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MICROCALORIMETRIC STUDIES OF THE INTERACTIONS BETWEEN CYTOCHROMES *c* AND c_1 AND OF THEIR INTERACTIONS WITH PHOSPHOLIPIDS

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Thermotropic properties of purified cytochrome c_1 and cytochrome *c* have been studied by differential scanning calorimetry under various conditions. Both cytochromes exhibit a single endothermodenaturation peak in the differential scanning calorimetric thermogram. Thermodenaturation temperatures are ionic strength, pH, and redox state dependent. The ferrocytochromes are more stable toward thermodenaturation than the ferricytochromes. The enthalpy changes of thermodenaturation of ferro- and ferricytochrome c_1 are markedly dependent on the ionic strength of the solution. The effect of the ionic strength of solution on the enthalpy change of thermodenaturation of cytochrome *c* is rather insignificant. The formation of a complex between cytochromes *c* and c_1 at lower ionic strength causes a significant destabilization of the former and a slight stabilization of the latter. The destabilization of cytochrome *c* upon mixing with cytochrome c_1 was also observed at high ionic strength, under which conditions no stable complex was detected by physical separation. This suggests formation of a transient complex between these two cytochromes. When cytochrome *c* was complexed with phospholipids, no change in the thermodenaturation temperature was observed, but a great increase in the enthalpy change of thermodenaturation resulted.

Introduction

Significant information on protein-protein, protein-lipid, and enzyme-substrate interactions has been generated through the use of differential scanning calorimetry (DSC), a technique which is particularly useful in detecting structural changes, as reflected in the change of enthalpy and transition temperatures [1].

Recent improvements on the sensitivity of commercial DSC have advanced thermotropic studies from lipid phase transitions in biological membranes [2,3] to individual components in the multiprotein complex of membrane-associated [4] or soluble [5] systems.

Study of the changes in thermotropic properties upon the interaction of two protein components during complex formation or lipid-protein interaction using DSC has provided important information about the nature of the interaction and the involvement of specific functional groups. The involvement of ionizable groups on proteins in formation of a transient or a stable complex, can be detected by changes in the DSC thermogram when complex formation is carried out at different pH or salt concentrations. Although the isolated cytochromes *c* and c_1 of the mitochondrial respiratory chain have been shown to form a stable complex at low ionic strengths [6], the nature of the interaction between these two cytochromes at higher ionic strength and the physiological meaning of the complex formed is not clear. DSC study of these two cytochromes, individually or in combination, would provide important information on their interaction. A change similar to that observed with a stable complex, but to a lesser extent, would indi-

cate formation of a transient complex between these two cytochromes. Our present DSC study confirmed that cytochrome c_1 was, indeed, somewhat stabilized when it was complexed with cytochrome *c*. However, the behavior of cytochrome *c* was less expected, as it became destabilized when complexed with cytochrome c_1. The destabilization of cytochrome *c* upon mixing with cytochrome c_1 was also observed at high ionic strength, suggesting formation of a transient complex between these two cytochromes.

In this paper, we report the thermotropic properties of cytochromes *c* and c_1 and the c_1-*c* complex under various conditions, and the effect of phospholipids on the thermotropic properties of these cytochromes. Part of this report has been presented previously [7].

