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EUGLENA GRACILIS CYTOCHROME 558

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SUMMARY

Euglena gracilis contains both the classical cytochrome *f* (cytochrome c_{552}) found in algae and plants, and an atypical cytochrome 558, which has covalently bound heme. We report here some of the chemical and physical properties of cytochrome 558, including amino acid composition, molecular size, and spectral properties. Further, the atypical nature of the pyridine ferrohemochrome of this cytochrome is considered and it is suggested that the heme is bound to the peptide chain *via* a single thioether link.

Two cytochromes were isolated from the eucaryotic alga *Euglena gracilis* by Gross and Wolken[1]. Cytochrome c_{552} was shown to be similar to the cytochrome *f* of photosynthetic plants and algae, but cytochrome 558, following partial purification by Perini *et al.*[2], was found to have anomalous properties. Cytochrome 558 was found[2] to have covalently bound heme, a characteristic of *c*-type cytochromes, but the alkaline pyridine ferrohemochrome spectrum was found[2] to be intermediate between that of *b*- and *c*-type cytochromes. Since the previous workers had not completely purified cytochrome 558, these results could be interpreted as an indication of a mixture of *b*- and *c*-type cytochromes.

Recently, a cytochrome "$c_{555.5}$" was isolated[3] from the mosquito parasite *Crithidia fasciculata* and found to have anomalous properties similar to those reported for *E. gracilis* cytochrome 558; that is, the *C. fasciculata* cytochrome has heme which is not extractable in acid-acetone and which gives an alkaline pyridine ferrohemochrome spectrum which is intermediate between those of *b*- and *c*-type cytochromes.

E. gracilis cytochrome 558 has been isolated and purified to homogeneity in order to substantiate the earlier reports and to compare with the *C. fasciculata* cytochrome "$c_{555.5}$." The results of this investigation support and extend the earlier findings of Perini *et al.*[2], and demonstrate considerable similarities to the *C. fasciculata* cytochrome.

E. gracilis strain Z was grown photosynthetically in an autotrophic medium as described by Evans and San Pietro[4]. Frozen cell paste was thawed in 0.1 M potassium phosphate buffer, pH 7.0, and the cells were disrupted in a Sorvall–Ribi Cell Fractionator (Ivan Sorvall, Inc.), operated at 20000 lb/inch^2 and 25 °C. Cell residues and chloroplast fragments were removed by centrifugation at 30000 × *g* for 10 min,

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followed by centrifugation at 205700 × *g* for 2 h. The supernatant solution was desalted on Sephadex G-25-C with buffer change to 1 mM Tris–HCl, pH 7.3, and adsorbed on CM-cellulose, equilibrated with 1 mM Tris–HCl, pH 7.3. The cytochrome was chromatographed using a stepwise NaCl gradient in 20 mM Tris–HCl, pH 7.3. Cytochrome 558 was eluted with 80 mM NaCl and then fractionated with $(NH_4)_2SO_4$, precipitating between 70 and 100% saturation. The cytochrome was desalted and chromatographed on Sephadex G-75, equilibrated with 20 mM Tris–HCl, pH 7.3. The final purification step was rechromatography on CM-cellulose as before. Cytochrome 558 was eluted from the final column as a symmetrical band with a constant ratio of 280 nm to 413 nm absorbance. 3 μmoles of cytochrome 558 were isolated per kg wet weight cells, assuming the absorptivity of the α peak (553 nm) of the alkaline pyridine complex to be the same as found for beef heart cytochrome *c*, *i.e.* 31.18 $mM^{-1} \cdot cm^{-1}$ (T. Flatmark, personal communication). The best ratio of 280 nm to 422 nm absorbance was 0.19.

