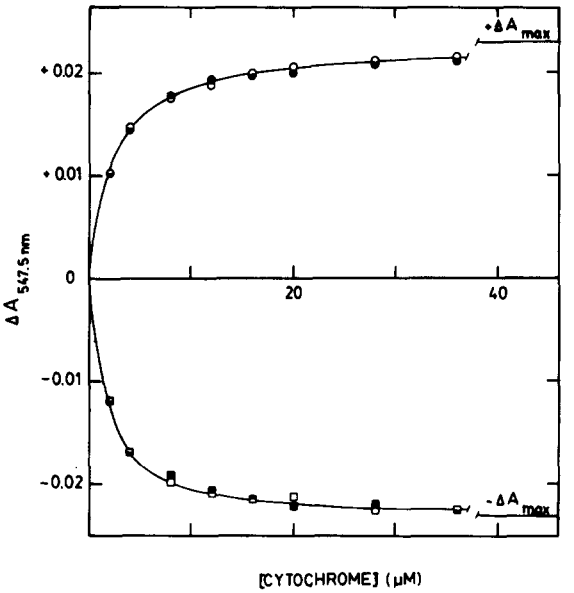


Fig. 5. Relationship between k' and the total concentration of cytochromes. Experiments were carried out at 10°C in a potassium phosphate buffer of 450 mM, pH 7.0, and 1% Tween 20. Absorbance changes were followed at 547.5 nm. For the reduction of cytochrome c_1 by cytochrome c, the k' values are represented by squares and for the reduction of cytochrome c by cytochrome c_1 circles are used. Determination of the k_1 values was as described in Materials and Methods. o———o, ferricytochrome c, 3.0–18.0 μM and ferrocytochrome c_1, 1.0 μM; •———•, ferricytochrome c, 1.0 μM and ferrocytochrome c_1, 3.0–18.0 μM; □———□, ferrocytochrome c, 3.0–18.0 μM and ferricytochrome c_1, 1.0 μM; ■———■, ferrocytochrome c, 1.0 μM and ferricytochrome c_1, 3.0–18.0 μM.

Fig. 6. Change in absorbance at 547.5 nm for the electron transfer between cytochrome c_1 and cytochrome c, as a function of the molar ratio of cytochromes. ΔA_{max} represents the transfer of 2.0 μM reducing equivalents, using an $\Delta\epsilon^{red\text{-}ox}_{547.5nm}$ value of 13.2 $mM^{-1} \cdot cm^{-1}$ and an optical pathway of 0.876 cm. The experiment was carried out as described in the legend of Fig. 1. Further conditions as described in Fig. 5. o———o, ferricytochrome c, 2.0–40.0 μM and ferrocytochrome c_1, 2.0 μM; •———•, ferricytochrome c, 2.0 μM and ferrocytochrome c_1, 2.0–40.0 μM; □———□, ferrocytochrome c, 2.0–40.0 μM and ferricytochrome c_1, 2.0 μM; ■———■, ferrocytochrome c, 2.0 μM and ferricytochrome c_1, 2.0–40.0 μM. The curves were computed using an equilibrium constant of 0.73.

ionic strength is also observed for the reduction of cytochrome c_1 by ferrocytochrome c. A plot of log k_{-1} vs. $\sqrt{I}$ (not shown) results in a line ($k_1/k_{-1} \approx 0.7$) parallel to that of log k_1 vs. $\sqrt{I}$ as shown in Fig. 4. From this result it is concluded that the kinetic equilibrium constant is independent of ionic strength.

Although the real equilibrium constant can be calculated from the extent of the stopped-flow traces, it is more accurate to determine this constant by registration of the absorbance changes at 547.5 nm with a Cary-219 spectrophotometer, before and after electron transfer. Fig. 6 shows the change in absorbance at 547.5 nm ($\Delta A_{547.5nm}$) as a function of the concentration of reactant present in excess. In the upper panel the reduction of ferricytochrome c and the oxidation of ferrocytochrome c_1 are presented, whereas in the lower panel the reduction of ferricytochrome c_1 and the oxidation of ferrocytochrome c are shown. It is clear that interchanging the concentrations does not affect the $\Delta A_{547.5nm}$ value (open and closed symbols). From the data, the oxidation-reduction degree of the cytochromes can be calculated and these are used to construct a 'Nernst-type' plot (log[cytochrome c_1^{3+}]/[cytochrome c_1^{2+}] vs. log[cytochrome c^{2+}]/[cytochrome c^{3+}]). From this plot (not shown) an equilibrium constant (K_{eq}) of 0.73 can be determined. This K_{eq} is used for simulating the curves in Fig. 6, which fit the experimental data well. Furthermore, it can be seen from this figure that at high concentrations of excess reactant the maximal absorbance change at 547.5 nm (ΔA_{max}) is reached. This indicates that the concentrations of intermediate complexes are very low. For this reason and because of the fact that the real equilibrium constant and the kinetic equilibrium constant are equal, the reaction between cytochrome c_1 and cytochrome c can be represented by a simple reaction scheme:

$$\text{cytochrome } c_1^{2+} + \text{cytochrome } c^{3+} \rightleftharpoons \text{cytochrome } c_1^{3+} + \text{cytochrome } c^{2+}$$

