Isolation of Monomeric Cytochrome f from Japanese Radish and a Mechanism of Autoreduction*

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Monomeric cytochrome f from Japanese radish (Raphanus sativus L. var acanthiformis Makino) leaves was isolated in a homogeneous state with an A420/405 of 7.6. Radish cytochrome f is a single polypeptide chain with a molecular weight of about 25,000. The midpoint potential is 350 mV. The amino acid analysis indicates the existence of 3 residues of half-cystine. Radish cytochrome f contains one thiol group which reacts with 5,5'-dithiobis(2-nitrobenzoic acid) only after denaturation by sodium dodecyl sulfate. Ferriyctochrome f is reduced by the superoxide radical at the rate of 6 × 10⁷ M⁻¹ s⁻¹ at pH 7.8. Radish ferricytochrome f is also reduced slowly without an exogenous electron donor. A kinetic study and the effect of the thiol reagent indicate that the autoreduction is an intramolecular reaction and that the thiol group is the electron donor.

Cytochrome f is a thylakoid-bound c-type cytochrome in higher plant chloroplasts. Its occurrence has been described by Yakushijii (1) and by Hill and Scarsbrick (2). This hemoprotein has since been shown to have a high midpoint potential of 360 mV and an α-band at 552 to 554 nm, distinguishing it from mitochondrial cytochrome c which has a potential of 270 mV and an α-band at 550 nm. Besides the differences in potential and α-band, plant cytochrome f has a higher molecular weight than cytochrome c. It has been demonstrated that algae contain soluble c-type cytochrome with a potential and α-band similar to that of plant cytochrome f but with a lower molecular weight. Recently, however, Wood (3) has reported that algal thylakoids have a tightly bound, high potential, c-type cytochrome with an α-band between 552 to 554 nm. The algal thylakoid-bound cytochrome is distinguished from the soluble cytochrome by its molecular weight, γ-band, and immunological properties (3). The high potential, c-type cytochrome in plants and algae is a single-electron carrier between plastocyanin and plastocyanin linking Photosystems I and II (4).

Unlike algae, no higher plant has the soluble c-type cytochrome, except for the mitochondrial cytochrome c (5). Cytochrome f has been purified from leaves of parsley (6), spinach (7, 8), Japanese radish (9), and Brassica rapa L. var. perriridis Bailey (10) after extraction with organic solvents or detergent. Cytochrome f from parsley (6) and spinach (7, 8) has been solubilized with methanol/ammonia or with butanol/Triton X-100 as a high molecular weight species of 62,000 to 270,000, which probably is oligomeric in form. Sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of spinach cytochrome f suggested that molecular weight of the monomeric form is 32,000 to 34,000 (7, 8). We (9) and Matsuzaki et al. (10) have isolated a monomeric form of cytochrome f from Cruciferae leaves using acetone or methyl ethyl ketone as the extractant.

This paper described an improved isolation procedure for monomeric cytochrome f from Japanese radish leaves and several properties of isolated cytochrome f with an A420/405 of 7.6. Radish ferricytochrome f is autoreduced, as is the spinach cytochrome (11). The present results show that this autoreduction is an intramolecular reaction and that the thiol group is the electron donor.

MATERIALS AND METHODS

All chemicals were reagent grade. Bovine serum albumin (Calbiochem, A grade), bovine pancreas chymotrypsinogen A (Sigma, type II), egg albumin (Sigma), horse heart cytochrome c (Sigma, type III), and bovine pancreas RNase A (Sigma, type I-A) were used as the standards of molecular weight. Japanese radish leaves (Raphanus sativus L. var. acanthiformis Makino) were obtained from a local farm.

The absorption spectra and autoreduction were recorded by a Shimadzu UV-200 spectrophotometer. Low temperature spectra were recorded with a Shimadzu MPS-5000 spectrophotometer with a low temperature attachment.

