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USE OF SPECIFIC TRIFLUOROACETYLATION OF LYSINE RESIDUES IN CYTOCHROME *c* TO STUDY THE REACTION WITH CYTOCHROME b_5, CYTOCHROME c_1, AND CYTOCHROME OXIDASE

MICHAEL B. SMITH, JACQUELINE STONEHUERNER, AFTAB J. AHMED, NICOLE STAUDENMAYER and FRANCIS MILLETT

Department of Chemistry, University of Arkansas, Fayetteville, AR 72701 (U.S.A.)

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Summary

The preparation, purification, and characterization of four new derivatives of cytochrome *c* trifluoroacetylated at lysines 72, 79, 87, and 88 are reported. The redox reaction rates of these derivatives with cytochrome b_5, cytochrome c_1 and cytochrome oxidase indicated that the interaction domain on cytochrome *c* for all three proteins involves the lysines immediately surrounding the heme crevice. Modification of lysines 72, 79, and 87 had a large effect on the rate of all three reactions, while modification of lysine 88 had a very small effect. Even though lysines 87 and 88 are adjacent to one another, lysine 87 is at the top left of the heme crevice oriented towards the front of cytochrome *c*, while lysine 88 is oriented more towards the back. Since the interaction sites for cytochrome c_1 and cytochrome oxidase are essentially identical, cytochrome *c* probably undergoes some type of rotational diffusion during electron transport.

Introduction

Specific chemical modification of cytochrome *c* lysines has recently proven to be a valuable tool in studying the mechanism of electron transport from cytochrome c_1 to cytochrome *c* oxidase in the mitochondrial respiratory chain, as

Abbreviations: $CF_3PhNHCO$-, trifluoromethylphenylcarbamoyl; Mops, 4-morpholinopropanesulfonic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride; Tos-PheCH_2Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone.

well as the reaction of cytochrome *c* with other enzymes such as cytochrome *c* peroxidase, sulfite oxidase, and cytochrome b_5 [1—10]. It has been found that the rate of the reaction of cytochrome oxidase with cytochrome *c* is decreased upon modification of the lysines immediately surrounding the heme crevice with trifluoroacetyl [9,10], trifluoromethylphenylcarbamyl ($CF_3PhNHCO$-) [6], or carboxydinitrophenyl [3] groups. Since the rate of the reaction of cytochrome c_1 with cytochrome *c* is equally affected by these modifications [1,8], it has been suggested that both cytochrome c_1 and cytochrome oxidase react with cytochrome *c* at the front of the heme crevice, and some type of rotational diffusion might be involved. The reaction site was also found to be very similar for cytochrome b_5 [5], cytochrome *c* peroxidase [4], and sulfite oxidase [2]. We report here the preparation and characterization of four singly trifluoroacetylated derivatives of cytochrome *c*. We have studied the kinetics of the four new derivatives with cytochrome *c* oxidase, succinate-cytochrome *c* reductase and cytochrome b_5.

Materials and Methods

Materials. Horse heart cytochrome *c* (type VI), Mops, sodium cholate, sodium deoxycholate, sodium ascorbate and TMPD were obtained from Sigma Chemical Company. Ethyl thioltrifluoroacetate was obtained from Pierce Chemical Co. Tris was obtained from Schwarz/Mann. Tos-PheCH_2Cl-treated trypsin was obtained from Worthington Biochemical Corporation.

Trifluoroacetylated cytochrome c derivatives. The derivatives were prepared according to the method previously published for the trifluoroacetyl-Lys-22 and trifluoroacetyl-Lys-25 derivatives [10]. Cytochrome *c* (250 mg) was dissolved in 2 ml of 0.14 M phosphate (pH 7.2) and adjusted to pH 8.0 with 1 M NaOH. Ethylthioltrifluoroacetate (7 μl) was added to the rapidly stirred solutin at room temperature, and the pH was continually maintained at 8.0 by addition of 1 M NaOH with a micrometer syringe. After 30 min a second 7 μl aliquot of reagent was added. After a second 30 min period base consumption had ceased, and the solution was passed through a small Bio-Gel P-4 column equilibrated with 0.03 M ammonium phosphate buffer, pH 7.2, to remove ethyl mercaptan and trifluoroacetate. The cytochrome was then applied to a 2 × 70 cm Bio-Rex 70 column equilibrated with 0.14 M ammonium phosphate, pH 7.2. The column was eluted with 0.14 M ammonium phosphate buffer at 25 ml/h. The chromatogram is shown in Fig. 1. The fractions for each peak were pooled (from half-height to half-height), concentrated on a small column of Bio-Rex 70, eluted in a small volume of 0.5 M ammonium phosphate, pH 7.2, and finally desalted by passing through a small Bio-Gel P-4 column equilibrated with 0.02 M sodium phosphate buffer, pH 6.0. The fractions were repurified on a 1.5 × 10 cm column of Whatman CM 32 carboxymethylcellulose eluted with 0.07 to 0.08 M phosphate buffer, pH 6.0, at a flow rate of 25 ml/h (Fig. 2). The fractions were pooled as shown in Fig. 2, and concentrated and desalted with 0.02 M sodium phosphate buffer, pH 7.2. They were never lyophilized at any point in the purification.