Discussion

The results of our experiments have shown that the reaction between isolated cytochrome c_1 and cytochrome c is dependent on ionic strength, in accordance with the findings of Yu et al. [9]. The same phenomenon has been observed for the reaction of cytochrome c with cytochrome c oxidase [3,5,6, 23,25], with the succinate-cytochrome c reductase complex [1,3,4,7,23] and with the bacterial cytochrome c-551 from *Pseudomonas fluorescens* [26]. Therefore, it is concluded that electrostatic interactions are essential for governing the rate of reactions in which cytochrome c participates. Electrostatic interactions are also important for the formation of a complex of cytochrome c with cytochrome c_1 [27, 28] or with cytochrome c oxidase [3,29,30] at low ionic strength.

In addition to a general ionic strength effect, phosphate, chloride and morpholinosulphonate exert a specific inhibitory effect on the reaction of cytochrome c_1 with cytochrome c, in contrast to cacodylate. The binding of anions to cytochrome c has been extensively described by Margoliash and coworkers [18,19,22–24] and Margalit and Schejter [20,21]. It has been demonstrated that these anions affect the reactivity of this enzyme towards cytochrome c oxidase [1,3,22–24] and the succinate-cytochrome c reductase complex [23]. Some anions are known to inhibit the reaction between cytochrome c and cytochrome c oxidase in a competitive way (chloride, citrate and acetate) or in a non-competitive way (phosphate, ADP and ATP) [22–24]. Some investigators were even able to localize the binding domain on the cytochrome c molecule for phosphate, ADP, ATP and citrate [24,31]. Therefore, the differences in reaction rate, found for media containing phosphate, chloride and morpholinosulphonate on the one hand and cacodylate on the other, are ascribed to the binding of the above-mentioned anions to cytochrome c. Although anion binding to cytochrome c decreases the net positive charge of the enzyme (cf. Ref. 20), it is remarkable that the value of the slope of the line in the Brønsted plot is not affected.

Yu et al. [9] and King [32] have reported a value for k_1 which is about half that which we measured at higher ionic strength (107 as against 223 mM). Extrapolation of the data presented in Fig. 4 to the same ionic strength, assuming a linear relationship between log k_1 and $\sqrt{I}$, increases the value of our k_1 by a factor of 2. The difference with the value reported [9,32] then amounts to a factor of 3.5. Similarly, it was calculated that k_{-1} is about 15-times greater than that reported by King and coworkers [9,32]. The use

of bovine cytochrome *c* instead of the enzyme from horse heart (cf. Ref. 9) cannot explain the difference in reactivity of the cytochrome c_1 preparations, since replacement of horse cytochrome *c* by bovine cytochrome *c* does not affect the k_1 value. For this reason and because of the fact that our cytochrome c_1 preparation is present in the monomeric state and possesses a single polypeptide [10], whereas the cytochrome c_1 preparation of Yu et al. [8] is a pentamer and contains two polypeptide chains, we suggest that the molecular state of the cytochrome c_1 preparation causes the differences in reactivity.

The plots of the observed pseudo first-order rate constant versus the total concentration of cytochromes reveal a linear relationship for cytochrome c_1 as well as cytochrome *c* being present in excess. This result indicates that the reaction is first-order both for cytochrome c_1 and cytochrome *c*. Since interchanging the concentration of reactants affects neither the rate constants nor the extent of the absorbance change, we conclude that the number of reactive sites on cytochromes c_1 and *c* are equal. An equal number of binding sites has also been found by Chiang et al. [27,28], who isolated a 1 : 1 complex of both cytochromes. From steady-state experiments for the cytochrome bc_1 complex, however, two binding sites for cytochrome *c* per cytochrome c_1 were postulated [6]. A two-site binding for cytochrome *c* was also observed in its reaction with cytochrome *c* oxidase [3].

The values of the equilibrium constant determined from equilibrium studies and calculated from the rate constants are equal. This result is also found by Yu et al. [9], although the absolute value for both constants differs by a factor of 3.5. From the equilibrium constant we measured, a difference in midpoint potential of 8 mV is determined, which implies that the midpoint potential of cytochrome c_1 is higher than that of cytochrome *c*. This value is in close agreement with the 5 mV difference found by Nicholls [2].

Although the kinetic results can be fitted in a simple model (cf. Results) we prefer the model as proposed by Chien [33], in which electron transfer proceeds via two complexes of cytochrome c_1 and cytochrome *c*:

$$\text{cytochrome } c_1^{2+} + \text{cytochrome } c^{3+} \rightleftharpoons \text{cytochrome } c_1^{2+} - \text{cytochrome } c^{3+} \rightleftharpoons \text{cytochrome } c_1^{3+} - \text{cytochrome } c^{2+} \rightleftharpoons \text{cytochrome } c_1^{3+} + \text{cytochrome } c^{2+}.$$

Since intramolecular electron transfer within the complex will be very rapid and the complex is only stable at low ionic strength [27,30], it is justified to simplify the reaction equation to the form presented in Results.