Content of heme c in cytochrome f was determined as follows. Pyridine, 1 ml, then 1 ml NaOH, 0.5 ml, were added to 4.5 ml of the cytochrome solution. The concentration of the pyridine hemechromo of cytochrome f was determined from the difference absorbance at 553.5 nm using an absorbance coefficient of 27.6 M⁻¹ cm⁻¹ after reduction with ascorbate. The concentration of the cytochrome at an early stage of purification was estimated from the absorbance coefficient of 22.2 M⁻¹ cm⁻¹ at 553.5 nm. Absolute and difference absorbance coefficient values at 553.5 nm were based on the values determined in this work.

Molecular weight was determined by three methods: gel filtration with Sephadex G-100 (13), sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis using the method of Weber and Osborn (14), and sedimentation equilibrium with an Hitachi UCA-IA analytical ultracentrifuge.

Amino acid analysis was carried out on an Hitachi KCA-3B amino acid analyzer. Each sample containing 7.35 mmol of cytochrome f was hydrolyzed in 1 ml of 6 M HCl in evacuated and sealed tubes for 22, 48, and 72 h at 110°C. Half-cystine was determined by performic acid oxidation followed by hydrolysis in 6 M HCl for 22 h according to Moore (15). Tryptophan was determined according to the method of Beaven and Holiday (16). The thiol group was assayed from an increase in absorbance at 410 nm in the presence of 5,5'-dithiobis(2-nitrobenzoic acid) (17).

Purification of cytochrome f was conducted at 0-5°C as follows. Unless otherwise stated, potassium phosphate buffer at pH 7.8 was used. Sixty kilograms of Japanese radish leaves were homogenized in a Waring Blendor with 60 liters of 0.1 M phosphate containing 2 mM 2-mercaptoethanol and 2 mM EDTA. The homogenate was filtered through cotton cloth using a basket centrifuge. The filtrate was

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centrifuged at 15,000 \times g in a CEPA continuous centrifuge and broken chloroplasts precipitated. These chloroplasts were suspended in 3 liters of the buffer to which 27 liters of methyl ethyl ketone (-20°C) was added with occasional stirring. After standing for an hour, the upper green layer was discarded by decantation. The aqueous phase was then mixed with 3-fold volume of chilled acetone (-20°C). The precipitate was collected by centrifugation at 6,000 \times g for 10 min and dissolved in and dialyzed against 10 mm phosphate containing 1 mm EDTA and 1 mm 2-mercaptoethanol for 18 h. The dark reddish dialyzed solution was clarified by centrifugation at 10,000 \times g for 30 min, then ammonium sulfate was added to make 90% saturation. The precipitate was dissolved in 200 ml of 10 mm phosphate containing 1 mm EDTA and 1 mm 2-mercaptoethanol, then dialyzed against the same buffer. In this step, the yield was 0.061 \mu mol of cytochrome f/kg of leaves. After centrifugation of the dialyzed solution, the supernatant was charged on a DEAE-Sephadex A-50 column (10 \times 50 cm) equilibrated with 10 mm phosphate containing 1 mm EDTA and 1 mm 2-mercaptoethanol. The column was washed with the equilibrating buffer until the absorbance at 280 nm decreased to 1.0. A pink band of cytochrome f appeared on the column on elution with 20 mm phosphate. Further elution with 30 mm phosphate separated two isozymes of cytochrome f (Fig. 1) whose major and minor peaks (cytochromes f II and I) were separately pooled and were brought to 90% saturation with ammonium sulfate. The yield of these two procedures and of our new procedure leaves with buffer and methyl ethyl ketone, afforded 0.076 \mu mol/kg of leaves. The yields of these two procedures and of our new procedure do not differ remarkably. However, the isolation of cytochrome f II and f I was not as easy as cytochrome f. The yield of electrophoretically homogeneous cytochrome f II was 0.4 \mu mol (13.2 mg). No remarkable differences in the molecular weights, spectroscopic properties, and oxidation reduction potentials of cytochromes f, I and II were detected. The following results are for cytochrome f II at the hydroxylapatite step.