^{19}F-NMR of derivatives. ^{19}F-NMR spectra of the trifluoroacetylated cytochrome *c* derivatives were obtained in the oxidized state at 84 MHz on a Bruker

HFX 90 spectrometer with a Nicolet NMR-80 Fourier transform accessory, using 5-mm NMR tubes. The only fractions which showed single ^{19}F-NMR resonances of greater than 95% purity were 3, 4A, 5A, 5B, 6, 8B, and 10 C. Fraction 3 has been previously identified as trifluoroacetyl-Lys 22 cytochrome [10], while fraction 5A was found to be identical to fraction 6, previously identified as trifluoroacetyl-Lys 25 cytochrome *c* [10]. The ^{19}F-NMR spectra of the new singly labeled fractions 4A, 5B, 8B, and 10C are shown in Fig. 3. None of the other fractions could be purified to the 95% level by further chromatography on CM 32 carboxymethylcellulose.

Peptide mapping. Rechromatographed singly modified cytochrome *c* derivatives at a concentration of 5 mg/ml in 0.05 M Tris buffer, pH 7.5, were digested with Tos-PheCH_2Cl-treated trypsin (0.5 mg/ml) at 37°C for 3 h. Peptide mapping was carried out on a 0.9 × 23 cm column of Aminex A-5 ion-exchange resin using a modified Phoenix amino acid analyzer with stream splitting according to the previously published procedure [9,10]. It was found that fractions 4A, 5B, 8B, and 10C contained singly modified cytochrome *c* derivatives trifluoroacetylated at lysines 79, 88, 72, and 87, respectively. The purity was greater than 95% in each case. Fractions 4B, 8A, and 10A were found to be singly trifluoroacetylated at lysines 7, 8, and 86, respectively but in each case the purity was only about 80%. These results are summarized in Table I.

Enzyme kinetics. The source of the cytochrome b_5 activity was a beef liver microsomal fraction [12]. The microsomal preparation was treated with 5% deoxycholate (1 mg/mg of protein) and diluted with buffer immediately prior to use. The assay medium contained 0.5 to 5.0 μM ferricytochrome *c*, 0.10 M Tris, pH 7.5, and 50 μM NADH. The rate of reduction of cytochrome *c* following addition of microsomes was followed on a Cary 14 spectrophotometer at 420 nm using 1-cm cells.

The source of the cytochrome *c* oxidase was cytochrome *c* depleted Keilin-Hartree particles [13,14]. Protein concentration was determined by the biuret method after solubilization of the Keilin-Hartree particles [15]. The cytochrome oxidase activity was measured polarographically with a Gilson Model KM Clark Electrode cell using the ascorbate — TMPD system [14]. Assays were run in 60 mM Mops, pH 7.5, 200 mM sucrose, 10 mM sodium ascorbate (from a stock solution of 0.5 M sodium ascorbate containing 1 mM EDTA) and 1 mM TMPD. The Keilin-Hartree particles were incubated with 5% deoxycholate (1 mg/mg protein) for 15 min and diluted with the buffer used in the assay. The low baseline rate consumption of oxygen was subtracted from the rate observed after addition of cytochrome *c*.

Beef heart succinate-cytochrome *c* reductase was prepared by a slight modification of the method of Yu et al. [16]. The extraction was done at pH 8.0 rather than pH 7.4 because the cytochrome reductase-cytochrome oxidase complex has been found to be much weaker at the higher pH [17]. The reductase preparation was treated with 5% cholate (1 mg/mg of protein) and diluted immediately prior to use with 0.2 M Tris buffer, pH 7.5, containing 10 mM succinate. The rate of reduction of cytochrome *c* following the addition of reductase was monitored at 420 nm on a Cary 14 spectrophotometer. The assay medium contained 0.1 to 12 μM ferricytochrome *c*, 10 mM succinate, and 0.2 M Tris-HCl, pH 7.5.

