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CHARACTERIZATION OF CYTOCHROME *c*-550 FROM CYANOBACTERIA

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Cytochrome *c*-550 has been purified from several cyanobacteria. It is a low-potential, auto-oxidizable cytochrome. This cytochrome should not be confused with a degradation product of cytochrome *f* which may be formed during the isolation of the latter protein. Cytochromes *c*-550 are distinctive in size, amino-acid composition and N-terminal amino-acid sequence.

Introduction

In 1963, Holton and Myers [1] described a low-potential, auto-oxidizable cytochrome *c*-549 in *Anacystis nidulans* and they later characterized this cytochrome in considerable detail [2,3]. Subsequently, there have been occasional reports of this cytochrome in cyanobacteria [4,5]. Several reports describing this cytochrome in eukaryotes, a red alga [6], a diatom [7], and a green alga [8], suggest a wider distribution of this protein. In 1981, Gomez-Lojero et al. [9] reported the conversion of cytochrome *f* into a low-potential, auto-oxidizable form with an absorption spectrum similar to that of cytochrome *c*-549. Since other *c*-type cytochromes can be converted to low-potential, auto-oxidizable forms by partial denaturation, we reinvestigated the identity of cytochrome *c*-549. Here, we call the cytochrome *c*-550 instead of *c*-549, since our measurements of the alpha band peak of the protein from several genera center on 550 nm but all the other properties of these proteins indicate they are the same as the cytochrome *c*-549 of Holton and Myers [1].

Materials and Methods

Aphanizomenon flos-aquae was collected from Lake Okoboji, IA and *Microcystist aeruginosa* from Lake Kegonsa, WI. *Spirulina maxima* was obtained from the commercial culture of Sosa Texcoco at Lake Texcoco, Mexico, D.F. *Anabaena variabilis* (B377) and *Anacystis nidulans* (625) were obtained from the culture collection of algae at the University of Texas and grown on medium C of Kratz and Myers [10]. *Porphyridium cruentum* was also obtained from this source and was grown on artificial sea water medium [11]. *Agmenellum quadruplicatum* (UTEX 2268) was a gift of Dr. E. Stevens, Pennsylvania State University. Cytochrome *c*-550 was prepared from these organisms as described by Ho et al. [5] and was subjected to a final purification by reversed-phase high performance liquid chromatography before carrying out the amino-acid composition and amino-acid sequence analyses. The isolation procedure prior to HPLC gives a 5000-fold purification over total cell protein and yields a protein which is 90% pure. A Varian 5000 Liquid Chromatograph with a built-in UV-100 detector was used for reversed-phase chromatography.

Spinach and parsley were obtained at the local

market, and cytochrome *f* was prepared by the procedure of Ho and Krogmann [12].

SDS-polyacrylamide gel electrophoresis was performed on slab gels using a Bio-Rad Protean 16 cm Slab Cell apparatus. The electrophoresis system of Laemmli [13] was employed and the molecular weight standards were purchased from Pharmacia Fine Chemicals. The standards used were α-lactalbumin, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin and phosphorylase *b*. Gels were stained by the method of Weber and Osborn [14]. The isoelectric points of cytochromes *c*-550 were determined by isoelectric focusing in polyacrylamide gels according to the procedure of Righetti and Drysdale [15] using Bio-Rad Bio-Lyte 3/10.

Antiserum to *A. flos-aquae* cytochrome *c*-550 was prepared using the procedure of Reichlin et al. [16]. Immunodiffusion tests were done on Ouchterlony plates according to Crowle [17].

Amino-acid analyses were performed on a Durrum D-500 amino-acid analyzer according to the manufacturer's instructions. Amino-acid sequences were determined by automated Edman degradation as described by Mahoney et al. [18].

Results

Cytochrome *c*-550 was purified from *M. aeruginosa* and *A. flos-aquae* using the procedure described earlier [5]. A final purification step was added using reversed-phase high performance liquid chromatography (Fig. 1). A gradient of 0–80% acetonitrile in water containing 0.1% (v/v) trifluoroacetic acid was used. The cytochrome is readily recognized by its brown color in the eluting solvent and is the major peak in the elution pattern. The cytochrome from *M. aeruginosa* showed nearly identical behavior to that of *A. flos-aquae* on the HPLC. This purification step denatures the cytochrome but improves its purity as reflected in the composition and sequence analyses. The yield and homogeneity of the sequenator analysis indicate a purity greater than 96%. Pyridine-hemochromogen analysis indicated a single heme per 15 500 Da polypeptide.