Purity—Cytochrome f at the hydroxylapatite step showed an A_{420.5}/A_{277} of 7.6 and an A_{553.5}/A_{277} of 1.1. The purified cytochrome f gave a single band on polyacrylamide disc gel electrophoresis at pH 8.3 in the absence and presence of sodium dodecyl sulfate. Homogeneity was also confirmed from the sedimentation pattern.

Stability—The cytochrome was obtained in a reduced form. Even when 2-mercaptoethanol was omitted during purification, about 90% was in the reduced form. The purified cytochrome f was stable for at least 3 months in 90% saturated ammonium sulfate containing 50 mm phosphate, pH 7.8, 0.1 mm EDTA, and 1 mm 2-mercaptoethanol at 5°C. The storage of cytochrome f in 50 mm phosphate, pH 7.8, in the absence of 2-mercaptoethanol at 5°C caused about 30% oxidation for a week; recovery of the a-peak by ascorbate was 90%. This is why we added 2-mercaptoethanol during purification. The freezing of cytochrome f in 50 mm phosphate, pH 7.8, in the presence and absence of 2-mercaptoethanol caused aggregation resulting in precipitation of the protein.

Spectral Properties—Absorption spectra of ferri- and ferrocyanochrome f are presented in Fig. 2. The a- and \( \beta \)-bands, respectively, occur at 553.5 and 524 nm with a subpeak at 532 nm. The Soret band of the reduced form at 420.5 nm is accompanied by a shoulder at 400 nm. In the ultraviolet region, peaks occur at 327, 283, and 277 nm. At 77 K, the a-band is split into three peaks, 564.0, 556.5, and 543.2 nm, and the \( \beta \)-band is separated into several peaks with the main one at 526.1 nm (Fig. 2B). The Soret band of ferrocyanochrome f is at 410.5 nm.

The absorbance coefficients of radish ferrocyanochrome f at 420.5, 524, and 565.5 nm were 149.9, 13.4, and 27.6 nm\(^{-1}\) cm\(^{-1}\), respectively, based on heme c which was determined as pyridine hemochrome. The coefficient of ferrocytochrome f at the Soret peak was 121.3 mm\(^{-1}\) cm\(^{-1}\). The oxidized-reduced difference absorbance coefficient at 553.5 nm was 22.2 mm\(^{-1}\) cm\(^{-1}\).

Molecular Weight—Subunit structure was analyzed using sodium dodecyl sulfate-polyacrylamide gel disc electrophoresis. The cytochrome was incubated at 37°C for 2 h in 50 mm potassium phosphate, pH 7.8, containing 1% sodium dodecyl sulfate with or without 2% 2-mercaptoethanol; then it was subjected to electrophoresis. In the presence or absence of 2-
mercaptoethanol, only one band was detected. This was true even when the cytochrome had been boiled in 1% sodium dodecyl sulfate for 20 min. The migration of this band corresponded to a molecular weight of 28,000 on calibration with bovine serum albumin, chymotrypsinogen A, egg albumin, cytochrome c, and RNase A as the molecular weight standards. From gel filtration, using Sephadex G-100 calibrated against the standard proteins, the molecular weight was estimated to be 33,000. The purified cytochrome f in 0.1 M NaCl containing 5 mM potassium phosphate, pH 7.8, was brought to sedimentation equilibrium at 19,923 rpm at 20°C. The resulting distribution of protein was analyzed following the absorbance at 280 nm. When In (absorbance at 280 nm) was plotted against the square of the distance from the center of rotation, a straight line was obtained (data not shown). The molecular weight was estimated to be 32,600 from the slope of the line and the partial specific volume of 0.73 ml calculated from the amino acid composition (Table II). The apparent discrepancy between the molecular weight calculated from sodium dodecyl sulfate-polyacrylamide gel disc electrophoresis and that calculated from sedimentation equilibrium and gel filtration is not yet understood, but purified cytochrome f is clearly a monomer not composed of subunits.