Materials and Methods

Preparation of cytochrome c_1 from the cytochrome b-c_1 III complex. The cytochrome *b*-c_1 III complex, prepared by the method of Yu and Yu [8] was dialyzed against 50 mM Tris-acetate buffer, pH 7.8, overnight, with one change of buffer. The dialyzed solution was then concentrated by ammonium sulfate precipitation (50% saturation). The precipitates were dissolved in 50 mM Tris-acetate buffer, pH 7.8 containing 1.5% Triton X-100 and 2 M urea to a protein concentration of approx. 10 mg per ml. The solution was then incubated at 0°C for 20 min, and frozen at −20°C for 2 h. The frozen solution was thawed and applied to a column which was packed with a 1:1 mixture of calcium phosphate [9] and cellulose, and equilibrated with 50 mM Tris-acetate buffer, pH 7.8 containing 2 M urea and 1.5% Triton X-100. The ratio of sample to column bed volume was about 1.0. The *b* cytochromes, iron-sulfur protein and other proteins were collected in the effluent, and cytochrome c_1 was retained on the column. The column was washed with three bed volumes of 50 mM phosphate buffer, pH 7.4, containing 0.5% sodium cholate, to replace the Triton X-100 in the column. Cytochrome c_1 was then eluted from the column with 0.2 M potassium phosphate buffer, pH 8.0, containing 1% sodium cholate. The collected cytochrome c_1 fraction was then frozen overnight in the presence of 0.1% β-mercaptoethanol at 20% ammonium sulfate saturation. After thawing, any precipitate formed was removed by centrifugation at 15000 rpm in a Beckman J2-21 centrifuge, rotor JA-20, and the supernatant solution was brought to 35% ammonium sulfate saturation with solid ammonium sulfate. After removing the precipitate by centrifugation, the solution was adjusted to pH 6.6 at 0°C with 1 M HCl, and the precipitate formed was removed by centrifugation. Purified cytochrome c_1, which was collected in the precipitate formed between pH 6.6 and 6.1, was dissolved in 50 mM phosphate buffer, pH 7.4, and kept in a deep freezer (−80°C) until use. About 40% of the cytochrome c_1 in the cytochrome *b*-c_1 III complex was recovered in the purified form. In the cytochrome c_1 thus obtained, the ratio between the absorptions at 418 nm and 278 nm equals 2.8. The preparation contains 28 nmol heme per mg protein. The intactness of the preparation is confirmed by its full ascorbate reducibility.

Preparation of a complex of cytochromes c_1 and c. It has been shown that purified cytochromes c_1 and *c* form a complex at low ionic strength [6]. Isolated cytochrome *c* (reduced or oxidized form) was dialyzed against 10 mM sodium/potassium phosphate buffer, pH 7.0, overnight, with two changes of buffer, to reduce the salt concentration. Cytochrome *c* was also dialyzed under the same conditions. The dialyzed cytochromes were then mixed in an equal molar ratio. For the purpose of checking the thermotropic properties of cytochromes c_1 and *c* under non-complex forming conditions, both proteins were adjusted to 0.1 M sodium/potassium phosphate buffer, pH 7.0, before they were mixed.

Preparation of cytochrome c_1-phospholipid and cytochrome c-phospholipid complexes. 2 mg of cytochrome c_1 as prepared, in 50 µl of 25 mM sodium/potassium phosphate buffer, pH 7.0, were mixed with 3 mg of asolectin in 1 ml of 1% sodium cholate solution in the same buffer and incubated at 0°C for 30 min before being dialyzed against 25 mM phosphate buffer, pH 7.0, for two days at 0°C with two changes of buffer. After dialysis, the cytochrome c_1-phospholipid complex was concentrated by ultrafiltration to a concentration of 450 µM cytochrome c_1. During this procedure, cytochrome c_1 became totally oxidized. Aliquots of

60 μl were used for DSC study. For preparation of the ferrocytochrome c_1-phospholipid complex, the concentrated ferricytochrome c_1-phospholipid complex was reduced with 1 mM sodium ascorbate. A similar procedure and the same protein : phospholipid ratio was used for preparation of the cytochrome *c*-phospholipid complex. However, after dialysis, the cytochrome *c*-phospholipid complex became precipitated. A suspension of the precipitate was used for the DSC study.

Differential scanning calorimetry. The thermotropic transition temperatures and the enthalpy changes of cytochrome c_1, cytochrome *c*, and their complex, under various conditions were determined with a Perkin-Elmer differential scanning calorimeter, model DSC-2C, equipped with a 0°C constant temperature bath. Scanning rates of 2.5 or 5 deg. per min (K/min) and sensitivities of either 0.1 or 0.2 mcal/s were routinely used. A 60 μl sample was placed in a steel large volume capsule (75 μl capacity) and the scanning was performed against the corresponding buffer system in the reference pan. The sample and reference capsules were weighed before and after each scan to ensure balance, and to detect possible leakage during scanning. The transition, or thermodenaturation temperature was taken at the peak of the endothermogram.

For determination of the enthalpy change, DSC was calibrated with Indium, and checked with the enthalpy change of the phase transition of a water suspension of dipalmitylphosphatidylcholine, whose enthalpy change of phase transition has been well documented [10], under conditions identical to those used for cytochromes c_1 and *c*.