TABLE I

AMINO ACID COMPOSITION

	Euglena gracilis Z cytochrome 558	*Crithidia fasciculata cytochrome "$c_{555.5}$"*[3]
Asp	9	10
Thr	5	4
Ser	6	4
Glu	9	9
Pro	4	9
Gly	10	15
Ala	12	14
Cys	(1)	(2)
Val	5	7
Met	2	3
Ile	4	2
Leu	5	7
Tyr	5	4
Phe	3	2
His	2	2
Lys	12	10
Arg	4	4
Trp	2–4	(1)
Total	100–102	109
Formula weight	11620	

Cytochrome 558 was sized by the procedure of Andrews[5] on a 1.4 cm × 50 cm Sephadex G-75 column, equilibrated with 0.1 M Tris–HCl, pH 7.3, 0.5 M NaCl. The proteins used as standards were: horse heart cytochrome *c*, formula weight 12400[6]; sperm whale myoglobin, formula weight 17800[7]; chymotrypsinogen, formula weight 25000[8]; and *Chromatium vinosum* ATCC 17899 cytochrome c_{552}, molecular weight 72000[9]. The standard proteins defined a straight line when their elution volumes were plotted against the logarithm of their formula weights or molecular weights. The size of the *Euglena* cytochrome 558 was found to be 12000 daltons.

The amino acid composition of cytochrome 558 was determined following

48-h hydrolysis in 6 M HCl at 107 °C, in deaerated, evacuated tubes. Analysis was conducted as described by Dus *et al*,[10] on a modified Beckman Spinco Amino Acid Analyzer. The amino acid composition was calculated relative to histidine and is given in Table I together with the reported amino acid composition of *C. fasciculata* cytochrome "$c_{555.5}$" for comparison. The cysteine yield was much less than the approximately 60 % of theoretical yield normally obtained from cytochromes *c* and could indicate the presence of only one cysteine residue in the protein. Two residues of tryptophan were recovered in amino acid analyses and judging by relatively low recoveries found by others[11] even when a reducing agent was added to the hydrolysis mixture, the content of tryptophan in cytochrome 558 could be as high as four. An unusually high content of tryptophan is also consistent with the relatively high 280-nm absorbance in the pure protein.

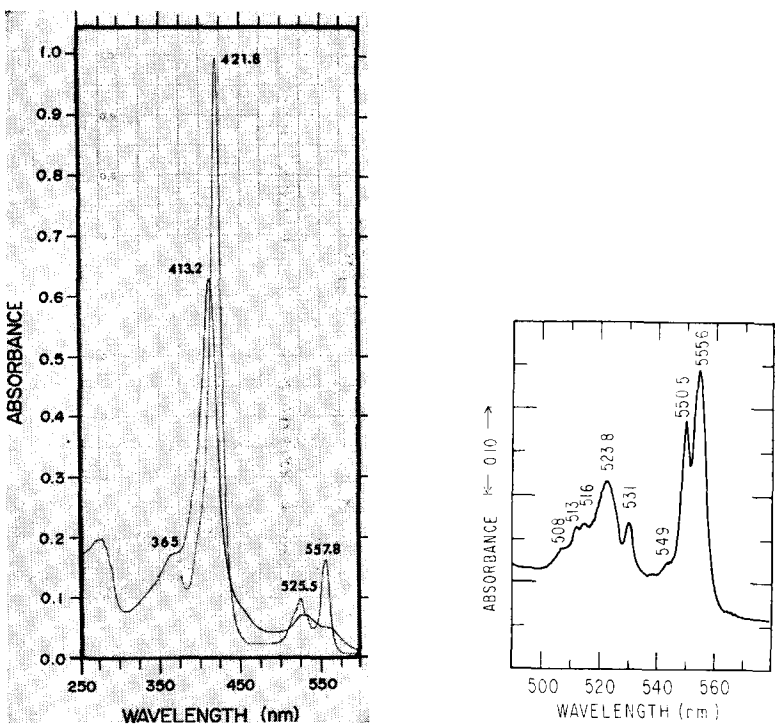

Fig. 1. Absorption spectra of *Euglena* cytochrome 558. The cytochrome was dissolved in 0.1 M phosphate buffer, pH 7.0. The oxidized sample was prepared by passing the salt-free solution of the cytochrome through an Amberlite IRA-400 anion exchange column charged with ferricyanide. The reduced spectrum was obtained by adding a few crystals of sodium dithionite to the oxidized sample.

Fig. 2. Absorption spectrum of *Euglena* ferrocytochrome 558 at 70 °K. The procedure and apparatus described by Cusanovich and Bartsch[13] were used to obtain this spectrum.