Oxidation-Reduction Potential—Assuming a midpoint potential (E°f) of 430 mV at pH 7.0 for the ferri- and ferrocyanide system, the standard oxidation-reduction potential of cytochrome f was determined to be 350 mV at pH 7.0 with an n of 1.16, according to Davenport and Hill (18).

Amino Acid Analysis—The amino acid composition is presented based on a molecular weight of 33,000. The values in Table II are averages of results of 22, 48, and 72 h of hydrolysis, except for serine and threonine, whose values are extrapolated to zero time of hydrolysis and for valine and isoleucine, whose values are for 72 h of hydrolysis.

Amino acid analysis shows 3 residues of half-cystine/mol of cytochrome f. Two residues are believed to bind with the heme in the thioether linkage, as do the c-type cytochromes (21). Titration with 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of 0.5% sodium dodecyl sulfate indicates 1.1 thiol group/mol of cytochrome f. In the absence of sodium dodecyl sulfate, the thiol reagent did not react, which suggests that the thiol group is buried in the molecule.

Reduction of Cytochrome f by Superoxide—Ferricytochrome c is reduced by the superoxide anion radicals and has been used to detect O2− (22). We tested the effect of the O2−-generating system on ferricytochrome f. Ferricytochrome f was prepared as described below and used immediately before autoreduction. Fig. 3 shows the reduction of ferricytochrome f by O2− generated with the aerobic xanthine-xanthine oxidase system and the inhibition by superoxide dismutase; this confirms the participation of O2− in the reduction. Reduction also occurred with the O2− generated from the

![Absorption spectra of radish cytochrome f at 25°C (A) and 77 K (B). A, the concentration was 3.2 μM in 50 mM potassium phosphate, pH 7.8. Solid line represents a reduced form, measured after reduction by ascorbate, which was removed by gel filtration using Sephadex G-25. Dotted line is an oxidized form, oxidized by ammonium persulfate. The reference cuvette also contained the same amount of oxidant. B, the concentration was 20 μM in 50 mM potassium phosphate, pH 7.8.](http://www.jbc.org/)

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**Table II**

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<th>Cytochrome c (horse)</th>
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a From Nelson and Racker (8).

b Calculated assuming a molecular weight of 33,000 from Davenport (19).

c From Cusanovich et al. (20).

d From Margoliash (21).
infusion of K$_2$O$_2$ dissolved in crown ether/dimethylsulfoxide.
Reduction depended on the concentration of cytochrome f as shown in Fig. 4. The reaction rate constant between ferricytochrome f and O$_2^-$ was determined from the competition of O$_2^-$ between superoxide dismutase and ferricytochrome f at 2.1 mM which prevents the spontaneous dismutation of O$_2^-$.
Crystalline spinach superoxide dismutase (SOD) was used. The reaction rate constant between O$_2^-$ and superoxide dismutase is assumed to be $2.3 \times 10^9$ M$^{-1}$ s$^{-1}$ (24). Using an equation described previously (25), we found a reaction rate constant of $6 \times 10^8$ M$^{-1}$ s$^{-1}$ at pH 7.8 for the reduction of cytochrome f by O$_2^-$.

This value is higher by 1 order of magnitude than that of cytochrome c at the same pH (26). Illuminated chloroplasts produce O$_2^-$ by autooxidation of the primary electron acceptor of Photosystem I (27). Further experiments are needed to see whether photochemically produced O$_2^-$ reduces cytochrome f in chloroplasts and whether O$_2^-$ links cyclic electron flow around Photosystem I. The ferrocytochrome f in the O$_2^-$-generating system did not produce any change in the spectrum. However, the addition of superoxide dismutase resulted in the oxidation of cytochrome f. This superoxide dismutase-dependent oxidation of cytochrome f by superoxide will be reported elsewhere.