Horse cytochrome *c*, type III and sodium cholate were obtained from Sigma and used without further treatment. Asolectin was a product of Associated Concentrate.

Results and Discussion

Thermotropic properties of cytochromes c and c_1

Fig. 1 shows the differential scanning calorimetry thermograms for the ferro- and ferricytochromes *c* and c_1 in the presence of 25 mM sodium/potassium phosphate buffer at pH 7.0. A relatively sharp transition or thermodenaturation peak was observed for both ferri- and ferrocytochrome *c*. Ferrocytochrome *c* is, however, much more stable than ferricytochrome *c*. The former underwent thermodenaturation at 376 K, whereas the latter denatured at 357.5 K. A small shoulder in the DSC thermogram of ferrocytochrome *c* at 360 K is due to partial oxidation of ferrocytochrome *c* at higher temperatures during scanning. Like cytochrome *c*, cytochrome c_1 shows a single endothermotransition peak in the DSC thermogram. The thermodenaturation peaks for ferri- and ferrocytochrome c_1 were found at 335.5 K and 338.5 K, respectively, in 25 mM phosphate buffer at pH 7.0. At the given conditions, the thermodenaturation temperatures of ferri- and ferrocytochrome c_1 differ by only 3 K, which is much less than the difference between ferri- and ferrocytochrome *c* (18 K). This indicates that the structural difference between the ferro- and ferricytochromes *c* is more distinct than that between the ferro- and ferricytochromes c_1. The peak width of the thermogram of cytochrome c_1 is dependent on the purity of the cytochrome c_1 preparation. The sharp peak in the thermogram shown in Fig. 1 indicates that the cytochrome c_1 used is in a highly purified form, even though the purified protein still contains some small molecular weight subunit (less than a stoichiometric amount to cytochrome c_1). This subunit, generally known as the cytochrome

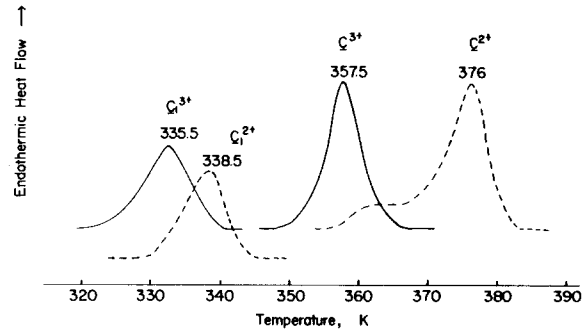

Fig. 1. DSC thermogram of ferri- and ferrocytochromes *c* and c_1 in 25 mM sodium/potassium phosphate buffer, pH 7.0. The concentration of cytochromes *c* and c_1 were 0.67 mM and 0.32 mM, respectively. 60-μl samples were scanned against the same volume of buffer with a heating rate of 5 K/min at a sensitivity of 0.1 mcal/s. The recorder chart speed was 100 s/inch. To ensure that the sample was in the fully reduced or oxidized state, 1 mM sodium ascorbate or potassium ferricyanide were included in the sample and buffer solutions.

c_1 associated polypeptide, has a molecular weight of less than 15000. The requirement for this subunit in the electron transfer reaction is currently the subject of controversy [11–13].

Although cytochromes c_1 and c are both known to be non-autoxidizable, the reduced form of both proteins usually contains a small amount of the oxidized form. To ensure that the preparation used was in the fully reduced state, 1 mM sodium ascorbate was routinely included in the sample for DSC study. In the same manner, 1 mM potassium ferricyanide was used in the ferric form samples. Sodium ascorbate and potassium ferricyanide, at the concentrations used, showed no thermotransition in the region of interest. No effect on the DSC thermograms of cytochromes c_1 or c was detected when these reagents were used.

