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# A HIGH POTENTIAL CYTOCHROME *c* FROM *CHROMATIUM* CHROMATOPHORES

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SUMMARY

A new cytochrome, C-553(550), with properties like those of several isolated algal cytochromes *f* has been isolated from membrane fractions of the photosynthetic bacterium, *Chromatium* Strain D. The minimum molecular weight of the cytochrome is approx. 13000, but the isolated protein is heterogeneous in size and appears to polymerize in solution. This cytochrome may be related to the membrane-bound cytochrome *c*-555 which is implicated as a primary reductant in light-induced reactions of the *Chromatium* chromatophore reaction center.

## INTRODUCTION

Of interest in the study of bacterial photosynthesis is the nature of the electron donors associated with active center bacteriochlorophyll. To date, cytochromes with a high oxidation–reduction potential have been implicated in that role through careful spectroscopic studies[1–3]. In *Chromatium*, cytochrome *c*-555 is of this type and appears to be tightly bound to the cell membrane and not freely extracted from disrupted cell preparations into aqueous buffer.

*Chromatium* Strain D has been intensively studied[1–4] and the membrane-bound members of the photosynthetic electron transport chain have been in part identified[2,5,6]. We have attempted to extract cytochrome *c*-555 and other components of the photoactive electron transport chain from *Chromatium* chromatophores, and we report here the isolation and characterization of a new *c*-type cytochrome with high oxidation–reduction potential from *Chromatium* extracts.

## METHODS

*Chromatium* Strain D was grown both photoautotrophically and photoheterotrophically as previously described[7,8]. Intact cells were suspended in 10% (w/v) sucrose *plus* either 0.1 M Tris (pH 7.3) or 0.1 M potassium phosphate (pH 7.5) and were disrupted by treatment in a Sorval-Ribi cell fractionator (Ivan Sorvall, Norwalk, Conn.) operated at 20000 lb/inch$^2$ and at sample temperatures less than 20°. The extract was

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centrifuged at 30000 × *g* for 60 min to remove cell debris (labeled large particles). Classical chromatophores were recovered by centrifuging the supernatant fraction at 100000 × *g* for 180 min. Light (low density) particles were prepared from the classical chromatophore preparation in the manner previously described[7].

Absorption spectra were measured with a Cary 14R recording spectrophotometer. To prepare the oxidized form of the cytochrome for spectrophotometry, a sample was passed through a small Dowex-2 anion-exchange column partly charged with ferricyanide. Absorption spectra at 80°K were measured with the use of a Pyrex Dewar assembly fitted with optical windows and mounted in the Cary spectrophotometer sample compartment. The sample cuvet, which consisted of a U-shaped copper frame fitted with 0.25-mm-thick cellulose acetate film windows spaced 1 mm apart, was mounted in a grooved copper block attached to a liquid $N_2$ charged copper vessel which was positioned in the Dewar. The solvent used for the cytochrome consisted of 50 % (v/v) glycerol in 50 mM Tris (pH 7.3), which froze to an optically clear glass when cooled to 80°K. The apparatus was constructed by Prof. H. Linshitz and M. A. Cusanovich.

Oxidation–reduction potentials were measured in a modified cuvet of 1-cm optical path which had a TS 24/25 ground glass joint joined to the top and a small side port which was closed with a gas chromatograph silicone rubber septum. The ground glass joint was closed with a tightly fitted silicone rubber stopper (size No. 6) through which were inserted a combination Pt and Ag–AgCl electrode (Instrumentation Laboratories No. 15020), a 24 gage (0.30 mm internal diameter) stainless steel syringe needle as a gas inlet and an 18 gage (0.84 mm internal diameter) needle as a gas outlet. The cytochrome or chromatophore preparation was diluted in 50 mM phosphate buffer (pH 7.0), which contained 100 $\mu$M $K_3Fe(CN)_6$. The protein solution was deaerated by bubbling with prepurified $N_2$ gas which had been passed through a column filled with MnO to remove traces of $O_2$ (ref. 9). The oxidation–reduction potential of the reaction mixture ($E_h$) was adjusted stepwise by adding a 1 % (w/v) solution of $Na_2S_2O_4$ dissolved in 50 mM phosphate (pH 7.0) from a microsyringe buret equipped with a needle inserted through the side port of the sealed cuvet. After each addition of titrant, the $E_h$ was measured with a millivolt–pH meter (Instrumentation Laboratories No. 145) equipped with an expanded scale. The reversibility of the reaction was checked by titrating in the reverse direction with 1 mM $K_3Fe(CN)_6$ solution. From the plots of $E_h$ *vs.* fraction of cytochrome reduced, the midpoint oxidation–reduction potential, $E_{m,7}$, was determined.

