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WATER-SOLUBLE CYTOCHROMES FROM A BLUE-GREEN ALGA

I. EXTRACTION, PURIFICATION, AND SPECTRAL PROPERTIES OF CYTOCHROMES C (549, 552, AND 554, *ANACYSTIS NIDULANS*)

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SUMMARY

1. Three water-soluble cytochromes were isolated from the blue-green alga, *Anacystis nidulans*, by an aqueous extraction of lyophilized cells, the most satisfactory technique of the several tried. Separation of the cytochromes was accomplished on a DEAE-cellullose column and 2 of the cytochromes were highly purified using $(NH_4)_2SO_4$ fractionation and further column chromatography.

2. The absorption spectra of the most highly purified samples of all 3 cytochromes resemble those of *c*-type cytochromes with α bands at 549, 552, and 554 mμ.

3. Cytochrome C (549) appears anomalous in its unusually low α peak at 549 mμ, and in being very autoxidizable.

4. The stability of cytochromes C (549 and 554) towards heat, and alkaline and acid pH is described.

INTRODUCTION

Although cytochromes have been isolated from many plant and microbial sources, prior to our work there was only a single report on isolation and partial purification from a blue-green alga[1]. In view of the supposed primitive evolutionary position of the Cyanophyta it is of comparative interest to examine the distribution of cytochromes within the group. Further, in several blue-green algae, notably *Anacystis nidulans*, absorption changes *in vivo* have been ascribed to turnover of a *c*- or *f*-type cytochrome during photosynthesis[2,3]. Hence we have proceeded to isolate and characterize the several *c*-type cytochromes of Anacystis[4] and present here an extended and detailed account of our work.

Abbreviations: cytochrome C (549), cytochrome C (549, *Anacystis nidulans*); cytochrome C (552), cytochrome C (552, *Anacystis nidulans*); cytochrome C (554), cytochrome C (554, *Anacystis nidulans*).

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METHODS AND MATERIALS

Algal culture

Anacystis nidulans Drouet was grown in large all-glass continuous culture chambers[5] which were manually controlled on a daily regimen of partial harvest and dilution. Growth was in Medium C of KRATZ AND MYERS[6] modified by increasing the KNO_3 concentration from 1.0 to 4.0 g/l. Aeration was provided by 5 % CO_2 in air and illumination by 30-W warm-white fluorescent lamps alone or in combination with tungsten lamps. The temperature was maintained at 35° or 37°. Immediately after harvest the algae were concentrated in a Sharples centrifuge, lyophilized (except where noted), and stored in brown bottles at 4°. Typical daily cell production from 2 chambers was about 8 g dry weight in 2.5 l harvest from a total culture volume of about 3.5 l.

Detection of cytochromes

For qualitative detection of cytochromes in the extracts, the visible spectrum was scanned using a Cary spectrophotometer (Model 14M). An aqueous extract of Anacystis contains other water-soluble pigments, notably phycocyanin with a broad absorption band at 620 mμ, chlorophyll *a* with peaks at 677 mμ and 433 mμ, and oxidized pteridines peaking at about 410 mμ. The broad phycocyanin band obscures detection of α-cytochrome bands near 550 mμ while the pteridine band at 410 mμ makes it impossible to observe the cytochrome Soret bands. Fortunately, sodium dithionite, reduces pteridines and removes their 410-mμ absorption so that the presence of reduced cytochrome Soret bands can be observed. Because of some residual interference by chlorophyll and pigments, observation of the Soret band in reduced extracts is useful only for the qualitative detection of cytochromes.

Extraction procedures

In order to compare the effectiveness of various extraction procedures, a semi-quantitative total cytochrome determination was devised. After extraction of the algae by any of the procedures described below, an $(NH_4)_2SO_4$ fractionation carried out at 4° was used to remove chlorophyll, phycocyanin, and pteridines. Both chlorophyll and phycocyanin were precipitated at 42 % of saturation with $(NH_4)_2SO_4$, the precipitate was removed by centrifugation and cytochromes remained in the supernatant. The cytochromes were precipitated at 70 % of saturation with the pteridines remaining in solution. The cytochrome precipitate was dissolved in water, dialyzed against distilled water, and the absorption spectrum run after addition of dithionite. Cytochrome concentration was estimated in terms of cytochrome *c* per g dry wt. algae using the ε_M (0.129) of the Soret absorption peak[7] and mol. wt. (12000) of horse-heart cytochrome *c*. Although the data obtained by this procedure have little significance on an absolute basis, they proved to be sufficiently reliable for comparison of extraction procedures.

Initial extraction procedures involved sonication of freshly harvested cells as used by KATOH[1] with Tolypothrix and HONDA, BAKER AND MUENSTER[8] with the green alga, *Chlorella vulgaris*. It soon became apparent that procedures giving maximum extraction of phycocyanin also gave maximum extraction of cytochromes. Therefore, sonification in acetate buffer, commonly used in phycocyanin preparation,

was studied under various conditions. Table I shows that with fresh cells, sonication in 1 M acetate buffer (pH 4.7), was the most effective extraction procedure of those tried.