Acknowledgements

The authors wish to thank Rolf Boelens and Enno Veerman for stimulating discussion, Henk Dekker for skilful technical assistance and Mrs. G.E.E. Van Noppen for correcting the English text. This work was supported by grants from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

References

1 Smith, L., Davies, H.C. and Nava, M. (1974) J. Biol. Chem. 249, 2904–2910
2 Nicholls, P. (1976) Biochim. Biophys. Acta 430, 30–45
3 Ferguson-Miller, S., Brautigan, D.L. and Margoliash, E. (1976) J. Biol. Chem. 251, 1104–1115
4 Ahmed, A.J., Smith, H.T., Smith, M.B. and Millett, F.S. (1978) Biochemistry 17, 2479–2483
5 Errede, B. and Kamen, M.D. (1978) Biochemistry 17, 1015–1027
6 Ferguson-Miller, S., Weiss, H., Speck, S.H., Brautigan, D.L., Osheroff, N. and Margoliash, E. (1979) in Cytochrome Oxidase (King, T.E., Orii, Y., Chance, B. and Okunuki, K., eds.), pp. 281–292, Elsevier/North-Holland, Amsterdam
7 Speck, S.H., Ferguson-Miller, S., Osheroff, N. and Margoliash, E. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 155–159
8 Yu, C.A., Yu, L. and King, T.E. (1972) J. Biol. Chem. 247, 1012–1019
9 Yu, C.A., Yu, L. and King, T.E. (1973) J. Biol. Chem. 248, 528–533
10 König, B.W., Schilder, L.T.M., Tervoort, M.J. and van Gelder, B.F. (1980) Biochim. Biophys. Acta 621, 283–295
11 König, B.W., Osheroff, N., Wilms, J., Muijsers, A.O., Dekker, H.L. and Margoliash, E. (1980) FEBS Lett. 111, 395–398
12 Margoliash, E. and Walasek, O.F. (1967) Methods Enzymol. 10, 339–348
13 Van Gelder, B.F. (1978) Methods Enzymol. 53D, 125–128

14 Van Gelder, B.F. and Slater, E.C. (1962) Biochim. Biophys. Acta 58, 593–595
15 Veerman, E.C.I., Wilms, J., Casteleijn, G. and van Gelder, B.F. (1980) Biochim. Biophys. Acta 590, 117–127
16 Clark, W.M. (1928) The Determination of Hydrogen Ions, 3rd edn., Williams and Wilkins, Baltimore
17 Breuning, G. (1970) in Biochemical Experiments (Breuning, G., Criddle, R., Preiss, J. and Rudert, F., eds.), pp. 31–70, Wiley Interscience, New York
18 Barlow, G.H. and Margoliash, E. (1966) J. Biol. Chem. 241, 1473–1477
19 Margoliash, E., Barlow, G.H. and Byers, V. (1970) Nature 228, 723–726
20 Margalit, R. and Schejter, A. (1973) Eur. J. Biochem. 32, 492–499
21 Margalit, R. and Schejter, A. (1973) Eur. J. Biochem. 32, 500–505
22 Osheroff, N., Koppenol, W.H. and Margoliash, E. (1978) in Frontiers of Biological Energetics (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), Vol. I, pp. 439–449, Academic Press, New York
23 Ferguson-Miller, S., Brautigan, D.L. and Margoliash, E. (1979) in The Porphyrins (Dolphin, D., ed.), Vol. VII, pp. 149–239, Academic Press, New York
24 Osheroff, N., Borden, D., Koppenol, W.H. and Margoliash, E. (1980) J. Biol. Chem. 255, 1689–1697
25 Wilms, J., van Rijn, J.L.M.L. and van Gelder, B.F. (1980) Biochim. Biophys. Acta 593, 17–23
26 Greenwood, C., Agro, A.F., Guerrieri, P., Avigliano, L., Mondovi, B. and Antonini, E. (1971) Eur. J. Biochem. 23, 321–327
27 Chiang, Y.-L., Kaminsky, L.S. and King, T.E. (1976) J. Biol. Chem. 251, 29–36
28 Chiang, Y.-L. and King, T.E. (1979) J. Biol. Chem. 254, 1845–1853
29 King, T.E., Kuboyama, M. and Takemori, S. (1965) in Oxidases and Related Redox Systems (King, T.E., Mason, H.S. and Morrison, M., eds.), Vol. II, pp. 707–736, John Wiley and Sons, New York
30 King, T.E. (1979) in Structure and Function of Biomembranes (Yagi, K., ed.), pp. 149–165, Japanese Science Society Press, Tokyo
31 Taborsky, G. and McCollum, K. (1979) J. Biol. Chem. 254, 7069–7075
32 King, T.E. (1978) Methods Enzymol. 53D, 181–191
33 Chien, J.C.W. (1978) J. Phys. Chem. 82, 2158–2171