Results

Preparation of specifically trifluoroacetylated cytochrome c derivatives

We have previously described the reaction of cytochrome *c* with ethylthiol-trifluoroacetate and the separation of the resulting derivatives using Biorex-70 cation exchange chromatography (Fig. 1) [10]. In the present study fractions separated by Biorex-70 chromatography were further purified on Whatman CM 32 carboxymethylcellulose (Fig. 2) to yield four new singly trifluoroacetylated cytochrome *c* derivatives of at least 95% purity, as summarized in Table I. The ^{19}F-NMR chemical shifts of these derivatives were well separated from one another (Fig. 3), probably because of different environments on the surface of cytochrome *c*.

Enzyme kinetics. The NADH-linked reduction of cytochrome *c* by liver microsomes results from the NADH-cytochrome b_5 reductase-cytochrome b_5 system [12]. We have previously established that the cytochrome b_5-cytochrome *c* step is rate-limiting only at low cytochrome *c* concentrations, and have found that V/K_m is a true measure of the cytochrome b_5 reduction of cytochrome *c* [5]. At low cytochrome *c* concentrations the time course for reduction was first order in cytochrome *c*, with a rate constant equal to V/K_m. Eadie-Hofstee plots are shown in Fig. 4 for the reaction of the four derivatives and native cytochrome *c* with cytochrome b_5. The value of V remained constant, but V/K_m was decreased by modification of the lysines surrounding the heme crevice, lysines 72, 79, and 87. The rate of reaction was unaffected by modification of Lys-88 at the upper left side of the heme crevice even though modification of the adjacent Lys-87 produced a 1.5-fold decrease in rate.

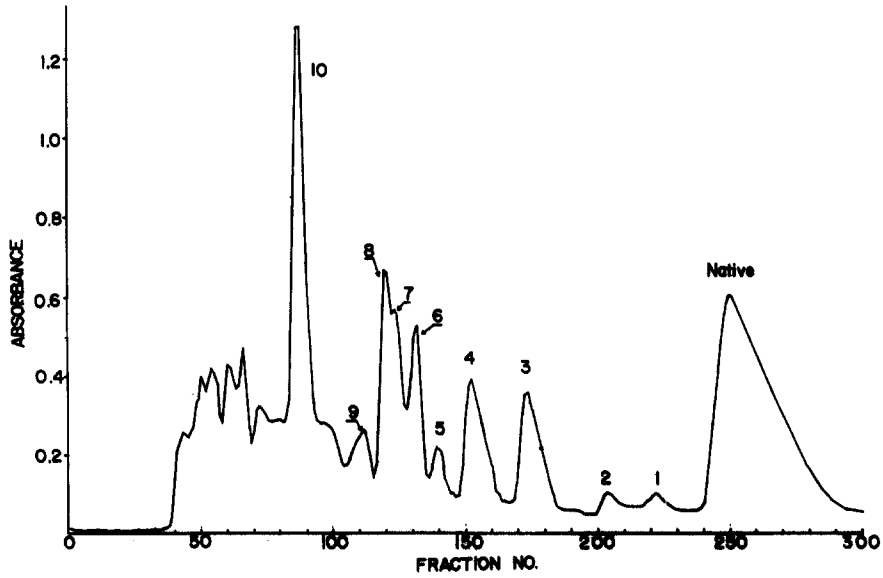


Fig. 1. Chromatogram of 250 mg of a crude reaction mixture of trifluoroacetylated cytochrome c eluted on a 2 × 70 cm Biorex 70 column with 0.14 M ammonium phosphate, pH 7.2, at a flow rate of 25 ml/h. The fraction size was 4 ml and the absorbance was measured at 430 nm.