Lyophilized cell material, often used for extraction of cytochromes from bacteria, also proved to be more suitable than fresh cells for Anacystis (Table II). Distilled water was the most effective extractant of those tried. Variations in ratio of ml water/g lyophilized cells of from 9 to 40 and extraction times varying from 2 to 25 h were almost equally effective and gave an average yield in 12 experiments of 1.3 mg/g with a range of 1.0–1.7 mg/g. An acetone powder preparation of cells was equally as good

TABLE I

SUMMARY OF CYTOCHROME EXTRACTION PROCEDURES USING FRESH CELLS

Expt. No.	*Cell disruption procedure*	*Extraction procedure*			*Extraction time (h)*	*Yield* (mg/g)*
		Medium	*Concn. (M)*	*pH*		
17	Sonication	Acetate	1.0	4.7	0.7	0.4**
18C	Sonication	Tris	0.2	8.1	0.5	0.1
19B	Sonication	Distilled water			0.7	0.1
20B	Sonication	Acetate	0.2	4.7	0.7	0.2
20C	Sonication	Acetate	1.0	5.5	0.7	0.2
22A	Frozen	Acetate	1.0	4.7	24	0.01
22B	None	Acetate	1.0	4.7	48	0.0

* Expressed in terms of mammalian cytochrome *c* per g dry wt. as described in METHODS AND MATERIALS.

** In 5 other similar experiments, the average was 0.28 mg/g and the range was 0.20–0.38 mg/g.

TABLE II

SUMMARY OF CYTOCHROME EXTRACTION PROCEDURES USING LYOPHILIZED CELLS

Expt. No.	*Cell disruption procedure*	*Extraction procedure*			*Extraction time (h)*	*Yield* (mg/g)*
		Medium	*Concn. (M)*	*pH*		
21A	Sonication	Acetate	1.0	4.7	0.7	0.5
23A	None	Acetate	1.0	4.7	4	0.9
23B	None	Acetate	0.2	4.7	4	1.0
23C	None	Acetate	1.0	5.4	4	1.0
23D	None	Water, 9 ml/g cells			4	1.5
23E	None	Tris	0.2	8.1	4	1.5
25A	None	Water, 40 ml/g cells			4	1.2
25B	None	Water, 27 ml/g cells			4	1.3
32B	None	Water, 9 ml/g cells			4	1.0
32A	None	Water, 6 ml/g cells			4	0.6
25C	None	Water, 15 ml/g cells			4	1.4
26A	None	Water, 15 ml/g cells			2	1.0
26B	None	Water, 15 ml/g cells			6	1.2
26C	None	Water, 15 ml/g cells			25	1.0
29	None**	Water, 13 ml/g cells			16	1.2

* Expressed in terms of mammalian cytochrome *c* per g dry wt. as described in METHODS AND MATERIALS.

** An acetone powder rather than lyophilized cells was used in Expt. No. 29.

as lyophilized cells for cytochrome extraction, but the latter were used routinely for reasons of convenience.

Separation of the cytochromes on DEAE-cellulose

From the extracts most of the cytochrome, the phycocyanin, the chlorophyll and various other pigments were adsorbed on a DEAE-cellulose column. DEAE-cellulose was prepared by suspending it in 0.005 M phosphate buffer (pH 7.0), titrating it to pH 7.0 with 0.2 M HCl, and washing it successively with several rinses each of 75 volumes of 0.005 M phosphate (pH 7.0), and of glass-distilled water. During elution from the first DEAE-cellulose columns it became evident that more than one cytochrome component was present. Low phosphate buffer concentrations, eluted a reduced cytochrome with an α band at 554. Somewhat higher buffer concentration eluted a cytochrome in the oxidized form which on reduction had an α band at 549. The elution pattern of a typical experiment is shown in Fig. 1. While elution was usually done with a pH 7.0 phosphate buffer, pH variation over the range of 6.85–7.4, as indicated in the figure, did not affect the elution pattern significantly. The buffer concentration at which the cytochrome fronts came off the column depended on the concentration of dissolved substances in the extract, the size of the column, and the rate of the step-wise gradient increase in buffer concentration used. As is evident