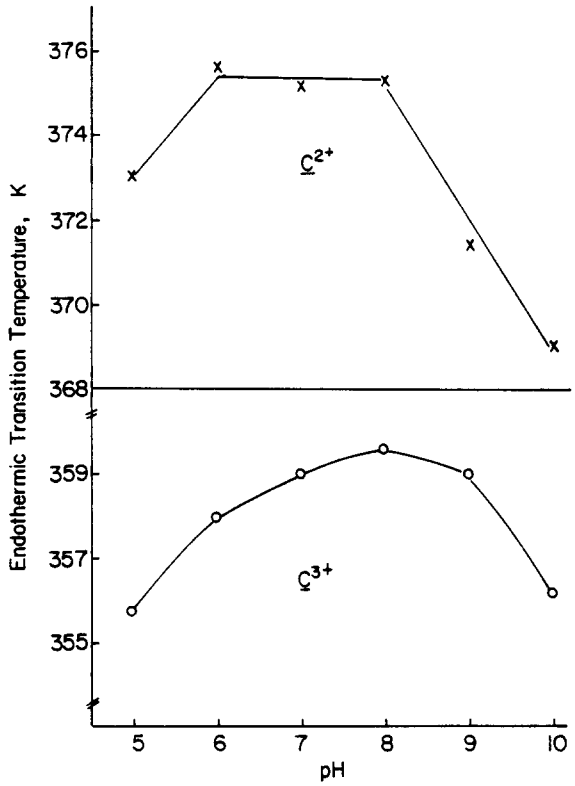

Fig. 2. pH dependence of the thermodenaturation of cytochrome c. The DSC settings were identical to those given in Fig. 1. The various pH conditions were obtained by diluting the concentrated cytochrome c in a buffer mixture composed of 50 mM acetate, phosphate and glycine, at the indicated pH. The concentration of cytochrome c was 1 mM.

Fig. 2 shows the effect of pH on the thermodenaturation temperature of cytochrome c. To avoid a possible effect from different species of ions on the thermodenaturation of cytochrome c or cytochrome c_1, a mixture of three buffer systems was used. The buffer mixture contains 50 mM each acetate, phosphate and glycine. This buffer mixture also has the benefit of a very low temperature coefficient below pH 8.0, with a change in pH of less than 0.05 pH unit with a change in temperature from room temperature to 90°C. At pH 10, however, the pH dropped about 0.8 pH unit from room temperature to 90°C. The pH values indicated in Fig. 2 and Fig. 3 are the pH values at room temperature, and no correction in the pH for temperature was made. The buffer mixture also contained either 1 mM sodium ascorbate or 2 mM potassium ferricyanide to ensure that cytochrome c was in the fully reduced or oxidized form. Cytochrome c was stable in both redox states as indicated by the thermodenaturation temperature. Although both ferro- and ferricytochrome c showed

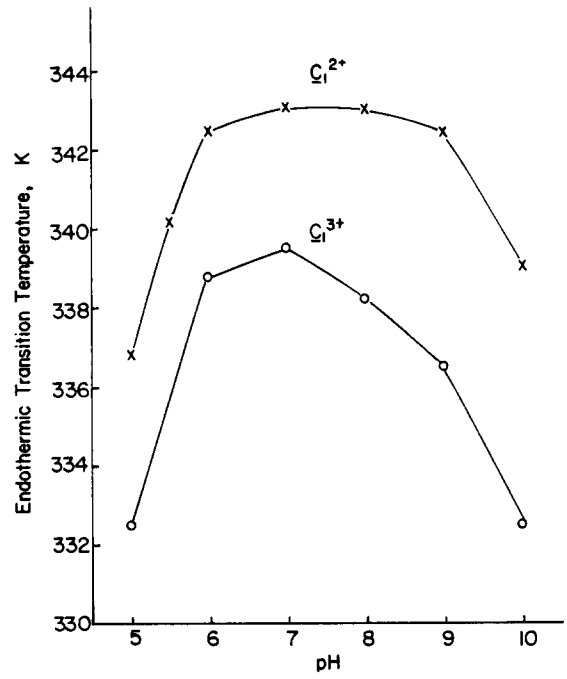

Fig. 3. pH dependence of the thermodenaturation of cytochrome c_1. The experimental conditions were identical to those in Fig. 2, except that the buffer concentration used was 0.1 M, and cytochrome c_1 was 0.43 mM.