**Autoreduction of Cytochrome f**—Even under aerobic conditions, the cytochrome f from radish leaves is obtained in reduced form because of its high oxidation-reduction potential. Garewal and Wasserman (11) found spontaneous reduction of purified spinach cytochrome f on removal of excess oxidants without the presence of an exogeneous electron donor or catalyst. We also found a similar “autoreduction” of purified radish ferricytochrome f in phosphate buffer.

Ferricytochrome f was prepared by adding ferricyanide to purified cytochrome f in 10 mM potassium phosphate, pH 7.8 at 5°C. Excess oxidant was removed by gel filtration using a short Sephadex G-25 column equilibrated with 10 mM potassium phosphate, pH 7.8. The time required to prepare ferrocytochrome f was within 5 min. Under these conditions, complete oxidation of cytochrome f occurred based on the disappearance of the a-peak and the shift of the Soret peak from 420.5 to 410.5 nm.

This ferricytochrome f was autoreduced at room temperature in phosphate buffer based on the increases of the a- and Soret peaks. After the remarkable increase of absorbance at 553.5 or 420.5 nm had ended, the addition of ascorbic acid brought about further increase of absorbance at both peaks. This indicates that 75% autoreduction took place within 1 h.

**Fig. 3.** Reduction of cytochrome f by O$_2^-$: The reaction mixture contained 0.1 mM xanthine, 25 µg of xanthine oxidase (Boehringer), 2.1 µM cytochrome f, 50 mM potassium phosphate, pH 7.8, 0.1 mM EDTA, and indicated amounts of spinach superoxide dismutase (SOD), in a total volume of 1 ml. The reaction was started by adding xanthine oxidase.

**Fig. 4.** Concentration dependence of cytochrome f (cyt. f) on the reduction by O$_2^-$ generated with a xanthine-xanthine oxidase system. Reaction conditions were the same as in Fig. 3, except for the concentration of cytochrome f.

**Fig. 5.** Time course of the autoreduction of cytochrome f. The reaction mixture contained 0.6 µM ferricytochrome f, 0.1 mM EDTA, and 100 mM phosphate buffer, pH 7.8. The reaction was started by a transfer from an ice bath to 30°C. Where indicated, ascorbate was added. Inset, replots of autoreduction. A$_0$ is the absorbance after the addition of ascorbate and A$_t$ is the absorbance at t min after the start of autoreduction.

A typical time course for autoreduction in phosphate, pH 7.8, at 30°C is shown in Fig. 5.

A replot of the time course of reduction indicates that the reaction proceeds with first order kinetics. The rate constant under the conditions of Fig. 5 is 0.12 min$^{-1}$ at 30°C. The first order reaction was also confirmed from Fig. 6 which shows that the initial rate of reduction is proportional to the initial concentrations of ferricytochrome f in the range tested and that the rate constants are 0.13 min$^{-1}$. The reduction rate increased with an increase in pH (Fig. 7). The effect of temperature on the autoreduction in Fig. 8 shows an increase in the rate up to 37°C and above that, a decline. At all the pH values and temperatures tested, the time course of reduction obeyed first order kinetics. The activation energy estimated from the Arrhenius plot for a range of 5°C and 37°C is 6.3 kcal/mol.

Autoreduction was not affected by the repeated evacuation and refilling with argon. Neither keeping the reaction vessel dark nor illumination with a projector (30,000 lux) affected the autoreduction. These observations do not include reduction by photochemical reaction or by photoproduced O$_2^-$.

The reaction kinetics suggest that an electron donor for
Japanese Radish Cytochrome $f$

FIG. 6 (left). Effect of cytochrome $f$ (cyt. $f$) concentration on the rate of autoreduction. Reaction conditions were the same as in Fig. 5, except for the concentrations of cytochrome $f$. The rate for the first 5 min was plotted for the concentration of cytochrome $f$.

FIG. 7 (center). Effect of pH on the autoreduction of cytochrome $f$. Autoreduction was started by mixing 0.6 $\mu$m ferricytochrome $f$ with the thermoregulated reaction mixture containing 0.1 mM EDTA and 100 mM phosphate buffer, the pH of which had been adjusted from 6 to 9.