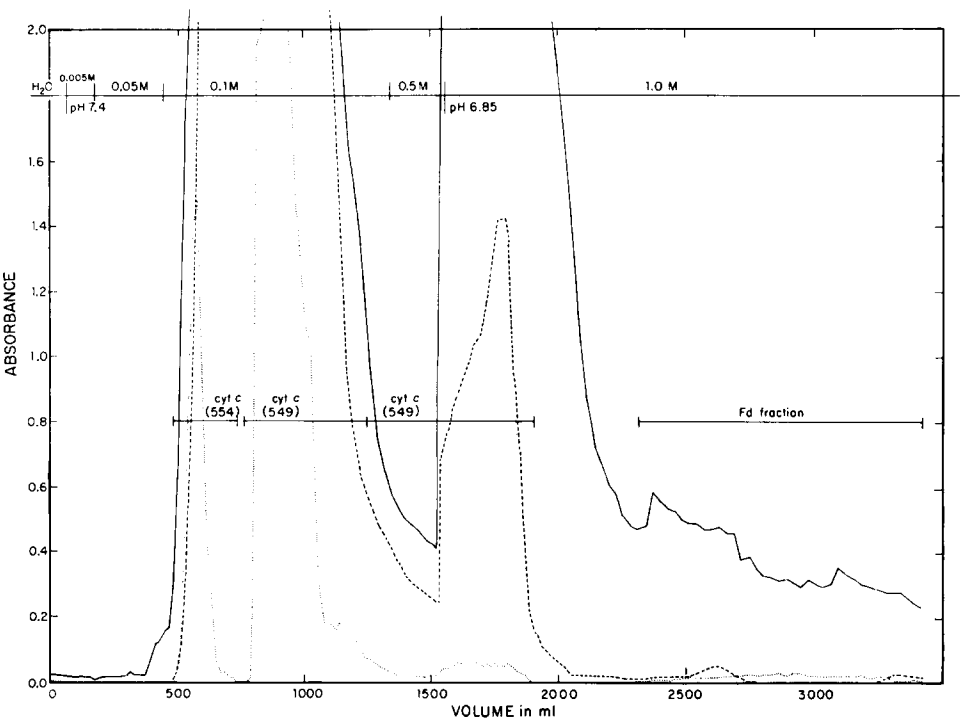

Fig. 1. A chromatographic elution pattern of Anacystis water-soluble pigments from DEAE-cellulose. Step-wise increases of phosphate buffer of the concentration and pH indicated in the upper horizontal line were used. ———, $A_{280\,m\mu}$; – – – –, $A_{620\,m\mu}$; ·····, absorbance in the Soret region (417 or 408 mμ) or at 420 mμ. The eluate was collected in fractions of about 15 ml and those pooled containing cytochrome C (554), cytochrome C (549), and ferredoxin (Fd) are indicated by the horizontal line in the middle of the figure.

in the figure, elution of phycocyanin (absorption at 620 mμ) begins very soon after elution of cytochrome C (554), and it is present to a greater or lesser extent in all cytochrome fractions collected. It may also be noted that cytochrome C (549) is eluted in a more heterogeneous manner than is cytochrome C (554) and several peaks at different phosphate buffer concentrations are obtained.

After concentration of the extract that passed through the DEAE-cellulose column a third *c*-type cytochrome with an α band at 552 mμ was detected but was present in much lower amounts than the 2 adsorbed cytochromes. Detailed procedure for extraction and separation of the cytochromes is summarized in the flow-sheet in Fig. 2.