a similar pH dependency, the optimum pH of ferrocytochrome *c* was shifted slightly to higher pH compared to ferricytochrome *c*. The pH dependence of the thermotransition temperature of cytochrome *c* between pH 1 and 5 has been reported [14]. The effect of pH on the thermodenaturation of cytochrome *c* was also studied using the same buffer system but at higher ionic strength (0.1 M). The pH profile of the thermodenaturation temperatures showed that cytochrome c_1 is also relatively stable at neutral pH (see Fig. 3). When the pH of the solution was higher than 7, ferricytochrome c_1 became less stable and underwent thermodenaturation at lowered temperatures. However, very little change in the thermodenaturation temperature was observed in ferro-cytochrome c_1 until the pH of the solution was higher than 9 or lower than 6. It should be mentioned here that the thermotransition of cytochrome *c*, but not that of cytochrome c_1, became reversible when the pH of the solution was lower than 5. A similar reversible thermal unfolding of ferricytochrome *c* at pH lower than 4 has been observed by Tsong [15]. He reported that ferricytochrome *c* at pH 4 in 0.015 N sodium perchlorate and 0.005 M sodium acetate buffer, underwent reversible thermo-unfolding at 340 K with an enthalpy change of transition of 35 kcal/mol, as deduced from the absorption change of the sample at various temperatures. However, using the present method (0.125 M sodium acetate, pH 4.0), the reversible thermotransition temperature of ferricytochrome *c* was at 350 K with an enthalpy change of 60 kcal/mol. Part of these discrepancies may be a result of the different buffer systems employed.

The thermodenaturation of cytochrome *c* is also affected by the ionic strength of the solution. Fig. 4 shows the effect of salt concentrations on the thermodenaturation temperatures of ferricytochrome *c* at pH 7.0. The effect of ionic strength on the thermodenaturation temperature of ferricytochrome *c* is more profound at low salt concentration and is ionic species dependent. When ferricytochrome *c* was dissolved in 20 mM sodium/potassium phosphate buffer, pH 7.0, the low concentrations of KCl stabilized cytochrome *c* significantly. However, the thermodenaturation temperature of ferricytochrome *c* decreased as the

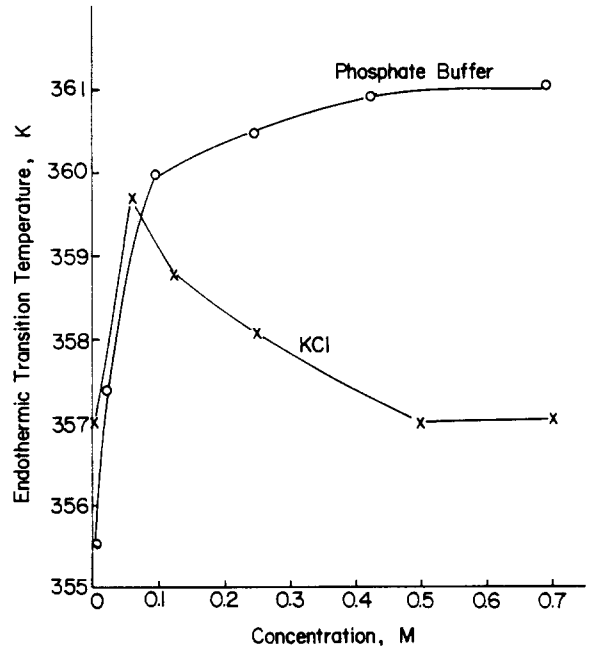

Fig. 4. Effect of ionic strength on the thermodenaturation temperature of ferricytochrome *c*. × ——— ×, cytochrome *c* dissolved in the indicated concentrations of KCl, containing 20 mM sodium/potassium phosphate buffer, pH 7.0; ○ ——— ○, cytochrome *c* dissolved in the indicated concentrations of sodium/potassium phosphate buffer, pH 7.0. The concentration of ferricytochrome *c* was 1 mM. The DSC settings were the same as described in Fig. 1.

KCl concentration increased from 75 to 500 mM. When only phosphate buffer was used in the system the thermodenaturation temperature of ferricytochrome *c* increased as the buffer concentration increased. A 5.3 K increase was observed with a change from 10 mM phosphate to 0.5 M phosphate buffer. These results suggest that the nature of the binding between phosphate and ferricytochrome *c* and the binding between chloride and ferricytochrome *c* are different. The latter may bind to a cationic group which may otherwise be involved in hydrogen bonding. The effect of ionic strength on the thermodenaturation temperature of ferrocytochrome *c* was negligible. In contrast to the effect of KCl on ferricytochrome *c*, the thermostability of cytochrome c_1 increased as the KCl concentration increased, up to 200 mM in the presence of 20 mM sodium/potassium phosphate buffer, pH 7.0. A similar stabilizing effect was observed when only phosphate buffer was used. At

