Studies on algal cytochromes II. 
Some physical and chemical properties of further purified Petalonia cytochrome c-553

Yasutomo Sugimura¹, Keishiro Wada² and Hiroshi Matsubara²

¹ Department of Biology, Faculty of Science, Toho University, Funabashi, Chiba 274 and 
² Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

(Received August 11, 1980)

A cytochrome c-553 was isolated from a brown alga, Petalonia fascia, and its physical and chemical properties were investigated. At liquid nitrogen temperature, the α-, β- and γ-band of this cytochrome shifted 1–2 nm to the shorter wavelength in comparison with those at room temperature. The α-band split into two peaks, 551.5 (major band) and 546.5 nm (minor band), at the low temperature. The cytochrome in high concentration showed a shoulder at 695 nm in its oxidized state, suggesting a methionine residue to be the sixth ligand of heme iron. The molecular weight was estimated to be about 10,000 containing one mole of heme c based on analyses of gel filtration, sodium dodecyl sulfate acrylamide gel electrophoresis, amino acid composition and iron content. The isoelectric points of the ferro- and ferricytochromes were estimated to be at pH 4.1 and 4.3, respectively, by the isoelectric focusing method. The amino acid composition of this cytochrome was Lys, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, Trp, with a total of 86 residues. The amino- and carboxyl-terminal sequences and four chymotryptic peptide sequences were compared with those of other cytochromes to show that these cytochromes were homologous to one another.

Key words: Cytochrome c-553 — Molecular properties — Partial sequence — Petalonia fascia.

Water-soluble algal c-type cytochrome was first isolated from a red alga, Porphyra tenera, by Yakushiji in 1935 (38) and similar cytochromes have been purified from several divisions of algae and their properties reported (7, 12, 15, 16, 19, 26, 27, 30, 31, 39, 40). During the past eighteen years, the soluble cytochromes of c-type, cytochrome c-553, had been considered to be cytochrome f which was discovered and isolated by Hill and coworkers (6, 9, 10), because of their similarity to one another in several properties: the asymmetric α-band, the sharp γ-band, the high midpoint potential, and the acidic nature. On the other hand, in 1977 Wood extracted a membrane-bound c-type cytochrome with a mixture of ethyl acetate, ethanol and ammonia from three divisions of algae, Chlamydomonas reinhardtii (Chlorophyta), Euglena gracilis (Euglenophyta) and Anacystis nidulans (Cyanophyta), and identified them as cytochrome f (36). indicating that the algal cytochrome f is

Abbreviations: SDS, sodium dodecyl sulfate; Em, midpoint oxidation-reduction potential; N- and C-terminal, amino- and carboxyl-terminal; CPase, carboxypeptidase; DEAE, diethylaminoethyl.
distinguishable from cytochrome c-553 in the absorption spectrum. A similar comparison was also carried out for Bryopsis maxima (Chlorophyta) (14). Cytochrome c-553 is thought to function as an electron transfer protein between cytochrome f and P700 in the photosynthetic transport system (37).

Several workers have determined the amino acid sequences of the cytochrome c-553 isolated from the five divisions of algae: Spirulina maxima (Cyanophyta) (1), Porphyra tenera (Rhodophyta) (1), Monochrysis lutheri (Chromophyta) (17), Alaria esculenta (Chromophyta) (18) and Euglena gracilis (Euglenophyta) (28). They were homologous to one another and also to mitochondrial cytochrome c. The sequence, Cys-X-Y-Cys-His, was common in all cytochromes c, and methionine-62 was one of the invariant residues, assumed to be the sixth ligand to the heme iron (3, 39).

We previously reported the crystallization of cytochrome c-553 from a brown alga, Petalonia fascia (Phaeophyceae), and its several properties such as absorption spectra, a molecular weight of $10.5 \times 10^3$ based on the iron analysis, and a midpoint potential of 0.36 volt (31). The present paper reports further purification and some physical and chemical properties of cytochrome c-553 from P. fascia.

**Materials and methods**

**Materials**

Standard molecular weight markers were obtained from either Pharmacia Fine Chemicals, Sweden, or Sigma Chemicals Co., U.S.A. Pharmalyte was from Pharmacia Fine Chemicals and all other reagents and enzymes were of analytical grade obtained from Wako Pure Chemicals Industries, Ltd. (Osaka) and Nakarai Chemicals Co. (Kyoto).