Molecular weights were determined by the short column sedimentation equilibrium method described by YPHANTIS[10] and calculated by a modification[11] of the method of VAN HOLDE AND BALDWIN[12] in which the synthetic boundary run was omitted. In analogy with mammalian cytochrome *c* for which $\bar{v} = 0.728$ has been determined[13], the value $\bar{v} = 0.72$ was assumed for cytochrome *c* 553 (550).

The isoelectric point, $I_p$, was determined by the isoelectric focusing procedure described by VESTERBERG AND SVENSSON[14], using the Ampholine column and carrier electrolyte (pH range 3–6) supplied by LKB Instruments. Amino acid compositions were determined on samples which were hydrolyzed at 110° with constant boiling HCl in sealed evacuated tubes. Analyses were performed on a modified Spinco automatic amino acid analyzer by the method of DUS *et al.*[15,16]. The amino-terminal amino acid was identified by the dinitrophenol labeling method of SANGER[17] and by the EDMAN[18] degradation procedure as practised by DOOLITTLE[19].

*Extraction and purification of bound proteins*

Whether whole cells, large particles, classical chromatophores or light particles were used, the fraction to be extracted was suspended in 10 mM Tris (pH 7.3) containing 100 $\mu$M 2-mercaptoethanol, in the proportion of 1 g wet weight cell material to 3 ml buffer. A homogeneous suspension was obtained by stirring the mixture overnight at 4°. An equal volume of acetone (−10°) was added to the suspension with vigorous stirring. The suspension was allowed to stand at −10° for 30 min and then centrifuged at 15000 × *g* for 10 min. The residue was resuspended in the same volume of buffer and the acetone extraction was repeated. The combined supernatant solutions, were passed through a DEAE-cellulose column (Selectacel-standard, Brown Co., 30 ml packed volume per 100 g wet weight starting material) equilibrated with the extraction buffer. The charged column was washed with 10 mM Tris (pH 7.3) and the unadsorbed solution and washings were discarded. If a particle fraction had been the starting material, the bulk of the adsorbed proteins were eluted with 0.5 M NaCl in 20 mM Tris (pH 7.3). If whole cells had been the starting material the column was first eluted with 0.2 M NaCl in the buffer to remove cytochromes and then the bacterial ferredoxin which remained adsorbed was eluted with 0.5 M NaCl in the same buffer. The cytochrome solution was passed through a column of Sephadex G-25 equilibrated with demineralized water to remove unwanted salts, the solution was adjusted to pH 7.3, and then charged onto a DEAE-cellulose column (Selectacel Type 20, 30 ml packed volume per 100 g starting material) equilibrated with 10 mM Tris (pH 7.3). A loosely bound red cytochrome band was eluted from the column with 20 mM Tris (pH 7.3). This cytochrome *c*-553(550) was further purified by rechromatography in the same manner.

Several additional colored proteins which were eluted from the column by buffer solutions of higher concentration are presently under investigation, but except for HiPIP[6] and ferredoxin[20], none of these components were recovered in greater quantity than that of C-553(550).

## RESULTS

The extraction procedure described was the best yet devised, although the amount of C-553(550) obtained was variable, apparently due in part to the lability of the cytochrome. Further extraction with 50% (v/v) acetone yielded additional cytochrome heavily contaminated with pigmented chromatophore fragments which could not be removed by centrifugation and which stopped the flow of the DEAE-cellulose columns. The best yields obtained from each of the various cellular fractions extracted are recorded in Table I.

*Spectral properties*

Absorption spectra of the oxidized and reduced cytochrome are presented in Fig. 1A. The most obvious features of these spectra are the asymmetric $\alpha$ peak in the reduced form, with an absorption maximum at 553 and a shoulder at 550 nm (see insert in Fig. 1A), and the relatively low absorption of the $\alpha$ peak relative to the $\gamma$- and $\beta$-absorption bands. The millimolar absorptivity coefficients together with the wavelength for C-553(550) are given in Table II.