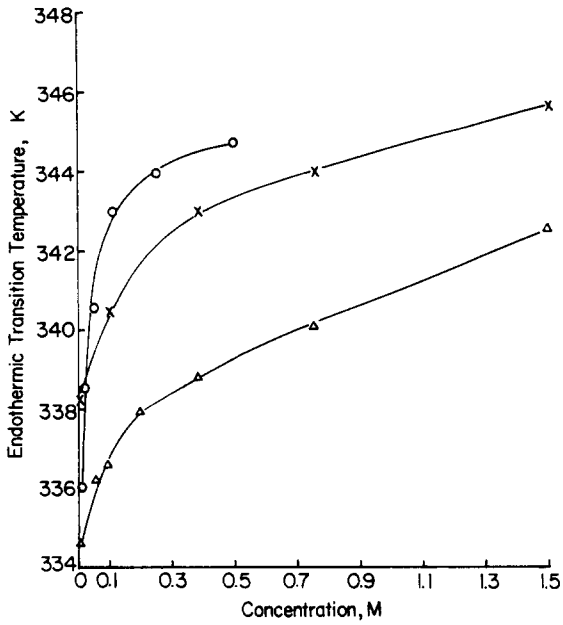

Fig. 5. Effect of ionic strength on the thermodenaturation temperatures of cytochromes c_1. Experimental conditions identical to those given in Fig. 4 were used, except the concentration of cytochrome c_1 was 0.32 mM. ×———×, ferrocytochrome c_1 dissolved in the indicated concentration of KCl, containing 20 mM sodium/potassium phosphate buffer, pH 7.0; △———△, ferricytochrome c_1 dissolved in the indicated concentration of KCl, containing 20 mM sodium/potassium phosphate buffer, pH 7.0; ○———○, ferrocytochrome c_1 dissolved in the indicated concentration of sodium/potassium phosphate buffer, pH 7.0.

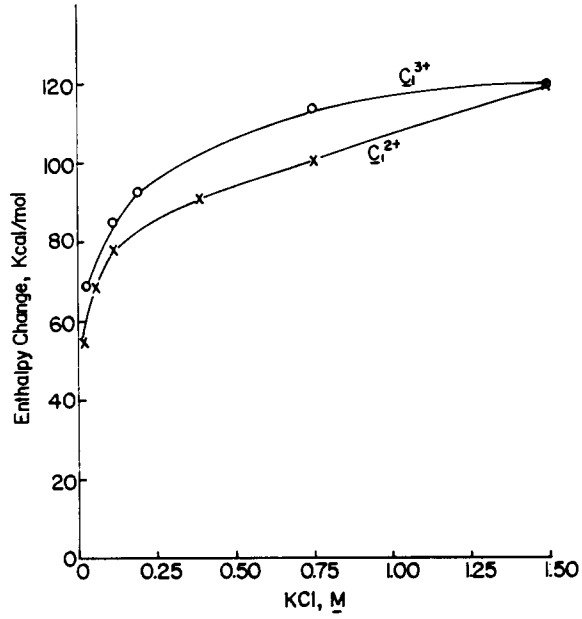

Fig. 6. Effect of the salt concentration on the change of enthalpy of the thermodenaturation of cytochrome c_1. The enthalpy change of thermodenaturation was obtained from integration of the endothermotransition peaks obtained in Fig. 5.

salt concentrations (either KCl or phosphate buffer) higher than 200 mM, the change in thermodenaturation temperatures with increasing salt concentrations is much less (see Fig. 5). A difference of more than 3 K was observed for both ferri- and ferrocytochrome c_1 when the solution was changed from 20 mM phosphate buffer to 20 mM phosphate buffer containing 250 mM KCl. Although phosphate buffer showed a slightly greater stabilizing effect on ferrocytochrome c_1, the difference between phosphate and KCl was rather small.