FIG. 8 (right). Temperature dependency of the autoreduction of cytochrome $f$. Assay conditions were the same as in Fig. 5, except that the temperature was varied as indicated.

FIG. 9 (left). Effect of p-chloromercuribenzoate on the autoreduction of cytochrome $f$ (cyt. $f$). P-Chloromercuribenzoate (pCMB) was added to the reaction mixture as in Fig. 5 just before the start of the reaction. The concentration of ferricytochrome $f$ was 0.3 $\mu$m.

FIG. 10 (center). Relationship between autoreduction and the thiol group of cytochrome $f$ (cyt. $f$). Ferricytochrome $f$ was autoreduced to the indicated degree. The thiol group of cytochrome $f$ was then determined by titration with 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of 0.5% sodium dodecyl sulfate. To calculate the degree of autoreduction, ascorbate-reduced cytochrome $f$ was regarded as 100% reduced and cytochrome $f$ just before the reaction started as 0%.

FIG. 11 (right). The restorative effect of 2-mercaptoethanol on repetitive autoreduction. Assay conditions are given in Fig. 5. The autoreduced cytochrome $f$ was reoxidized by ferricyanide directly, or after addition with 1 mM 2-mercaptoethanol. Excess oxidant was removed by gel filtration.

Heme comes from cytochrome $f$ itself or, although unlikely, from a contaminating excess reductant. The following experiments excluded the latter mechanism and indicate that autoreduction is an intramolecular reaction and that the residue of half-cystine is an electron donor.

Titration of ferricytochrome $f$ with p-chloromercuribenzoate inhibited autoreduction. Fig. 9 indicates that the cytochrome was not autoreduced by blocking 1 mol of the thiol group with the thiol reagent. As stated previously, cytochrome $f$ contains 3 residues of half-cystine, one of which exists as the thiol group. Inhibition by p-chloromercuribenzoate suggests that the thiol group participates in the autoreduction.

Other thiol reagents such as monooiodoacetate, 5,5'-dithiobis(2-nitrobenzoic acid), and N-ethylmaleimide did not affect autoreduction at 10 mM. The ineffectiveness of 5,5'-dithiobis(2-nitrobenzoic acid) on the autoreduction is probably due to the fact that the reagent did not react with the thiol group of native cytochrome $f$. As shown above, the thiol group of cytochrome $f$ reacts only after denaturation with sodium dodecyl sulfate. Thus, intramolecular electron transfer from the thiol group to heme is a possible mechanism for the autoreduction of cytochrome $f$.

Further support for this mechanism comes from the decrease of the thiol group as autoreduction progresses (Fig. 10). The contents of the thiol group were determined using 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of sodium dodecyl sulfate at different times after the onset of autoreduction. Disappearance of the thiol group in the autoreduced cytochrome $f$ confirmed that an electron had been donated by the thiol group to heme. The autoreduced cytochrome $f$ was reoxidized by ferricyanide; excess oxidant was removed by gel filtration. As seen in Fig. 11, the reoxidized cytochrome $f$ showed no autoreduction probably because of oxidation of the thiol group. However, when autoreduced cytochrome $f$ was treated with 2-mercaptoethanol then oxidized by ferricyanide, the potential for autoreduction was restored. Stimulation of autoreduction at a high pH suggests that donation of an electron by RS$^-$ is faster than that by RSH. It should also be noted that ferricytochrome $f$ was reduced by 2-mercaptoethanol in phosphate buffer, pH 7.8, with no additions, although mammalian cytochrome $c$ is only reduced with thiols on the addition of an external catalyst such as selenium (28).