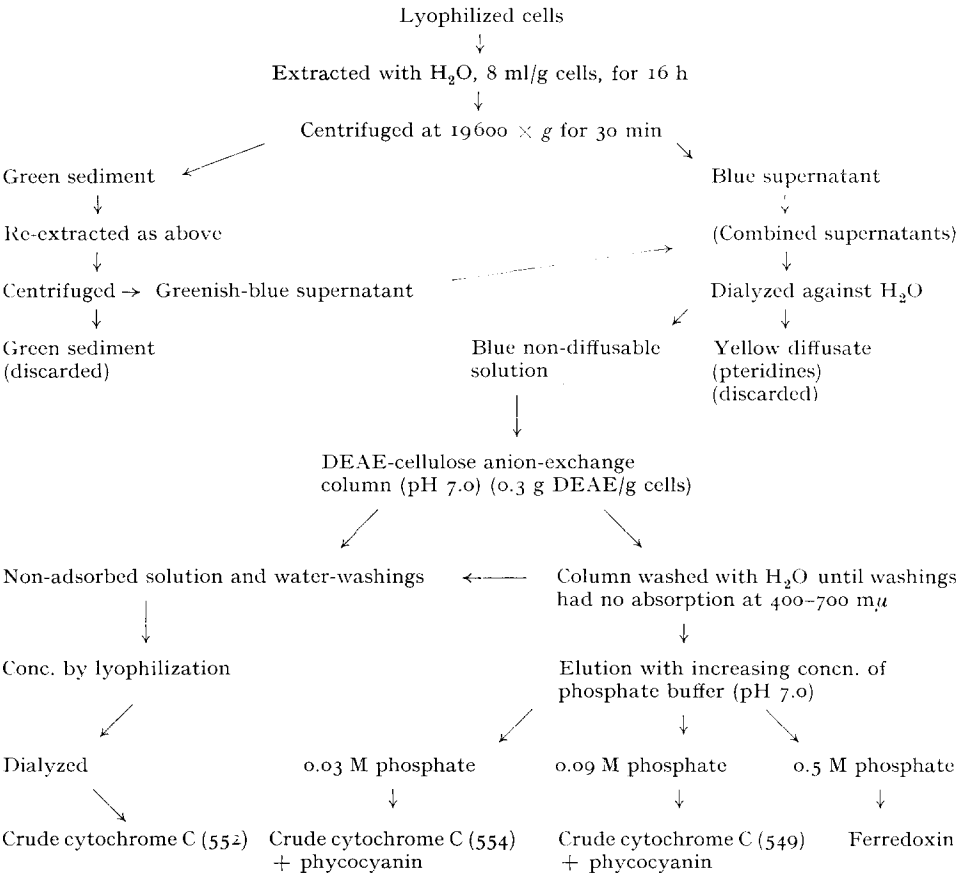

Fig. 2. Flow-sheet for the extraction and separation of crude cytochrome fractions from Anacystis.

Purification of cytochrome C (549)

The fractions collected from the DEAE-cellulose column that contained cytochrome C (549) were dialyzed against glass-distilled water and concentrated by lyophilization. Much of the phycocyanin was removed by precipitation with solid $(NH_4)_2SO_4$ added to 35 % of saturation and the precipitate removed by decantation and filtration through a millipore filter 0.45 μ pore size. Recovery of traces of cyto-

chrome from the phycocyanin precipitate was attempted but found to be impracticable. After addition of $(NH_4)_2SO_4$, the pH was about 5.5; adjustment of pH with NH_4OH to pH 7.0 or 8.5 resulted in less precipitation of phycocyanin without affecting the precipitation of the cytochrome, so routinely no pH adjustment was made.

The cytochrome was precipitated from the supernatant fraction by addition of solid $(NH_4)_2SO_4$ to 56 % of saturation. Further purification was effected on DEAE-cellulose using first a pH 6.0 phosphate buffer as an eluting agent with the concentration increased in a step-wise manner (Fig. 3). Most cytochrome came off at 0.025 M

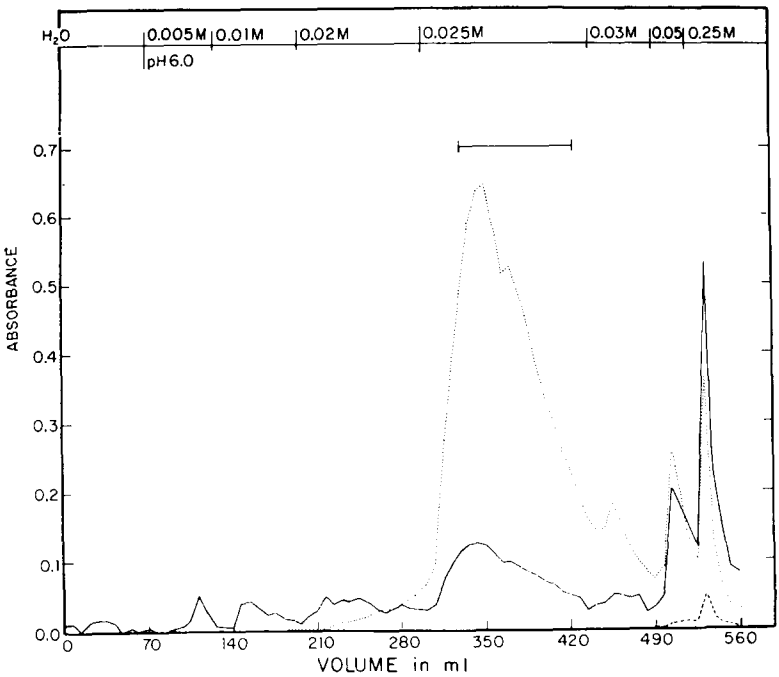

Fig. 3. Chromatography of cytochrome C (549) on DEAE-cellulose. Phosphate buffer (pH 6.0) of the indicated concentrations was used as a step-wise eluting agent. The horizontal bar indicates the fractions pooled with ratios of $A_{408\,m\mu}$ (· · · · · ·) to $A_{280\,m\mu}$ (- - - - -) of 4.2 or above. The shoulder on the main peak is considered to be an artifact since the ratio did not change at that point. Phycocyanin absorbance (- - - - -) appears only after most of the cytochrome is eluted.

buffer but several other cytochrome bands were removed at higher concentrations. Phycocyanin present was not eluted until buffer concentrations of about 0.05 M were reached so that this procedure was useful in freeing the cytochrome solution of traces of that pigment. The next step was another fractionation on DEAE-cellulose, but this time a pH 7.0 phosphate buffer was used for elution. At this pH the cytochrome was eluted at about 0.04 M buffer concentration. Step-wise concentration increases during elution were more useful than gradient elution because each fraction could be scanned in the spectrophotometer after collection and the decision then made as to whether or not to go on to a higher buffer concentration. Often it was necessary to repeat the fractionations at pH's 6 and 7 in order to get the cytochrome free or nearly free of contaminating proteins as determined by the criteria used (see below). Other

chromatographic adsorbents including Sephadex G-100, Amberlite XE-64, and CM-cellulose were tried but did not prove to be useful.