The isoelectric points of cytochromes *c*-550 which we isolated from several cyanobacteria and the eukaryotic red alga *P. cruentum* are shown in

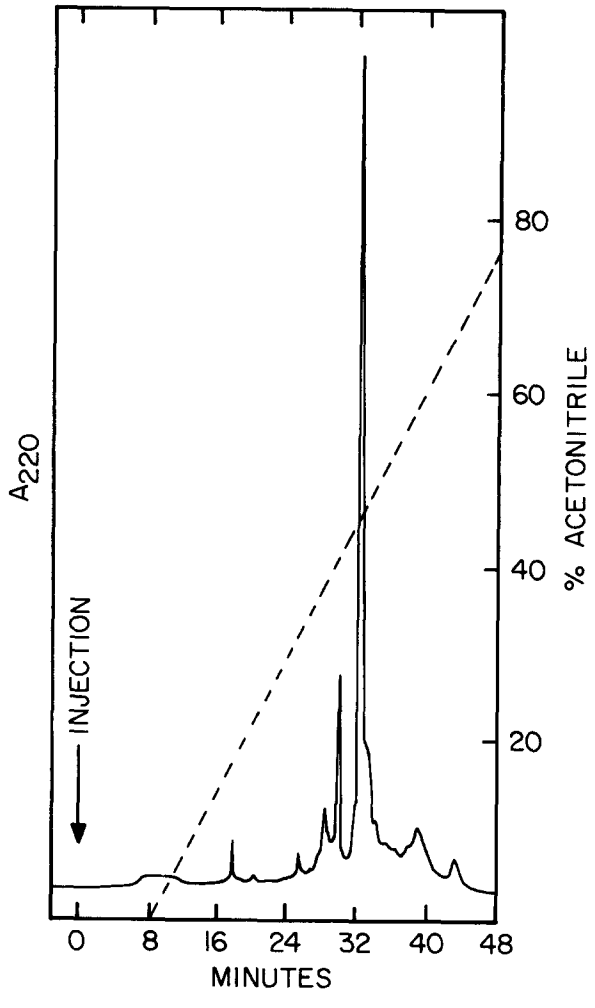

Fig. 1. Reversed-phase HPLC of *M. aeruginosa* cytochrome *c*-550. Approx. 10 mg protein was applied to a Synchropak RP-P (Synchrom Inc., Linden, IN) column (250×4.1 mm) and eluted at ambient temperature at a flow-rate of 0.7 ml/min with a gradient from 0–80% acetonitrile in water. Both solvents contained 0.1% (v/v) trifluoroacetic acid. Absorbance of the effluent was measured at 220 nm at 2 absorbance units full-scale.

Table I. Values for the p*I* of cytochrome *c*-553 are also included, since this protein shows unusual variation, depending on the genus from which it is isolated. Cytochrome *c*-553 obtained from the two filamentous cyanobacteria, *A. variabilis* and *A. flos-aquae* is a basic protein, but that obtained from other cyanobacteria and eukaryotic algae is acidic. The isoelectric points of cytochromes *c*-550 show less variation. The amino-acid compositions

TABLE I

ISOELECTRIC POINTS OF CYTOCHROMES *c*-550 AND *c*-553

	c-550	*c*-553
A. variabilis	4.2	8.9
A. flos-aquae	4.4	9.3
A. quadruplicatum	4.7	–
M. aeruginosa	4.3	5.5
S. maxima	4.4	5.2
A. nidulans	4.8	3.8
P. cruentum	4.1	4.4

of cytochromes *c*-550 from *M. aeruginosa* and *A. flos-aquae* are quite similar (Table II), except for the higher histidine and cysteine in the protein from *A. flos-aquae*. The amino-acid composition of cytochrome *f* from *A. flos-aquae* is given for comparison and clearly differs from that of cytochrome *c*-550. Fig. 2 shows the N-terminal amino-acid sequences for these three cytochromes. The two cytochromes *c*-550 have ten identical residues in the first twenty residues of the N-terminal sequence. The cytochrome *f* is apparently unrelated. The molecular weight of 15 500 for *M. aeruginosa* cytochrome *c*-550 determined by SDS-gel electrophoresis is in good agreement with the molecular weight of this cytochrome from *A. flos-aquae* obtained by ultracentrifuge measurements [5] and by estimates of molecular weight using calibrated gel filtration columns.

An antiserum prepared against *A. flos-aquae* cytochrome *c*-550 was tested for its ability to cross react with various cytochromes. The antiserum reacted with the originating antigen and with the cytochrome *c*-550 from *M. aeruginosa*. The antiserum did not react with the cytochromes *f* from several sources. Cytochrome *c*-553 from *A. flos-aquae* and from several other cyanobacteria did not react with this antiserum, nor did antisera prepared against cytochrome *c*-553 or cytochrome *f* react with cytochrome *c*-550.