Determination of change in enthalpy of cytochrome c_1

The enthalpy change involved in thermodenaturation of the sample can be calculated from the area under the endothermogram peak. Fig. 6 shows the salt dependence of the enthalpy change of thermodenaturation of cytochrome c_1. Ferrocytochrome c_1 undergoes thermodenaturation with an enthalpy change of 55 kcal/mol at low ionic strength (25 mM phosphate buffer) and 120 kcal/mol at higher ionic strength (1.5 M KCl in 20 mM phosphate buffer). Although the thermodenaturation temperature of ferricytochrome c_1 is about 3 K lower than that of the ferroprotein, a similar enthalpy change for denaturation was observed in both. Ferricytochrome c_1 also showed a salt concentration dependent enthalpy change. It is of interest to note that the enthalpy change for thermodenaturation of ferrocytochrome c_1 is quite similar to the activation energy calculated from the solvent denaturation of ferrocytochrome c_1 [16], especially considering the large difference in protein concentration involved between the two systems. The Arrhenius activation energy for solvent denaturation of cytochrome c_1 of 46 kcal/mol was obtained from the autoxidation rate induced by solvent at various temperatures, with cytochrome c_1 at a concentration of 13 μM, in 5.6% propanol. The enthalpy change of cytochrome *c* thermode-

naturation is about 60 kcal/mol and is not salt concentration dependent.

The thermotropic properties of the cytochrome c_1 and cytochrome c complex

It is known that isolated cytochromes c_1 and c form a stable stoichiometric complex at low ionic strength (10 mM phosphate buffer, at neutral pH), which is separable from the uncomplexed cytochrome c_1 or cytochrome c by molecular sieving column chromatography. DSC studies of a mixture of cytochromes c_1 and c in 10 mM phosphate buffer, pH 7.0, confirmed the formation of a complex. The formation of a complex was evident from the change in the DSC pattern, as it stabilized cytochrome c_1, but caused cytochrome c to become more labile toward thermodenaturation. The formation of the cytochromes c_1 and c complex also resulted in an increase of about 70% in the enthalpy change. Fig. 7 shows the DSC thermograms of an equimolar mixture of ferricytochromes c_1 and c in 10 mM (Fig. 7A) and 100 mM phosphate buffer, pH 7.0 (Fig. 7B). The endothermic transition temperature of the ferricytochromes c-c_1 complex is about 15 K lower than that of free ferrocytochrome c, and is about 4 K higher than that of free ferricytochrome c_1. Although no stable complex can be formed at high ionic strengths (100 mM phosphate), an interaction between these two cytochromes still exists (see Fig. 7B). Under such conditions, cytochrome c was still destabilized upon mixing with ferrocytochrome c_1, as it denatured at 350 K. However, the change in the thermodenaturation temperature of ferricytochrome c_1 upon mixing with ferricytochrome c was rather small.

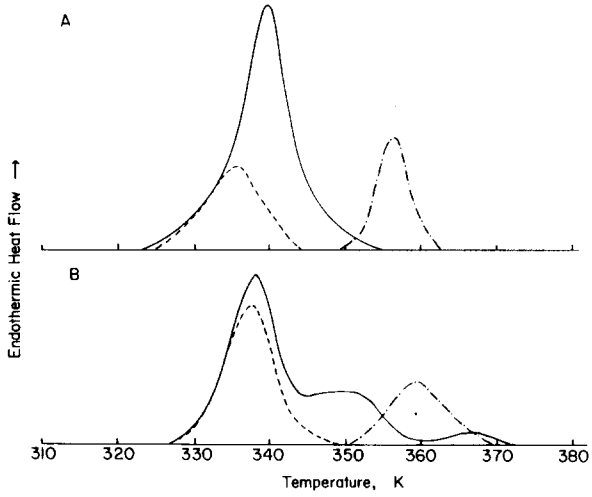

Fig. 7. Effect of the complex formation on the thermodenaturation of ferricytochromes c_1 and c. The solid lines (———) represent an equal molar concentration (0.32 mM) of ferricytochromes c_1 and c mixed in (A), 10 mM, and (B) 100 mM sodium/potassium phosphate buffer, pH 7.0, and incubated at 0°C for 30 min before the DSC thermograms were measured. Other conditions were the same as those given in Fig. 1. The broken line (— — —) and dashed line (·–·–·) represent cytochromes c_1 and c, respectively, measured separately under the same conditions.