Purification of cytochrome C (554)

The pooled, dialyzed fractions from the original DEAE-cellulose columns containing cytochrome C (554) also contained some phycocyanin and colorless proteins. $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography (as used with cytochrome C (549)) freed this cytochrome of phycocyanin but disc-electrophoretic analysis (see below) showed that several other proteins were present in low concentration. Various other column-chromatographic procedures including DEAE-Sephadex G-50, Amberlite XE-64, CM-cellulose, and Sephadex G-100, did not further purify this cytochrome. Oxidation of the cytochrome before DEAE-cellulose chromatography did not improve the separation.

The final purification step was disc electrophoresis on polyacrylamide gel scaled up from the usual analytical procedure (see below) in a lucite tube 21 mm in diameter and 15 cm long. Twenty ml of the lower fine-pore gel, 10 ml of spacer gel and 10 ml of upper sample gel (5 ml of cytochrome solution and 5 ml upper gel) were added and polymerized in that order. A current of approx. 15 mA was applied and the movement of the red cytochrome band followed visually. Moving at about 3.3 mm/h, it reached the bottom of the gel in about 30 h at 4°. The cytochrome band was then electrophoresed into a dialysis bag slipped over the bottom of the column. Contaminating substances (non-protein) obtained from the gel column were removed by subsequent dialysis and chromatography on a small DEAE-cellulose column. A small amount of brown pigment remained at the top of the cellulose column after elution of the red cytochrome C (554) with 0.025 M phosphate buffer (pH 7). The final cytochrome preparation appeared to be free of other proteins by the criteria used.

Partial purification of cytochrome C (552)

Because only small amounts of this cytochrome are present in Anacystis extracts, very little was available and only partial purification has been carried out. This cytochrome was adsorbed on Amberlite XE-64 but not on CM-cellulose at pH 7.0. The XE-64 was used to partially purify this cytochrome. Disc electrophoresis of the partially purified preparation indicated that at least 5 other proteins were present.

Criteria for purity

Disc electrophoresis on polyacrylamide-gel columns was used. A special modification allowed staining of the same column by both the general protein stain and a specific heme stain. In a lucite column (6 mm inside diameter) holes were drilled on either side at a point about 1 mm above one end. A fine platinum wire was inserted in the hole and cemented in place. This end was used as the bottom and the gel layers prepared and the column run in the usual manner. At the end of the run, the gel was extruded from the bottom by application of water pressure from a syringe attached to the top of the column. As the gel column passed out the bottom, the wire cut it into 2 equal halves, one of which was added to the protein stain (amido-schwartz) and the second to a freshly prepared heme-specific benzidine stain (5 parts 0.2 % benzidine in ethanol, 10 parts 3 % acetic acid, 1 part 1 % sodium nitroprusside, and

1 part 3 % H_2O_2). After about 1 h, the excess heme stain was poured off and the gel stored in 7.5 % acetic acid. From this half of the gel column, any heme proteins on the amido-schwartz-stained half could be located. When at high concentrations the heme proteins could be followed visually as they moved down the column during electrophoresis.

An analytical ultracentrifuge was also used to check for protein homogeneity. A Spinco Model E ultracentrifuge at 20° with a synthetic boundary cell was used for the determination of sedimentation data at 59780 rev./min in 0.05 M potassium phosphate buffer (pH 7.0).

Conventional starch-gel electrophoresis was also used. Samples were dialyzed against the Tris–borate buffer (pH 8.6) used in the preparation of the gel. Experiments were run for about 2 h with a potential difference of 400 V. At the end of the run, the gel was sliced in two and one half stained with the general protein stain and the other with a heme-specific benzidine stain described above.

RESULTS

Spectroscopic properties

The absorption spectrum of the most highly purified cytochrome C (549) is shown in Fig. 4. The spectrum is similar to that of other *c*-type cytochromes and the absorption maxima are summarized in Table III. Isobestic points in the visible region were found at 557, 540, 529, 507.5, 430.5, and 411.5 mμ. Because of autoxidizability of this cytochrome, the absorption maxima of the reduced form were generally ob-