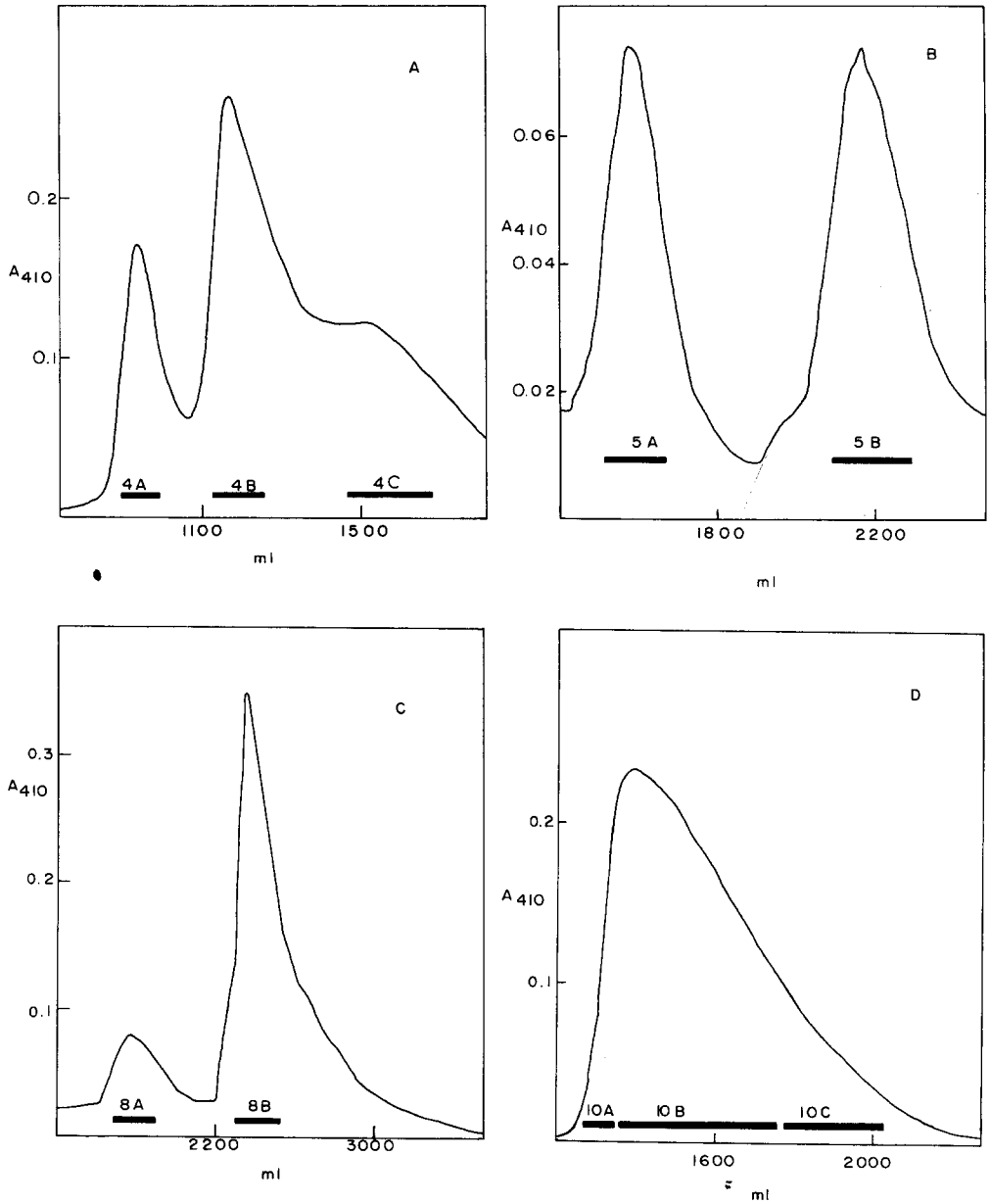


Fig. 2. Chromatograms from individual fractions shown in Fig. 1 eluted on a 1.5 X 10 cm Whatman CM 32 carboxymethyl cellulose column with 0.08 M phosphate buffer, pH 6.0 at a flow rate of 25 ml/h. (A) Fraction 4 of Fig. 1. (B) Fraction 5. (C) Fraction 8. (D) Fraction 10. The solid bars indicate how the fractions were pooled for further study.

The reduction of cytochrome *c* by succinate-cytochrome *c* reductase is rate-limited by the cytochrome c_1-cytochrome *c* step only at low cytochrome *c* concentrations where the time course is first order in cytochrome *c*, and thus V/K_m is the relevant kinetic parameter [1]. Modification of lysines 72, 79, and

TABLE I

PROPERTIES OF TRIFLUOROACETYLATED CYTOCHROME *c* DERIVATIVES

Fraction	Lysine modified	^{19}F-NMR chemical shift *	Purity (%) **
3 ***	22	—0.191	+95
4A	79	0.551	+95
4B	7	—0.093	80
5B	88	0.168	+95
5A,6 ***	25	0.142	+95
8A	25	0.192	80
8B	72	—0.090	+95
10A	86	0.215	80
10C	87	0.253	+95

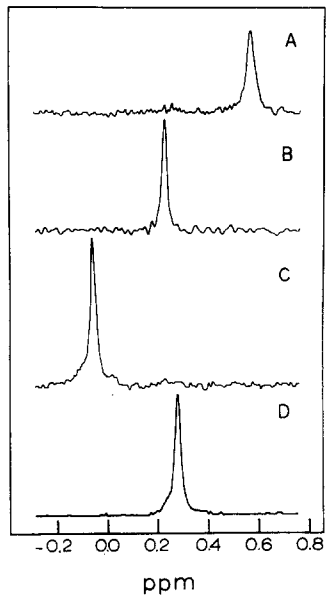
* ^{19}F-NMR chemical shift in parts per million from trifluoroacetate. The ^{19}F chemical shift was obtained for the oxidized protein in 0.5 M ammonium phosphate, pH 7.2 at 20°C.