The alkaline pyridine hemochrome spectrum of C-553(550) is typical for *c*-type cytochromes, with the $\alpha$-peak maximum located at 550 nm. The heme group of the

TABLE I

YIELDS OF C-553 (550) FROM VARIOUS PARTICULATE FRACTIONS

| *Fraction extracted with 50% acetone* | *μmoles heme as C-553 (550) per 100 g wet wt. material* |
|---|---|
| Whole cells (photoautotrophic) | 0.2 |
| Classical chromatophores (photoautotrophic) | 0.4–0.6 |
| Classical chromatophores (photoheterotrophic) | 0.3–0.4 |
| Light particles (photoheterotrophic) | 1.5 |

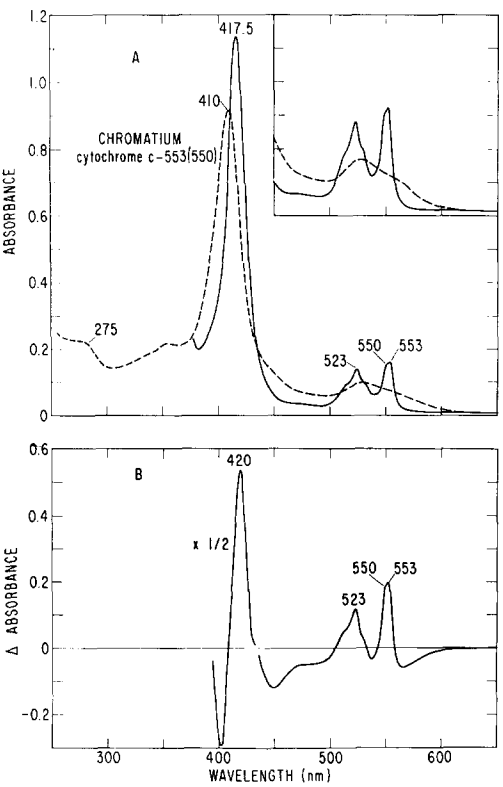


Fig. 1. A. Absorption spectra of oxidized and reduced *Chromatium* C-553 (550) (10 μM; inset, 20 μM). The cytochrome was dissolved in 0.1 M Tris (pH 7.3). – – –, oxidized cytochrome (prepared as described in METHODS); ——, reduced cytochrome ($Na_2S_2O_4$). B. Absorption difference spectrum of Chromatium cytochrome C-553 (550) (20 μM), reduced-*minus*-oxidized. The conditions are the same as described under A.

cytochrome could not be extracted into acidic methyl ethyl ketone[21]. Therefore C-553 (550) may be classified as *c*-type cytochrome. At neutral pH the absorption spectrum of the reduced cytochrome was not changed under one atmosphere of CO, suggesting that the cytochrome is unreactive with CO.

TABLE II

ABSORPTIVITY VALUES OF *Chromatium* CYTOCHROME *c*-553 (550) AT pH 7.0, BASED ON ALKALINE PYRIDINE FERROHEMOCHROME SPECTRA*

| *Oxidized* | | *Reduced* | | *Reduced-minus-oxidized* | |
|---|---|---|---|---|---|
| $\lambda_{max}$ | $\varepsilon_{mM}$ | $\lambda_{max}$ | $\varepsilon_{mM}$ | $\lambda_{max}$ | $\varepsilon_{mM}$ |
| 529.0 | 8.8 | 553.0 | 16.3 | 566 | — 2.9 |
| 410.0 | 93.5 | 550.0 | 15.7 | 553 | 10.0 |
| 355.0 | 22.2 | 522.8 | 14.3 | 550 | 9.4 |
| 275.0 | 14.1 | 417.5 | 115.0 | 523 | 8.9 |
| | | | | 450 | — 6.1 |
| | | | | 420 | 55 |
| | | | | 403 | — 32 |

* Determined using $\varepsilon_{mM} = 31.18$ for the $\alpha$ peak of the pyridine hemochrome spectra of cytochrome *c* (T. FLATMARK, unpublished observations).