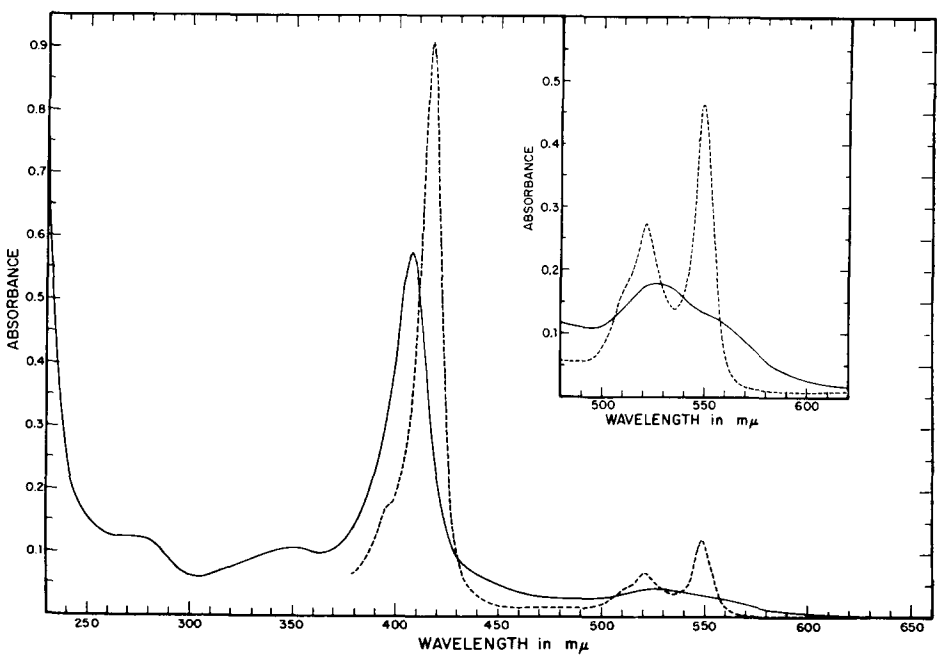

Fig. 4. Absorption spectrum of highly purified cytochrome C (549) at room temperature. ———, oxidized form; - - - - -, reduced form ($Na_2S_2O_4$). Protein concn., 0.09 mg/ml. Inset: A more concentrated sample showing 500–600-mμ region.

TABLE III

SPECTROSCOPIC PROPERTIES OF WATER-SOLUBLE CYTOCHROMES FROM ANACYSTIS

Absorption peaks (mμ)	*Cytochrome C (549)*	*Cytochrome C (554)*	*Cytochrome C (552)*
Reduced form (α, β, Soret, δ, protein)	549, 521.5, 417.5 322, 279	554, 522.5, 416.5 316, 276	552, 523.5, 420
Oxidized form (α, β, Soret, protein)	525–30, 408, 351, 277	525–30, 411, 360, 275	526, 410, 352, 279
Absorbance ratios			
β/α	0.58	0.68	0.64
Soret/α	7.5	7.1	7.3
α/protein	0.97	0.84	(0.21) *
Soret (red.)/Soret (ox.)	1.6	1.4	1.5

* Contaminating proteins present contribute to absorbance at protein peak.

tained using sodium dithionite in the visible region and borohydride below 380 mμ where the dithionite absorbs. The absorbance ratios (Table III) are averages for 2 different highly purified preparations, one of which showed no other proteins by disc and starch electrophoresis and the second of which showed traces of other proteins. Because the α/protein absorbance ratios were essentially the same in these 2 samples, the contaminants were assumed to contribute only negligibly to absorption.

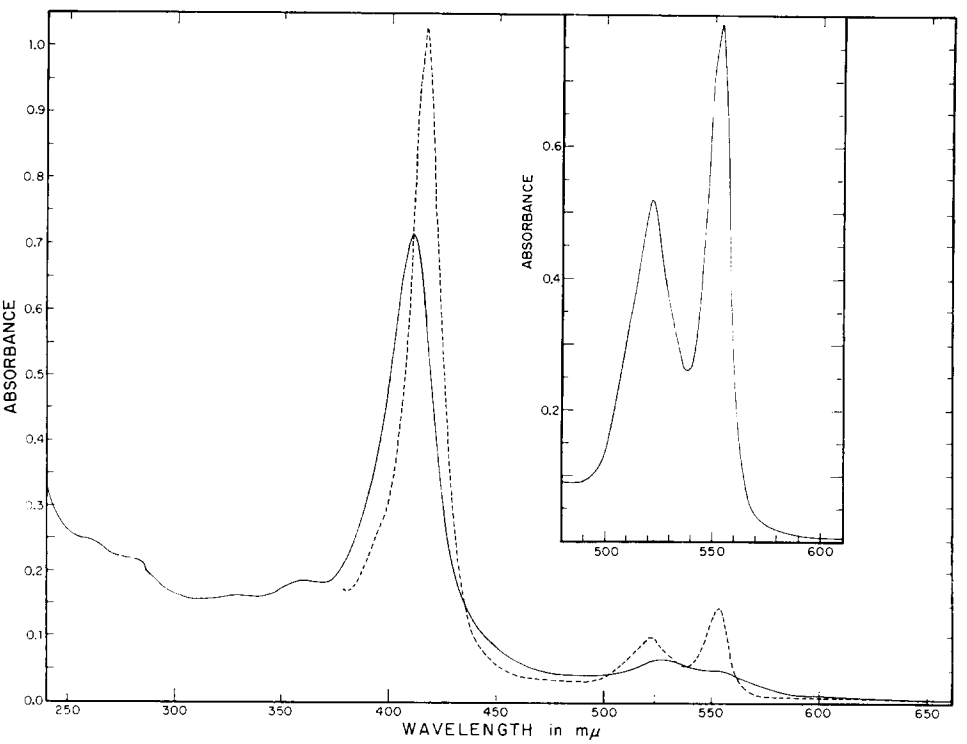

Fig. 5. Absorption spectrum of highly purified cytochrome C (554) at room temperature. ———, oxidized form; – – – –, reduced form. Inset: A more concentrated reduced sample showing the 500–600-mμ region.