This observed change in thermotropic properties of the mixture of ferricytochromes c_1 and c at high ionic strength could be due to the formation of a transient complex between these two cytochromes through electrostatic interaction. This suggests that a transient complex may form between these two cytochromes during electron transfer, because the salt concentration under physiological conditions is higher than 10 mM. The transient complex formed between ferrocytochrome c_1 and ferricytochrome c would be expected to be more favorable in the in vivo electron transfer process than the transient complex formed by both ferricytochromes. Unfortunately, such a transient complex of ferrocytochrome c_1 and ferricytochrome c cannot be detected by DSC, as the electron transfer from ferrocytochrome c_1 to ferricytochrome c takes place in less than a microsecond [11,17]. The study of the effect of ionic strength on the rate of electron transfer between the isolated cytochromes c_1 and c [17] has revealed that the rate of electron transfer decreased only slightly as salt concentration increased, up to 0.15 M. A more profound decrease in the rate of electron transfer was observed at salt concentrations higher than 0.15 M, suggesting that at salt concentrations higher than 0.15 M, the transient complex formation between these two cytochromes is greatly hindered. Although the DSC thermogram of the interaction between ferrocytochromes c_1 and c is complicated by the presence of some ferricytochrome complex, resulting from partial autoxidation at higher temperatures, the stabilization and destabilization of cytochrome c_1 and c upon complex formation, respectively, were still observed. No effort was made to perform the DSC of ferro-

cytochromes c_1 and c under anaerobic conditions, which would eliminate the complication of autoxidation from the thermogram.

Effect of phospholipids on the thermotropic properties of cytochromes c_1 and c

It has been shown that when isolated cytochrome c_1 is mixed with phospholipid vesicles, a complex between phospholipid and cytochrome c_1 is formed [6]. One molecule of cytochrome c_1 is incorporated with about 200 molecules of phospholipids. The physical interaction of cytochrome c_1 and phospholipids is also evident from the change of molar ellipticity of cytochrome c_1 in the ultraviolet light region of the circular dichroism spectra upon interaction [6]. Therefore, phospholipids might be expected to exert an effect on the thermotropic properties of cytochrome c_1. When cytochrome c_1 was embedded in phospholipid vesicles, no significant change in the thermodenaturation temperature was observed in either ferro- or ferricytochrome c_1, but a significant increase in the enthalpy change was observed in both cases (see Table I). When ferrocytochrome c_1 was incorporated into phospholipid vesicles by the method described in Materials and Methods, it became oxidized during the process of preparation. It could be fully reduced by sodium ascorbate, indicating that the cytochrome c_1 in the prepared ferricytochrome c_1-phospholipid complex was not denatured by the procedure employed. The ferrocytochrome c_1-phospholipid complex can be obtained by simply reducing the ferricytochrome c_1-phospholipid complex with sodium ascorbate. The effect of phospholipids on the thermotropic properties of cytochrome c_1 was observed only when cytochrome c_1 was incorporated into phospholipid vesicles by the method described. If cytochrome c_1 was simply incubated with the preformed phospholipid vesicles, no effect on the thermotropic properties of cytochrome c_1 was observed, indicating that cytochrome c_1 as prepared is in the aggregated pentamer form, and could not be incorporated into the phospholipid vesicles. The effect of phospholipids on the thermotropic properties of cytochrome c was expected to be less profound than that on cytochrome c_1 or cytochrome oxidase, because cytochrome c is only loosely attached to the surface of the membrane, through mainly ionic interactions. It was surprising, therefore, to observe that the interaction between cytochrome c and phospholipids is very complex. When ferricytochrome c was mixed with phospholipids in the presence of 1% sodium cholate and followed by dialysis, the cytochrome became precipitated, and the DSC thermogram of the dialyzed cytochrome c-phospholipid complex showed a large exothermotransition peak at 352 K with a minor endothermotransition peak at 325 K. Phospholipid vesicles alone, however, showed no thermotransition in the temperature range measured. Further investigation is needed in order to understand the nature of the interaction between cytochrome c and phospholipids.

TABLE I

THERMAL DENATURATION TEMPERATURES AND CHANGE IN ENTHALPY OF CYTOCHROME c_1 AND ITS PHOSPHOLIPID COMPLEX IN 25 mM SODIUM/POTASSIUM PHOSPHATE, pH 7.06

Preparation	T_m (K)	H (kcal/mol)
Ferrocytochrome c_1	338.5	55
Ferrocytochrome c_1/phospholipids	338.5	97
Ferricytochrome c_1	335.5	67
Ferricytochrome c_1/phospholipids	335.5	149

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