The reduced-*minus*-oxidized difference spectrum for C-553(550) is given in Fig. 1B. This spectrum does not correspond to any found with the other cytochromes extracted from *Chromatium*[5] nor to any spectral forms observed in the light-induced difference spectra obtained with *Chromatium* light particles[2].

The absorptivity ratios for the cytochrome and several other heme proteins from photosynthetic organisms are compared in Table III. The ratio $A_{\gamma(red)}/A_{\alpha(red)} = 7.1$ is similar to the corresponding ratio reported for certain cytochromes with highly positive oxidation-reduction potentials from algal and higher plant photosynthetic tissues. The absorption spectrum of reduced C-553(550) at 80°K is given in Fig. 2.

*Stability*

As shown in Fig. 1A, the spectrum in the protein region near 275 nm is broad and unresolved. However, freshly prepared samples less than 2 days old show a sharp absorption peak in this region, centered at 275 nm with $A_{275\,nm}/A_{417.5\,nm} = 0.15$, rather than the value of 0.25 shown in Fig. 1A. The increase of the ratio takes place within a few days after preparation, even when the sample is stored at −20°. Because there was

TABLE III

ABSORPTIVITY RATIOS OF C-553 (550) AND OTHER *c*-TYPE CYTOCHROMES

| *Cytochrome* | *Ref. No.* | $\frac{A_{\gamma(red)}}{A_{\gamma(ox)}}$ | $\frac{A_{\gamma(red)}}{A_{\alpha(red)}}$ |
|---|---|---|---|
| *Chromatium* C-553(550) | This study | 1.24 | 7.1 |
| *Chlorobium thiosulfatophilum* C-555 | 22,23 | 1.12 | 7.1 |
| *Rhodospirillum rubrum c* | 24 | 1.24 | 5.1 |
| Parsley cytochrome *f* | 25 | 1.5 | 7.0 |
| *Anacystis nidulans* C-554 | 25 | 1.40 | 7.1 |
| *Petalonia fuscia* C-553 | 27 | 1.45 | 6.9 |
| *Euglena gracilis* C-552 | 28 | 1.26 | 5.7 |

no detectable loss of absorption in the Soret or visible region of a stored sample, the change in the $A_{275\ nm}/A_{417.5\ nm}$ ratio appeared to be due to an increase in $A_{275\ nm}$.

*Oxidation–reduction potential*

Cytochrome *c*-553(550) was isolated in the nearly completely reduced state and was only slowly autooxidizable. Careful oxidation–reduction titrations as shown in Fig. 3 give a midpoint potential at pH 7.0 of $E_{m,7} = 330$ mV, fitting nicely the theoretical curve for an $n = 1$ electron transfer reaction. This oxidation–reduction potential is similar to that found for the particle-bound photoactive cytochrome *c*-555, $E_{m,7.5} = 319$ mV (ref. 2), and unlike the potentials of *Chromatium* cytochrome *c*-552, $E_{m,7} = 10$ mV, and cytochrome *cc'*, $E_{m,7} = -5$ mV (ref. 5).

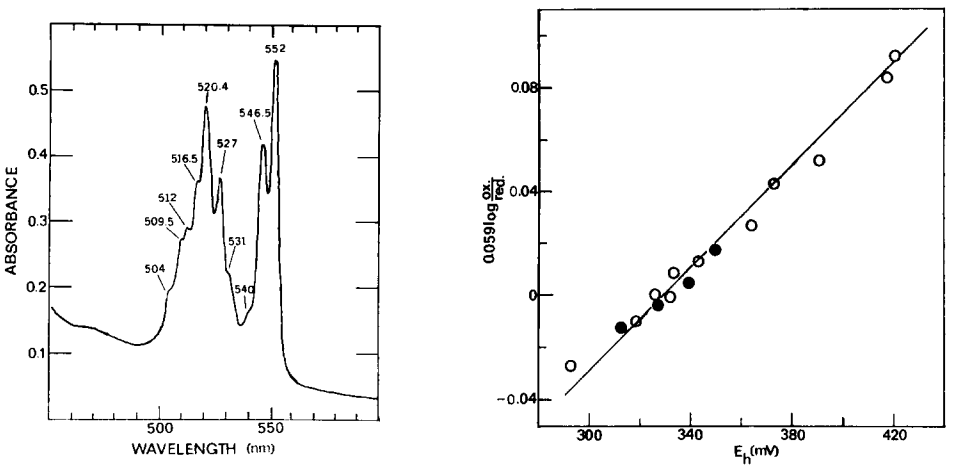


Fig. 2. Absorption spectrum of reduced *Chromatium* cytochrome *c*-553 (550) (200 $\mu$M, 1 mm optical path) at 80°K. The reduced cytochrome was dissolved in 50% (v/v) glycerol *plus* 50 mM Tris (pH 7.3).