** Determined by both ^{19}F-NMR and peptide mapping.

*** Ref. 10.

87 decreased the value of V/K_m significantly, while modification of lysine 88 caused only a small decrease (Fig. 5).

The reaction of cytochrome *c* oxidase with cytochrome *c* was studied at



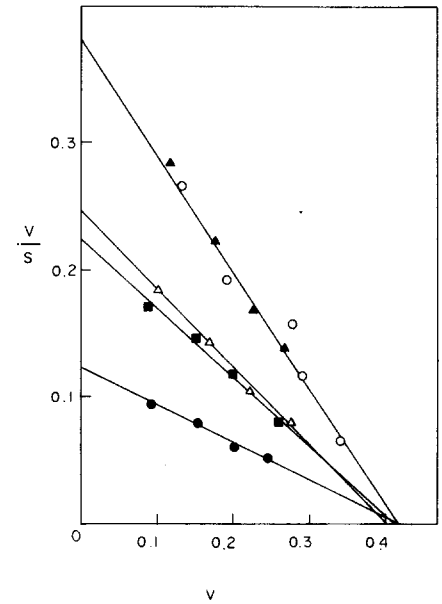


Fig. 3. ^{19}F-NMR spectra of trifluoroacetylated cytochrome *c* derivatives in 0.5 M ammonium phosphate, pH 7.2. Each spectrum is the Fourier transform of 1000 0.5-s free induction decays. The chemical shifts are in parts per million from trifluoroacetate. (A) Fraction 4A (CF_3CO-Lys-79). (B) Fraction 5B (CF_3CO-Lys-88). (C) Fraction 8B (CF_3CO-Lys-72). (D) Fraction 10C (CF_3CO-Lys-87).

Fig. 4. Rate of reduction of cytochrome *c* derivatives by cytochrome b_5. The initial velocity, v, was measured in μM cytochrome *c* reduced per minute following addition of beef liver microsomes to a 1 ml solution containing 0.3 to 5.0 μM cytochrome *c*, 50 μM NADH in 0.10 M Tris-HCl buffer, pH 7.5. The final cytochrome b_5 concentration was 0.12 nM, and the reduction rate was monitored at 420 nm, 25°C. The symbols used for cytochrome *c* trifluoroacetylated at the indicated lysing are: unmodified native (○), 72 (●), 79 (■), 87 (△), and 88 (▲).

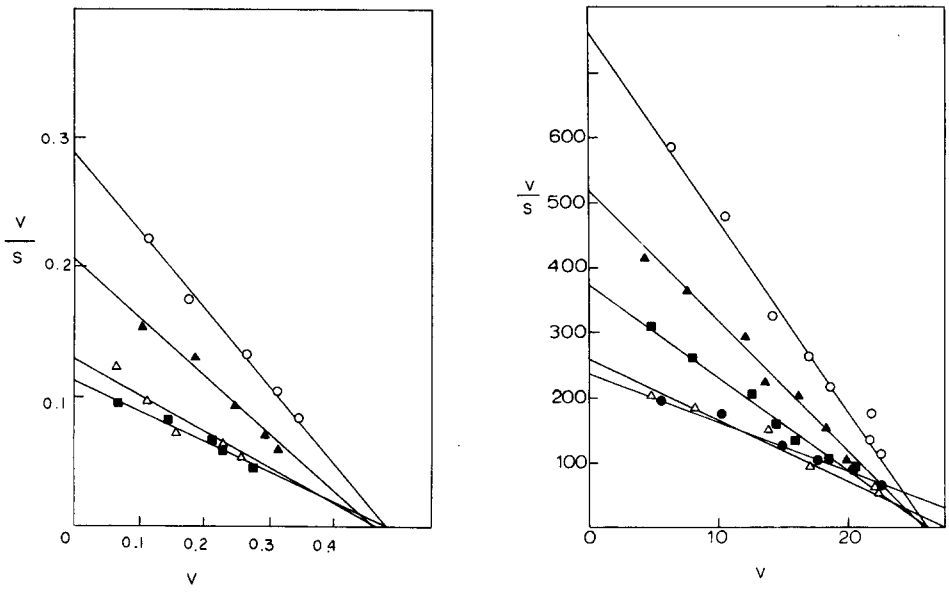


Fig. 5. The rate of reduction of cytochrome *c* derivatives by cytochrome c_1. The initial velocity, v, was measured in μM cytochrome *c* reduced per min following addition of succinate-cytochrome *c* reductase to a 2 ml solution containing 0.1 to 4 μM ferricytochrome *c*, 10 mM succinate in 0.2 M Tris-HCl buffer, pH 7.5, 25°C. The final cytochrome c_1 concentration was 0.11 nM and the reduction rate was monitored at 420 nm. The symbols used for cytochrome *c* trifluoroacetylated at the indicated lysine are: unmodified native (○), 79 (■), 87 (△), and 88 (▲).