Cytochrome C (549) appears to possess the rather unique property of an α absorption peak at lower wavelength than that of horse-heart cytochrome *c* whose stated α peak at 550 mμ was obtained also with the same spectrophotometer. The α peak is sharp and symmetrical suggesting that no contaminating cytochromes were present. The β peak has a slight shoulder at about 510 mμ. The protein peak in the most highly purified preparation is not sharp and is distinguished only by a broad shoulder before the end absorption begins.

The spectrum of a sample of cytochrome C (554) that was freed of contaminating proteins using preparative disc electrophoresis is shown in Fig. 5. Cytochrome C (554) is not appreciably autoxidizable but to insure complete reduction, a small amount of dithionite was added and the excess was removed by aeration or dialysis. Cytochrome C (554) also has a sharp and nearly symmetrical α absorption peak. Concentrated solutions appear to have a slight shoulder on the blue side at about 550 mμ and similar shoulders are found in other *f*-type cytochromes[8,9].

Fig. 6 shows the absorption spectrum of a sample of cytochrome C (552). While disc electrophoresis demonstrated that no contaminating cytochromes were present, at least 5 other protein bands were detected as might be expected from the relatively strong protein absorption band in relation to the α absorption band at 552 mμ. Because of the small amount of material available, little has been done with this cytochrome. The spectroscopic properties resemble those of other *c*-type cytochromes.

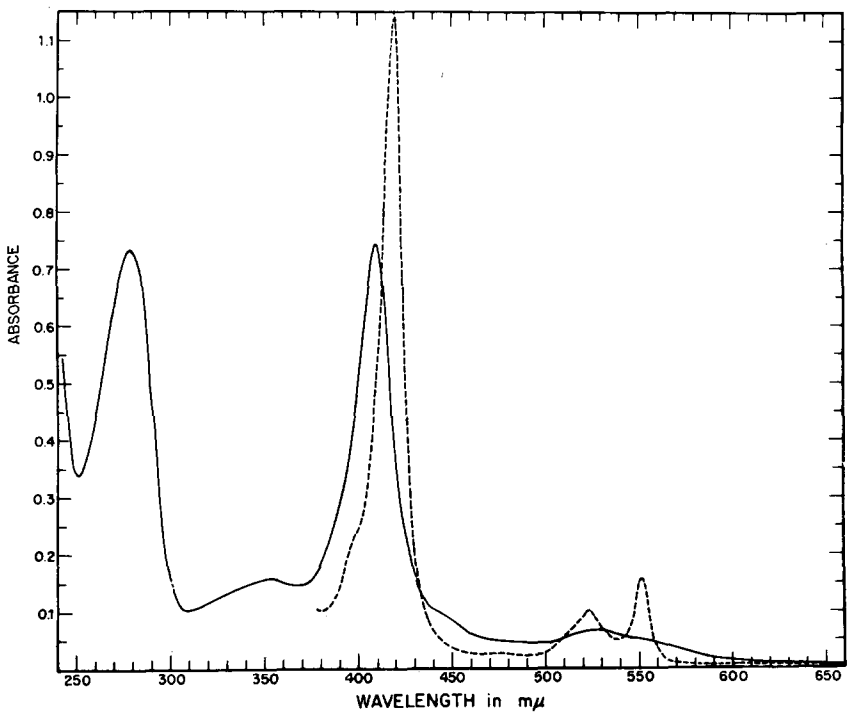

Fig. 6. Absorption spectrum of purified cytochrome C (552) at room temp. ———, oxidized form; – – – –, reduced form.

Stability

The effects of various denaturing agents were examined in terms of changes in the absorption spectra. Cytochrome C (549) in pH 7.0 phosphate buffer was exposed to 60° for 5 min and loss in absorption of both the Soret peaks in the oxidized and reduced forms and the appearance of turbidity in the cuvette were taken as indications that denaturation had occurred.

When a solution of reduced cytochrome C (554) in pH 7.0 phosphate buffer was kept at 60° for 5 min, some autoxidation of the reduced cytochrome occurred. Further autoxidation of this cytochrome solution occurred after standing overnight suggesting that some heat denaturation had occurred.