Fig. 3. Redox titration of *Chromatium* cytochrome *c*-553 (550) (20 $\mu$M). The cytochrome was dissolved in 100 $\mu$M $K_3Fe(CN)_6$ *plus* 0.1 M potassium phosphate (pH 7.0). ○, reductive titration with 1% (w/v) $Na_2S_2O_4$ in 50 mM phosphate buffer (pH 7.0); ●, oxidative titration with 1 mM $K_3Fe(CN)_6$ in 50 mM phosphate buffer (pH 7.0).

*Isoelectric point determination*

By the electrofocusing technique in the presence of the carrier electrolytes covering the pH range 3–6, the apparent isoelectric point, $I_p = 4.38$, was found. No heterogeneity of colored components was noted. This $I_p$ value is slightly more acidic than the reported values[5] measured by electrophoresis in free solution for *Chromatium* cytochrome *c*-552 ($I_p = 5.1$) and cytochrome *cc'* ($I_p = 5.0$).

*Amino acid composition*

The amino acid composition of C-553(550) is presented in Table IV. Each entry represents the average of at least two determinations made with cytochrome prepared from photoheterotrophically grown cells. In parentheses are those values which differ from the previous values for the cytochrome prepared from cells grown photoauto-

TABLE IV

AMINO ACID COMPOSITION OF C-553 (550)

| *Amino acid* | *Time of hydrolysis* | | | *Integral number of residues** |
|---|---|---|---|---|
| | *24 h* | *48 h* | *72 h* | |
| $CySO_3H$ | 1.0** | | | 1.0 |
| Asp | 3.9 | 4.0 | 3.9 | 4.0 |
| Thr | 2.6 | 2.3 | 2.4 | 2.5 |
| Ser | 2.5 | 2.2 | 2.2 | 2.5 |
| Glu | 8.5 (5.9)*** | 9.0 (6.0) | 8.8 (7.8) | 9.0 (8.0) |
| Pro | 3.7 | 3.5 | 3.6 | 4.0 |
| Gly | 5.7 | 5.4 | 5.3 | 5.5 |
| Ala | 8.5 (6.4) | 8.4 (6.8) | 8.6 (6.5) | 8.5 (6.5) |
| Val | 2.8 | 2.5 | 2.5 | 2.5 |
| Met | 1.7 | 1.6 | 1.9 | 2.0 |
| Ile | 1.5 | 1.3 | 1.4 | 1.5 |
| Leu | 3.0 | 2.8 | 3.0 | 3.0 |
| Tyr | 2.9 (1.7) | 2.3 (1.7) | 2.7 (1.6) | 3.0 (2.0) |
| Phe | 1.0 | 1.2 | 1.1 | 1.0 |
| His | 1.0 | 1.0 | 1.0 | 1.0 |
| Lys | 2.2 | 2.4 | 2.6 | 2.5 |
| Arg | 2.7 | 2.1 | 2.6 | 3.0 |
| Trp | | | | 0.5§ |
| Total | | | | 57 (53) |

* Stated relative to 1 His residue. The cytochrome contains twice the total number of amino acid residues indicated per heme.

** Determined after performic acid oxidation.

*** Values in parentheses are those values for C-553 (550) from photoautotrophically grown cells which differ significantly from the results of the protein prepared from photoheterotrophically grown cells.

§ Trp was inferred from the magnitude of the 275-nm peak relative to that of cytochromes with a known tryptophan content.

trophically. Except for the three amino acids, alanine, glutamic acid and tyrosine, the amino acid composition of the two preparations is identical.

To calculate the amino acid composition in terms of the number of residues per mole of heme in the sample hydrolyzed, the number of residues tabulated must be multiplied by two, giving a total of 114 residues for the photoheterotrophic cytochrome and 106 residues for the photoautotrophic cytochrome. By summing the residue mole weights *plus* the mole weight of protoheme, the minimum formula weight = 12989 is obtained for the photoheterotrophic cytochrome, assuming one heme per molecule.