TABLE II

AMINO ACID COMPOSITIONS OF CYANOBACTERIAL CYTOCHROMES

Amino acid	*M. aeruginosa* Cyt *c*-550	*A. flos-aquae* Cyt *c*-550	*A. flos-aquae* Cyt *f*
Asx	18	17	39
Thr	11	8	21
Ser	6	6	15
Glx	16	13	48
Pro	7	8	23
Gly	8	8	28
Ala	10	8	34
Val	8	6	34
Met	1	2	3
Ile	4	4	18
Leu	19	16	21
Tyr	4	4	7
Phe	3	4	12
His	3	5	3
Lys	6	6	19
Arg	5	4	8
Cys	2	6	2
M_r from sum of individual amino acids	14000	14000	36000

Table III shows data on the visible absorption spectra of oxidized and reduced cytochromes. Holton and Myers [2] found that the reduced cytochrome *c*-550 of *A. nidulans* had absorption maxima at 549, 521.5 and 417.5. We found the same peak positions for the cytochrome from *A. nidulans* and slightly shifted maxima at 550, 519 and 414 for the cytochromes from *M. aeruginosa* and *A. flos-aquae*. The cytochrome is reduced by dithionite but not by ascorbate. The reduced form is auto-oxidizable. Gomez-Lojero et al. [9] described the conversion of cytochrome *f* in extracts of *S. maxima* into an auto-oxidizable form in which the absorption maxima of the reduced form

M. aeruginosa cyt c_{550} Leu Glu Leu Asp Glu Lys Thr Leu Thr Ile Thr Leu Asn Asp Ala Gly Glu Ser Val Thr Leu

A. flos-aquae cyt c_{550} Leu Glu Leu Asp Glu Thr Ile Arg Thr Val Pro Leu Asn Asp Lys Gly Gly Thr Val Val Leu

A. flos-aquae cyt f Tyr Pro Phe Trp Ala Gln Glu Thr Ala Pro Glu Thr Pro Arg Glu Ala Thr Gly Arg Ile Val

Fig. 2. N-terminal amino-acid sequences of cyanobacterial cytochromes.

TABLE III

SPECTRAL CHARACTERISTICS OF CYANOBACTERIAL CYTOCHROMES

Cytochrome	Wavelength of peak of reduced cytochrome (nm)			Ratio of reduced/oxidized γ peaks
	α	β	γ	
Native cytochrome *c*-550 *M. aeruginosa*, *A. flos-aquae*	550	519	414	1.37
Partially denatured cytochrome *f*, low-potential form, *M. aeruginosa*, *S. maxima*	550	520	415	0.99
Native cytochrome *f* *M. aeruginosa*, *S. maxima*	556	523	422	1.55

had shifted from 556, 523 and 442 to 550, 520 and 415 nm. We readily confirmed this observation. When partially purified cytochrome *f* from one of the several cyanobacteria or from spinach was stored at 3°C for 24 h, the cytochrome became auto-oxidizable and showed the new spectrum. Table III gives the absorption peaks of the low potential form of cytochrome *f*, and those of the native form of this cytochrome. The auto-oxidizability and the positions of the alpha and beta peaks of cytochrome *c*-550 and the low-potential form of cytochrome *f* are very similar. However, there is a difference in spectral properties in the Soret region. Cytochrome *c*-550 shows a marked increase in absorbance in the Soret region on reduction as is typical of native '*c*' type cytochromes. In contrast, the Soret peak of the low-potential form of cytochrome *f* is not increased in absorbance by reduction. Since some cytochromes are altered by extremes of pH, we incubated purified cytochrome *f* at various pH values and looked for the appearance of the low-potential form. The absorption spectrum of reduced cytochrome *f* was not altered by acidification to pH 4.2. Below this point, the protein precipitated. At alkaline pH, a very slow conversion to the low-potential form occurred. After 141 h, the absorption had diminished at all wavelengths and the cytochrome auto-oxidized. The alpha band had shifted to 550 nm, and the Soret shifted from 422 to 415 nm in the reduced form. The much larger molecular weight of cytochrome *f* was unchanged in samples in which there was complete conversion of the cytochrome to its low-potential form.

Discussion

Low-potential cytochrome *c*-550 from cyanobacteria and eukaryotic algae is not a degradation product of cytochrome *f*. The molecular weight of 15 500 is unique among the known *c*-type cytochromes in photosynthetic organisms. The N-terminal 20 residue sequence does not resemble those of any other cytochrome. Of the many cytochromes that have been described, cyanobacterial cytochrome *c*-550 most resembles the cytochrome *c*-551 which Gray et al. [19] purified from *Chromatium vinosum*. This cytochrome has a molecular weight of 18 800, a redox potential of −0.299 V and a reduced absorption spectrum with an α peak at 551 nm, a β peak at 523 nm and a γ peak at 418 nm. While the absorption spectrum of reduced cytochrome *c*-550 resembles the spectrum of a low-potential form of cytochrome *f* generated by aging, the former does not arise from the latter.

The function of cytochrome *c*-550 is not known. Redlinger and Gantt [20] have found that this cytochrome is attached to membranes in the red alga *P. cruentum* when the cells are broken under rather mild conditions. Bowes and co-workers [21] have found this cytochrome in Photosystem II particles of *Phormidium luridum*. Kienzl and Peschek [22] suggested the cytochrome is an endogenous cofactor of cyclic photophosphorylation. We have obtained preliminary evidence that this cytochrome is more abundant in cells grown on high levels of nitrate and is absent from cells grown on ammonia. The low potential of −0.26 V

found by Holton and Myers [3] makes this cytochrome a powerful reductant and thus a possible participant in the reduction of nitrate to ammonia.

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