The effect of pH on the stability of cytochromes C (549 and 554) was examined by placing them in buffers of pH's 2.9 and 4.9 (phthalate), 7.0 (phosphate), 8.4 and 9.3 (Tris), and 11.2 (β-alanine). Cytochrome C (549) at pH 4.9 and 7.0 showed no change in spectra of the reduced or oxidized forms during the 6 h of observation. At pH 2.9, precipitation of the cytochrome occurred and no peaks could be observed. At pH's 8.4 and 9.3, the reduced Soret peak decreased in height by 50 % in 4 h at pH 8.4 and 70 % in 2.5 h at pH 9.3. At pH 11.2, the α absorption peak disappeared completely and only a slight Soret peak could be noted. Cytochrome C (549) appears to be more sensitive to alkaline than to acid conditions.

Cytochrome C (554) showed no change in its spectrum in the reduced form in buffers at pH's 4.9, 7.0, and 9.3. At pH 2.9, some precipitation occurred but the principle absorption peaks remained visible. At pH 11.2, the reduced Soret peak decreased by 50 % in height in 24 h and its wavelength shifted from 417 to 414 mμ while the α absorption band disappeared. Addition of dithionite did not bring back the usual reduced spectrum suggesting that denaturation under alkaline conditions was not due to a simple conversion to an autoxidizable form of the protein. In summary, cytochrome C (554) is considerably more stable to acid and basic pH than is cytochrome C (549).

Purity

While inferences as to purity could be obtained from the chromatographic patterns and absorption spectra, the final tests for purity were 'disc' electrophoresis, starch-gel electrophoresis and preliminary ultracentrifugal analyses.

As is mentioned above, only cytochromes C (549 and 554) were highly purified. Disc-electrophoresis patterns of the most highly purified samples of each of these cytochromes appeared free of other proteins. In starch-gel electrophoresis, these cytochromes moved towards the anode but at very similar migration rates. The results of starch-gel electrophoresis indicated little if any contaminating proteins but because of the similar migration rates, the patterns were not highly discriminating.

With cytochrome C (549), a sedimentation run in the ultracentrifuge was homogeneous but the sample was quite dilute. A second run with a different more concentrated sample, showed minor contaminants. No runs were made with cytochrome C (554) samples. Attempts at crystallization of cytochromes C (549 and 554) were unsuccessful with the quantities at hand.

The best samples are therefore considered 'highly purified' and free of contaminating cytochromes but with the possibility that traces of other proteins are present.

DISCUSSION

Our knowledge of the water-soluble cytochromes, particularly cytochrome *c*, has reached such a highly refined state that suggestions as to the evolution of this molecule in heterotrophic organisms are possible[10]. In chlorophyll-containing organisms, the search for information about the cytochrome complement is complicated by both the presence of other cytochromes known to be involved in photosynthesis and by the presence of other pigments which make the detection and isolation more difficult. With the blue-green alga, Anacystis, an aqueous extraction of lyophilized cells has proved to be effective means of removing the cytochromes while DEAE-cellulose chromatography is useful in separating the cytochromes from other pigments. These general procedures may be useful in the extraction of cytochromes from other photosynthetic organisms.

Spectrally, cytochromes C (549, 552 and 554) appear to be *c*-type cytochromes and this belief is confirmed by chemical data in an accompanying paper[11]. The cytochromes with α peaks at 549 and 552 mμ resemble mammalian cytochrome *c*, while the cytochrome with the α peak at 554 mμ is similar to the photosynthetic cytochrome which is also a *c* type. However, an α peak at less than 550 mμ as in Anacystis cytochrome C (549) appears to be very rare. The only other similar cytochrome *c* reported is that from *Candida krusei*[12] also with an α peak at 549 mμ. The possible significance of the α peak at this wavelength is unknown.

In mammalian cytochrome *c*, the ratio of the reduced Soret absorbance to that of the α band is usually about 5. In the Candida cytochrome *c*, this ratio is 4.1 (see ref. 12), while in Anacystis cytochrome C (549), the ratio is 7.5. This high absorbance ratio of the reduced Soret to the α band is typical of the *f*-type cytochromes of leaves[13] and algae[8,9,14]. However, it should be noted that in Euglena, a somewhat lower ratio was found by PERINI, KAMEN AND SCHIFF[15] than that of WOLKEN AND GROSS[14] and the difference was attributed by the former to a higher purification and elimination of impurities absorbing in the blue-violet region. Thus Anacystis cytochrome C (549) more closely resembles the photosynthetic *c* (or *f*-type) cytochromes in this way in spite of the fact that the α peak is close to the wavelength of that of the mammalian cytochrome *c* of the respiratory chain. Anacystis cytochrome C (554) clearly is like other photosynthetic cytochromes both in the wavelength of the α absorbance band at 554 mμ and in the 7.1 absorbance ratio of the reduced Soret to the α band.

In conclusion, it is clear that the absorption spectra of the 3 Anacystis cytochromes resemble those of other *c*-type cytochromes. Detailed physicochemical studies[11] confirm these conclusions and contribute further information about the anomalous cytochrome C (549).

ACKNOWLEDGEMENTS

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