The absorption spectra (Fig. 1) of Euglena cytochrome 558 in 0.1 M potassium phosphate, pH 7.0, are different from any known *b* or *c*-type cytochrome. There is some similarity to cytochrome b_5[12], although at liquid nitrogen temperature (Fig. 2) the α band of cytochrome 558 is split into three components with relative intensities

different from those of cytochrome b_5[12]. The alkaline pyridine ferrohemochrome spectrum of cytochrome 558 (0.1 M NaOH, 25 % pyridine) reduced with sodium dithionite shows an alpha peak maximum at 553 nm, in accord with the results of Perini *et al.*[2] and different from that found with typical *c*-type cytochromes (550 nm) or *b*-type cytochromes (556 nm). Since cytochrome 558 has been purified to apparent homogeneity and is not a mixture of *b*- and *c*-type cytochromes, it is suggested that the heme is either different from that in typical *c*-type cytochromes or bound in a different manner. The heme of cytochrome 558 cannot be removed by treatment with acid–acetone, and thus is presumed to be covalently bound.

The observations of Perini *et al.*[2] with partially purified *E. gracilis* cytochrome 558 have been borne out with the purified protein and it is also clear that this cytochrome is similar in several respects to *Crithidia fasciculata* cytochrome "$c_{555.5}$". The atypical alkaline pyridine ferrohemochrome spectrum of these two cytochromes is so far unique. The absorption spectra at pH 7.0 have similar maxima, although a close comparison is impossible since the original spectra of the *Crithidia* cytochrome have not been published. The redox potential of *Euglena* cytochrome 558 is reported to be 307 mV[2] as compared to 280 mV[3] for *Crithidia* cytochrome "$c_{555.5}$". The amino acid compositions of the two proteins are very similar. From a functional standpoint, the *Euglena* cytochrome was found to be more closely associated with the aerobic pathways than with the photosynthetic pathways of electron transfer[14], and thus could serve a role similar to that of the *Crithidia* cytochrome.

The amino acid sequences of the two proteins should show whether they are indeed homologous and whether there may be a link between the *Euglenophytes* and *Trypanosomes*. The amino acid sequence might also provide an answer to the question of the mode of heme binding. The alkaline pyridine ferrohemochrome spectrum of cytochrome 558 is similar to that of 2(4)-vinyl 4(2)-ethyl substituted heme[15] and suggests the possibility that the anomolous properties of this cytochrome arise from the substitution of a non-functional amino acid for one of the two heme binding cysteine residues. In support of this hypothesis, the heme of *Crithidia oncopelti* cytochrome 557 has been shown to be covalently bound and to give a positive test for unsaturated side chains. Further, the pure protein and the tryptic heme peptide have only one cysteine residue[16].

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REFERENCES

1 J. Gross and J. Wolken, *Science*, 132 (1960) 357.
2 F. Perini, M. D. Kamen and J. A. Schiff, *Biochim. Biophys. Acta*, 88 (1964) 74.
3 J. P. Kusel, J. R. Suriano and M. M. Weber, *Arch. Biochem. Biophys.*, 133 (1969) 293.
4 W. R. Evans and A. San Pietro, *Arch. Biochem. Biophys.*, 113 (1966) 236.
5 P. Andrews, *Biochem. J.*, 91 (1964) 222.
6 E. Margoliash, E. L. Smith, G. Kreil and H. Tuppy, *Nature*, 192 (1961) 1125.
7 A. B. Edmundson, *Nature*, 205 (1965) 883.

8 J. R. Brown and B. S. Hartley, *Biochem. J.*, 101 (1966) 214.
9 R. G. Bartsch, T. E. Meyer and A. B. Robinson, in K. Okunuki, M. D. Kamen and I. Sekuzu, *Structure and Function of Cytochromes*, University of Tokyo Press, 1968, p. 443.
10 K. Dus, S. Lindroth, R. Pabst and R. Smith, *Anal. Biochem.*, 14 (1966) 41.
11 H. Matsubara and R. M. Sasaki, *Biochem. Biophys. Res. Commun.*, 35 (1969) 175.
12 Y. Okada and R. Okunuki, *J. Biochem.*, 65 (1969) 581.
13 M. A. Cusanovich and R. G. Bartsch, *Biochim. Biophys. Acta*, 189 (1969) 245.
14 F. Perini, J. A. Schiff and M. D. Kamen, *Biochem. Biophys. Acta*, 88 (1964) 91.
15 J. E. Falk, *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1964.
16 G. Pettigrew and T. E. Meyer, *Biochem. J.*, 125, (1971) 46P.