*Molecular weight*

To determine the hydrodynamic molecular weight by sedimentation–equilibrium centrifugation in a Spinco Model E ultracentrifuge operated at 20°, protein concentrations between 2 and 4 mg/ml were used in the following buffers: 10 mM and 20 mM Tris (pH 7.3) containing 0.1, 0.2 or 0.5 M NaCl. The different NaCl concentrations were tested in an unsuccessful effort to repress the polymerization of the protein. Both photoautotrophic and photoheterotrophic cytochrome samples were analyzed with equivalent results. The rotor was operated at either 20440 or 23150 rev./min in differ-

ent runs and the attainment of equilibrium was checked by calculating the molecular weight at two different times of centrifugation, usually 18 and 24 h. The average molecular weight was calculated as well as the molecular weight at various distances from the top of the centrifuge cell.

Photoheterotrophic C-553(550) gave an average molecular weight of 19000 (two runs) with a distribution from the top to the bottom of the centrifuge cell from 13000 to 30000, indicating gross heterogeneity. Five separate determinations with the photoautotrophic sample gave an average molecular weight of 28000 with a distribution across the cell from 18000 to 50000. Horse heart cytochrome *c* (Sigma Type III which had been purified by gel filtration on Sephadex G 75 (ref. 29) gave an average molecular weight of 12000 ± 1300, indicating that the heterogeneity observed with C-553(550) was not the result of the analytical technique.

*End group analysis*

The N-terminal amino acid, as determined by the method of SANGER[17], was alanine. This result was confirmed by the EDMAN[18] degradation procedure which gave the N-terminal sequence for the photoheterotrophic sample as Ala–Glu–Glu–Leu.

*Light-induced reactions of light particles extracted with 50 % (v/v) acetone*

Light particles were twice extracted with cold 50 % (v/v) acetone–10 mM Tris (pH 7.3). A maximum of 25 % of the total heme of the particles was solubilized, as assayed as alkaline pyridine ferrohemochrome, and of this soluble heme, 7–8 % could be accounted for as C-553(550). The treated particles were not agglomerated or denatured in any obvious way, even though considerable cytochrome and some bacteriochlorophyll had been removed.

The extent of the endogenous light-induced absorbance change of bound cytochrome *c*-555, $\Delta A_{555\,nm}/\mu$mole bacteriochlorophyll, measured with treated particles, was 50–60 % of that measured with the untreated particles[2]. When the oxidation-reduction potential of the suspension of treated particles was poised at $E_h = 250$ mV, the $E_h$ at which maximal extent of reaction of cytochrome *c*-555 in untreated particles is induced, the extent of the reaction was increased to only 70 % of the normal level. Evidently the endogenous reduction of the cytochrome *c*-555 remaining in the extracted particles was essentially unimpaired and the reduced cytochrome was effectively oxidized by a light-induced reaction. Efforts to further increase the reaction by adding reduced cytochrome *c*-553(550) (26–128 $\mu$moles/$\mu$mole bacteriochlorophyll) to the treated particle suspension were unsuccessful. The light-induced absorbance changes in both the native particles and the acetone-extracted particles occurred at comparable rates in so far as could be measured with the Cary spectrophotometer and with comparable intensities of exciting light ($2.2 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$sec$^{-1}$). A careful redox titration of the light-induced oxidation of bound cytochrome *c*-555 gave the midpoint potential, $E_{m,7.5} = 317$ mV, as compared with the value for untreated particles, $E_{m,7.5} = 319$ mV (ref. 2). It must be concluded that if a portion of the cytochrome *c*-555 was removed from the reactive bound state in the treated chromatophores, the cytochrome could not be replaced with purified cytochrome *c*-553(550). Furthermore, the light-induced oxidation of the cytochrome *c*-555 which remained in the treated particles was not impaired.