Fig. 6. Steady-state cytochrome oxidase activities of cytochrome *c* derivatives. The velocity v was measured in μM O_2 reduced per min following addition of 0.01 to 0.5 μM cytochrome *c* to a 1.8 ml solution containing 10 mM ascorbate, 1.0 mM TMPD, 0.03 mg/ml protein Keilin-Hartree particles in 200 mM sucrose, 0.06 M Mops buffer, pH 7.5, 25°C. The low affinity phase of the reaction at higher cytochrome *c* concentrations is not shown. The symbols used for cytochrome *c* trifluoroacetylated at the indicated lysine are: unmodified native (○), 72 (●), 79 (■), 87 (△), 88 (▲).

cytochrome *c* concentrations of 0.01 to 0.3 μM where the reaction was in the high-affinity phase of the observed biphasic kinetics [6,14]. While all four of the derivatives showed an increase in K_m with no change in V, the change observed for trifluoroacetyl-Lys-88 cytochrome *c* was considerably smaller than for the other three derivatives modified at lysines surrounding the edge of the heme crevice (Fig. 6).

Discussion

The chemical modification studies reported here and elsewhere indicate that the reaction between cytochrome *c* and other electron transport proteins is facilitated by electrostatic interactions between lysines surrounding the heme crevice of cytochrome *c* and negatively charged groups on the other proteins (Fig. 7). The decrease in reaction rate caused by modification of a specific lysine could be due either to elimination of the electrostatic interaction involving the modified lysine, or to steric interference of the modified group with the reaction. One way to distinguish between these effects is to compare the decrease in reaction rate caused by modification with the small trifluoroacetyl-

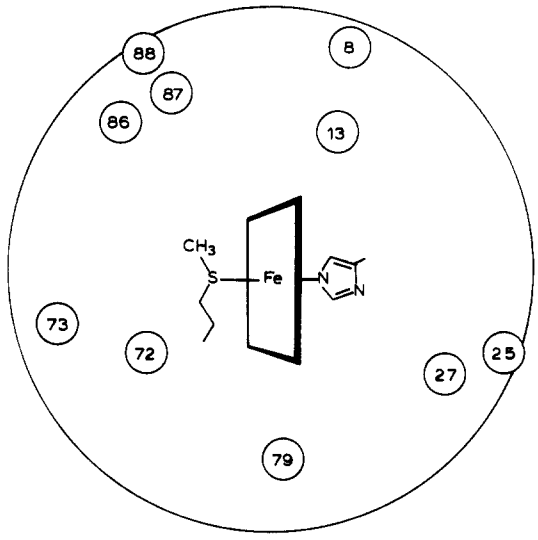


Fig. 7. A schematic diagram of horse heart cytochrome *c* showing the approximate positions of the lysine groups surrounding the heme crevice. The view is from the front of the heme crevice (23).

group to that caused by the bulky $CF_3PhNHCO$- group. If both types of modification cause the same effect, the magnitude of the decrease in reaction rate probably provides a quantitative estimate of the importance of the positive charge on the lysine to the overall electrostatic interaction between the two proteins. Table II summarizes the effect of chemical modification on the reaction of cytochrome *c* with cytochromes b_5, c_1, and oxidase. In all three reactions modification of lysine 72 with trifluoroacetyl- caused nearly the same decrease in rate as modification with $CF_3PhNHCO$-, indicating that lysine 72 at the left of the heme crevice is involved in the electrostatic interaction. The decrease in reaction rate caused by modification of lysine 72 with carboxydinitrophenyl was much larger because of electrostatic repulsion between the negatively charged group and negatively charged groups on the other proteins [3,8]. Similarly, modification of lysine 79 with trifluoroacetyl- caused nearly the same decrease in reaction rate as modification with $CF_3PhNHCO$-, indicating that lysine 79 at the bottom of the heme crevice is involved in the electrostatic interaction between cytochrome *c* and all three of the other cytochromes.