DISCUSSION

Our preparation of cytochrome $f$ from Japanese radish has a monomeric form with a molecular weight of about 33,000.
Its ratio of $A_{260}/A_{280}$ is the highest among the cytochromes $f$ isolated so far from plants. Some proteins resist dissociation into subunits in sodium dodecyl sulfate at room temperature; thus, for dissociation, a high temperature is required (29). Even with treatment with sodium dodecyl sulfate at 100°C, radish cytochrome $f$ does not give a band of smaller molecular weights, which confirms that a molecular weight of about 33,000 is the ultimate unit of plant cytochrome $f$. The subunit weight of the oligomeric cytochrome $f$ from spinach (7, 8) and the molecular weight of the monomeric cytochrome $f$ from *Brassica rapa* (10) also support this conclusion.

No remarkable differences in absorption peaks and midpoint potential are found between the oligomeric cytochrome $f$ from parsley (6) and spinach, but it is not identical; 2 residues of half-cystine are reported for spinach cytochrome $f$ in contrast to 3 residues for the radish cytochrome. The contents of histidine and methionine in radish cytochrome $f$ are the same as in mammalian cytochrome $c$. The histidine and methionine residues in cytochrome $c$ have been shown to be ligands of heme (30).

Most soluble c-type cytochromes found in mitochondria and algae contain 2 residues of half-cystine (Table II) which form thioether linkages with heme $c$ (20, 21). Only yeast cytochrome $c$ contains 3 residues of half-cystine (31). Radish cytochrome $f$ has 3 residues of half-cystine, therefore, one should be a free thiol group. Thiol determination indicates that this is the case and that the thiol group reacts with the thiol reagent only after denaturation by sodium dodecyl sulfate. Beef heart cytochrome $c_1$ has been reported to contain 11 or 12 residues of half-cystine (32).

Another feature of the amino acid composition of radish cytochrome $f$ is its high content of nonpolar amino acids. Isoleucine, leucine, valine, phenylalanine, proline, alanine, cysteine, methionine, and tryptophan account for about 40% of the total amino acid residues. This reflects the membrane-bound property of cytochrome $f$ (33).

Auto-reduction of cytochrome $f$ was discovered by Garewal and Wasserman (11). Similar reduction has been observed with monomeric *Brassica* cytochrome $f$ (10), the degree of auto-reduction being 13%. Radish ferricytochrome $f$ also exhibits reduction without the addition of an exogenous electron donor: reduction proceeds up to 75%. Kinetic study suggests that the auto-reduction is an intramolecular reaction. Inhibition of auto-reduction by p-chloromercuribenzoate (Fig. 9) and the decrease of the numbers of the thiol group as auto-reduction progresses (Fig. 10) clearly demonstrate that the thiol group, which may be buried in the molecule of cytochrome $f$, is an electron donor for auto-reduction. The thiol group may be situated near heme $c$ in the cytochrome $f$ molecule. p-Chloromercuribenzoate-treated cytochrome $f$ could be reduced by ascorbate which suggests that the thiol group is not essential for electron donation from low molecular reductants like ascorbic acid. At present, the state of the thiol group after auto-reduction is too slow for electron transport in chloroplasts. Yu et al. (32) reported the photoreduction of cytochrome $c_1$ from beef heart and the participation of the thiol group in this reaction. Neither light nor darkness affected the auto-reduction of cytochrome $f$. Recently Wood (3) has proposed that mitochondrial cytochrome $c_1$ and algal and plant thylakoid-bound cytochromes $f$ are homologous proteins based on their similar molecular weights and function in the electron transport system. The membrane-bound cytochrome $c_1$ or $f$ forms a link between a lipophilic cytochrome $b$, cytochrome $b_6$, or plastocyanin and a peripheral cytochrome $c$ or plastocyanin. The heme-containing subunit of cytochrome $c_1$ from beef heart and from yeast have respective molecular weights of 30,600 and 31,000 (34, 35). Present results confirm the similarity of the molecular weights of cytochrome $c_1$ and cytochrome $f$ and that the thiol group of cysteine residue is situated near heme $c$ and can donate an electron.

Note Added in Proof—After we submitted this paper, a report which documents the isolation of monomeric cytochrome $f$ from chardlock has appeared (36).

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Isolation of monomeric cytochrome f from Japanese radish and a mechanism of autoreduction.
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