## DISCUSSION

The goal of this work was to extract and characterize membrane-bound cytochromes of the light-induced electron-transport chain of *Chromatium*. To this end one new component, C-553(550), was isolated. A variety of other extraction procedures which were tried using aqueous buffers *plus* various concentration of sodium dodecyl sulfate, sodium cholate, and sodium deoxycholate and phospholipase A as solubilizing agents failed to yield new heme proteins (M. A. CUSANOVICH, unpublished observations). Observation of small amounts of C-553(550) isolated from extracts of acetone powders (T. HORIO, private communication) and from aqueous extracts of *Chromatium* cells had been neglected in the past.

Although there is no direct evidence to relate C-553(550) to any of the bound proteins previously detected in *Chromatium*, some speculations may be made concerning this possible relationship. On the basis of amino acid composition, absorption spectra and oxidation–reduction potentials, it can be concluded that C-553(550) is not related to any of the *Chromatium* heme proteins previously characterized. The yield of C-553(550) suggests that it is not a major component, although not all the C-553(550) which can be solubilized is recovered and treatment with 50 % (v/v) acetone may not solubilize all of the bound C-553(550). Therefore the total amount of the cytochrome or its bound precursor may be significantly higher than that recovered in soluble form.

Based on the essentially identical oxidation–reduction potentials of C-553(550) and bound cytochrome *c*-555, it is tempting to equate the two. Unfortunately the oxidized-*minus*-reduced difference spectrum of C-553(550) is not identical with the light-induced difference spectrum of bound cytochrome *c*-555, nor will addition of isolated C-553(550) to particles depleted by acetone extraction cause an increase in the apparent magnitude of the light-induced absorbance change of cytochrome *c*-555. It may be suggested that upon solubilization, the bound cytochrome is converted to the C-553(550) form with preservation of only part of the properties of the bound form. Upon solubilization, the absorption spectrum may be changed, and perhaps because of the tendency to polymerize, as indicated by the ultracentrifuge results, the cytochrome is unable to recombine with the photoactive center of the depleted membrane particles.

Certain of the properties of C-553(550) are strikingly similar to those of highly positive oxidation–reduction potential cytochromes *f* isolated from plants and algae and thought to be part of photosystem I in these organisms. The similar absorptivity ratios of high potential *Chromatium* C-553(550) and *Chlorobium thiosulfatophilum* C-555 and various cytochromes *f* are contrasted with those of *Rhodospirillum rubrum* cytochrome $c_2$ in Table III. The cytochromes which resemble cytochrome *f* all have an asymmetrical $\alpha_{(red)}$ peak with the exception of *Euglena gracilis* cytochrome *c*-552, but all those tested, including the Euglena protein, show pronounced splitting into multiple $\alpha$ peaks when cooled to 80°K. The low temperature splitting of the absorption spectrum of C-553(550) is illustrated in Fig. 2. This behavior was first reported by BONNER[30] for parsley cytochrome *f*. The similarity between the absorption spectra of cytochrome *c*-555 of *C. thiosulfatophilum* and algal cytochromes *f* has been reported by YAMANAKA AND OKUNUKI[23].

With respect to spectral properties, oxidation–reduction potentials, and the possible implication as a primary participant in light activated electron-transfer reactions, C-553(550) may be grouped in the cytochrome *f* class.

Cytochrome *c*-553(550) contains a single covalently bound heme in a molecule of approx. 13000 minimum molecular weight as determined by amino acid analysis, but in solution the cytochrome appears to exist as a mixture of a small amount of monomer *plus* polymers containing two, three or possible four monomer units. The presence of but one N-terminal amino acid indicates that the cytochrome consists of but one peptide chain and that the preparations were probably homogeneous. Homogeneity is also indicated by the electrophoretic behavior of the cytochrome. The changes in the absorptivity ratios of the ultraviolet and $\gamma$ absorption bonds as a function of the length of time of storage may be related to the possible progressive increase in the degree of polymerization after release of the cytochrome from the binding site in the chromatophore.

Of a variety of solubilization techniques tried, only the treatment with 50% (v/v) acetone–buffer, a method very similar to that used to solubilize *Chromatium* bacterial ferredoxin[31,19], has yielded an appreciable amount of a soluble form of what was probably a membrane-bound cytochrome of *Chromatium* chromatophores. Unfortunately the solubilized cytochrome *c*-553(550) was not identified with certainty with the membrane-bound cytochrome *c*-555 which appears to be primary reductant of reaction center bacteriochlorophyll in the *Chromatium* light-activated electron-transport system.

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