Trifluoroacetylation of lysine 87 caused a significant decrease in the rate of all three reactions, confirming previous studies on the reaction of carboxydinitrophenyl 87 cytochrome *c* with cytochromes c_1 and oxidase [3,8]. It is very interesting that trifluoroacetylation of the adjacent lysine 88 had no effect on the reaction with cytochrome b_5 and only a slight effect on the reaction with cytochromes c_1 and oxidase. Evidently, lysine 87 which is at the top left of the heme crevice oriented towards the front of cytochrome *c* is involved in the reactions, while lysine 88 which is oriented more towards the rear is not.

Comparison of the reaction rates for all the derivatives studied in this laboratory (Table II) gives the following order of effect on the reaction with cyto-

TABLE II

EFFECT OF SPECIFIC LYSINE MODIFICATIONS ON THE REDOX REACTIONS OF CYTOCHROME *c*

The relative reaction rates are presented as $(V/K_m)_{native}/(V/K_m)_{derivative}$. The cytochrome c_1 reaction rate was determined from the initial velocity of reduction of cytochrome *c* by succinate-cytochrome *c* reductase at 25°C in 10 mM succinate, 0.2 M Tris-HCl, pH 7.5. The cytochrome oxidase reaction rate was measured polarigraphically in 10 mM ascorbate, 1.0 mM TMPD, 0.2 M sucrose, and 60 mM potassium Mops, pH 7.5. The cytochrome b_5 reaction rate was determined from the initial velocity of reduction of cytochrome *c* by calf liver microsomes in 50 μM NADH, 0.1 M Tris-HCl, pH 7.5. CF_3CO-, trifluoroacetyl-. References are given in brackets.

Derivative	Cytochrome c_1	Cytochrome oxidase	Cytochrome b_5
$CF_3PhNHCO$-Lys-8	1.7 [1]	2.4 [6]	1.4 [5]
CF_3CO-Lys-13	4.2 [1]	4.9 [9]	2.7 [5]
$CF_3PhNHCO$-Lys-13	3.8 [1]	6.9 [6]	2.7 [5]
CF_3CO-Lys-22	1.0 [1]	1.1 [10]	1.0 [5]
CF_3CO-Lys-25	0.94 [1]	2.7 [10]	1.8 [5]
$CF_3PhNHCO$-Lys-27	2.1 [1]	1.6 [6]	1.8 [5]
CF_3CO-Lys-55	0.97 [1]	1.0 [9]	1.0 [5]
CF_3CO-Lys-72	2.1	3.1	3.0
$CF_3PhNHCO$-Lys-72	2.1 [1]	2.7 [6]	2.5 [5]
CF_3CO-Lys-79	2.5	1.9	1.8
$CF_3PhNHCO$-Lys-79	2.3 [1]	1.8 [6]	1.9 [5]
CF_3CO-Lys-87	2.0	2.8	1.5
CF_3CO-Lys-88	1.5	1.4	1.0
CF_3CO-Lys-99	1.0 [1]	0.90 [9]	0.97 [5]
$CF_3PhNHCO$-Lys-100	1.0 [1]	0.94 [6]	1.0 [5]

chrome c_1: $13 > 72 \approx 79 \approx 27 \approx 87 > 8 > 88$. The same comparison for cytochrome oxidase indicates: $13 > 72 \approx 25 \approx 87 > 8 > 79 > 27$ and for cytochrome b_5: $13 \approx 72 > 25 \approx 79 \approx 27 > 87 \approx 8$. In all cases modification of lysines 13, 72, 79, 87 and 27 surrounding the heme crevice of cytochrome *c* produced a definite decrease in the reaction rate while modification of lysines 22, 55, 99, and 100 produced no effect. Modification of lysines slightly removed from the heme crevice, lysines 8, 25, and 88, had a small or no effect on the reaction rate.

Salemme [18] proposed a model for the cytochrome b_5-cytochrome *c* interaction based on examination of the crystal structures of the two proteins. In it the positively charged cytochrome *c* lysines 13, 27, 72, and 79 form complementary charge interactions with the cytochrome b_5 carboxyl groups of Glu-52, Glu-48, Asp-64, and the most exposed heme proprionate, respectively. Our studies of singly-labeled derivatives have shown that these four lysines are indeed directly involved in the reaction of cytochrome *c* with cytochrome b_5 as well as with the other proteins. The similarity of the interaction domain of cytochrome *c* with the other protein systems indicated that they probably also have a group of negatively charged carboxyl groups available for complementary charge interactions with cytochrome *c* lysines.

Another technique, differential chemical modification [19], has recently been used to define the binding site of cytochrome *c*. The binding domain is assumed to include those lysines which are susceptible to chemical modification in the uncomplexed cytochrome *c*, but which are protected and therefore

not modified in the complex of cytochrome *c* with another protein. This method has the advantage that the chemical modification is done after the complex is completely formed and therefore should not be influenced by subtle changes in the conformation which could occur upon modification. Although it has been shown that modification of single lysine residues does not induce any gross conformational changes in cytochrome *c* [6,9,10,20], small localized changes in the orientation of the lysine side chain could affect the results of our kinetic studies. However, the results of differential chemical modification studies are pertinent to product complex formation and not directly to the electron transfer reaction. This technique has recently been applied to study complex formation of cytochrome *c* with cytochromes oxidase [21] and bc_1 [22], and shows that in both cases the binding domain on cytochrome *c* involves those lysines surrounding the heme crevice. Quantitative estimates of the importance of the individual lysines to the interaction agree well with the kinetic studies. Since the shortcomings of the differential chemical modification approach and the kinetic approach are mutually exclusive, the evidence strongly suggests that cytochrome c_1 and cytochrome oxidase bind and react at the same site on the front of cytochrome *c*. If these conclusions about isolated components of the electron transport chain can be extrapolated to the intact mitochondrial membrane, then cytochrome *c* might undergo some type of diffusion during electron transport. Chiang and King have recently found that purified cytochromes c_1 and oxidase form a dimer to which cytochome *c* can bind to form a 1 : 1 : 1 complex [24]. It is thus possible that the cytochrome c_1-cytochrome oxidase complex might form a single continuous binding site for cytochrome *c* composed of two adjacent subsites provided by cytochrome c_1 and cytochrome oxidase, respectively. Cytochrome *c* might then undergo a low amplitude rotational diffusion between the two subsites during electron transport, without completely dissociating from the complex.

Acknowledgement

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References

1 Ahmed, A., Smith, H.T., Smith, M.B. and Millett, F.S. (1978) Biochemistry 17, 2479—2483
2 Dethmers, J., Margoliash, E. and Rajagopalan, K.V. (1979) International Congress of Biochemistry, 1979, Abst. No. 04-2—S64
3 Ferguson-Miller, S., Brautigan, D.L. and Margoliash, E. (1978) J. Biol. Chem. 253, 149—159
4 Kang, C.H., Brautigan, D.L., Osheroff, N. and Margoliash, E. (1978) J. Biol. Chem. 253, 6502—6510
5 Ng, S., Smith, M.B., Smith, H.T. and Millett, F. (1977) Biochemistry 16, 4975—4978
6 Smith, H.T., Staudenmayer, N. and Millett, F. (1977) Biochemistry 16, 4971—4974
7 Boon, P.J., Van Raay, A.J.M., Fesser, G.I. and Nivard, R.J.F. (1979) FEBS Lett. 108, 131—135
8 Speck, S.H., Ferguson-Miller, S., Osheroff, N. and Margoliash, E. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 155—159
9 Staudenmayer, N., Ng, S., Smith, M.B. and Millett, F. (1977) Biochemistry 16, 600—604
10 Staudenmayer, N., Smith, H.T., Spies, F.K., Jr. and Millett, F. (1976) Biochemistry 15, 3198—3205
11 Margoliash, E. (1962) J. Biol. Chem. 237, 2161—2174
12 Strittmatter, P., Rogers, M.J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188—7194
13 Smith, L. and Camerino, P.W. (1963) Biochemistry 2, 1482—1432
14 Ferguson-Miller, S., Brautigan, D.L. and Margoliash, E. (1976) J. Biol. Chem. 251, 1104—1115

15 Jacobs, E.E., Jacob, M., Sanadi, D.R. and Bradley, L.B. (1956) J. Biol. Chem. 223, 147—156
16 Yu, C.A., Yu, L. and King, T.E. (1974) J. Biol. Chem. 249, 4905—4910
17 Chiang, Y.L. and King, T.E. (1978) J. Biol. Chem. 254, 1845—1853
18 Salemme, F.R. (1976) J. Mol. Biol. 102, 563—568
19 Bosshard, H.R. (1979) in Methods of Biochemical Analysis (Glick, D., ed.), Vol. 25, pp. 273—301, John Wiley, New York
20 Brautigan, D.L., Ferguson-Miller, S., Tarr, G.E. and Margoliash, E. (1978) J. Biol. Chem. 253, 140—148
21 Rieder, R. and Bosshard, H.R. (1978) J. Biol. Chem. 253, 6045—6053
22 Rieder, R. and Bosshard, H.R. (1978) FEBS Lett. 92, 223—226
23 Swanson, R., Trus, B.L., Mandel, N., Mandel, G., Kallai, O. and Dickerson, R.P. (1977) J. Biol. Chem. 252, 759—775
24 Chiang, Y. and King, T.E. (1979) J. Biol. Chem. 254, 1845—1853