**Preparation of Petalonia cytochrome c-553**

Petalonia cytochrome c-553 was prepared as described previously (31), except for the introduction of DEAE-Sephadex column chromatography and Sephadex G-75 gel filtration after the acrilin treatment and ammonium sulfate fractionation. The thalli of Petalonia fascia were harvested at Shizugawa Bay, Miyagi, and stocked in a freezer at $-20^\circ$C. The cytochrome was extracted at $20^\circ$C with 20 mM ammonia water from the thalli, and purified by ammonium sulfate fractionation and acrilin treatment (31). The crude preparation (200 ml) containing about 40 mg of the cytochrome with a purity index ($A_{554}/A_{278}$) of 0.4 was obtained from 2.5 kg of the thalli. After dialysis overnight against 5 mM K-phosphate buffer, pH 7.0, the solution was reduced with 1 mg of solid Na-ascorbate and applied to a DEAE-Sephadex A-25 column (2.6 × 45 cm) equilibrated with the same buffer. After washing with 200 ml of 10 mM phosphate buffer, pH 7.0, the column was eluted with a linear gradient of 0 to 0.25 mM NaCl in 1.2 liters of the same buffer. The eluate was collected in 5-ml fractions. The fractions with a purity index of above 0.9 were combined and saturated with ammonium sulfate. The precipitate was collected by centrifugation at 15,000 × g for 10 min and dissolved in 3 ml of 0.125 mM NaCl containing 10 mM phosphate buffer, pH 7.0. This solution was applied to a Sephadex G-75 column (2.6 × 45 cm) equilibrated with the same buffer and the cytochrome was developed with the same buffer. The fractions with a purity index of above 1.0 were combined, saturated with ammonium sulfate, centrifuged, and
dissolved in 3 ml of 10 mM phosphate buffer. After centrifugation at 2,300 \times g for 10 min, the supernatant was dialyzed against 0.2 M Na$_2$HPO$_4$ saturated with ammonium sulfate. Crystallization of the cytochrome was almost completed within a few days. After recrystallization twice under the same conditions, about 20 mg of crystalline cytochrome c-553 was obtained with a purity index of 1.10.

Absorption spectra

The absorption spectra of cytochrome c-553 were measured with a Hitachi 200 spectrophotometer at room temperature. Ferricytochrome was prepared by adding about 0.3 mg of K-ferricyanide to 0.05 mM cytochrome solution in 50 mM K-phosphate buffer, pH 7.0. The spectrum of ferrocyanochrome was measured by adding about 0.5 mg of Na-ascorbate to the ferricytochrome solution. The spectrum of ferrocyanochrome in 0.5 mM phosphate buffer, pH 7.0, at liquid nitrogen temperature was measured in a Hitachi 356 spectrophotometer.

Molecular weight

The molecular weight of cytochrome c-553 was estimated by gel filtration on a Sephadex G-75 column by the method of Andrews (2) with modifications (32) and by polyacrylamide gel electrophoresis in the presence of SDS (35) using rabbit muscle phosphorylase b (molecular weight of 94,000), bovine serum albumin (67,000), egg white ovalbumin (43,000), bovine erythrocyte carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), bovine milk a-lactalbumin (14,400) and bovine pancreatic insulin (5,700) as molecular weight markers. The cytochrome (0.02 \mu mole) and these marker proteins (0.02 \mu mole each) were pretreated at 100°C for 5 min in 60 \mu l of the mixture of 25% glycerol, 5% \beta-mercaptoethanol and 1% SDS, and 10-\mu l portions of the resulting mixture were applied to the 15% polyacrylamide gels (0.5 \times 9 cm) containing 0.1% SDS and 0.1 mM phosphate buffer, pH 7.2. The electrophoresis was carried out at 7 mA and 25°C for 6 hr. Its molecular weight was also calculated from the amino acid composition.

Isoelectric points

The isoelectric points of Petalonia ferri- and ferrocyanochromes c-553 were determined by a density gradient isoelectric focusing method (33) using Pharmalyte carrier ampholyte of the pH range of 2.5 to 5.0 in a 110-ml column (24). A linear density gradient was prepared in a column by adding a thin solution (2.5 g of glycerol, 51 ml of water and 1.4 ml of Pharmalyte) to a more dense solution (27 g of glycerol, 34 ml of water and 1.4 ml of Pharmalyte) with constant mixing. The cytochrome c-553 (about 0.02 \mu mole) dissolved in 1.5 ml of a mixture of the dense and the thin solutions (1:1) was 50% oxidized by the addition of 1 mM potassium ferricyanide solution (about 10 \mu l). The cytochrome solution was applied to the middle of the column. Electrophoresis was performed at 800 v (10-1.5 mA) and 0°C for 30 hr. The solution was collected in 1-ml fractions after the electrophoresis. The pH of each fraction was measured at 0°C. The absorbances at 410 and 415.5 nm were monitored at 20°C for ferri- and ferrocyanochromes, respectively.

Amino acid composition and sequence analysis

The amino acid compositions of Petalonia cytochrome c-553 and its peptides
were determined with an amino acid analyzer (Beckman Model 120B) after acid hydrolysis as described (34). Tryptophan was estimated by thioglycolic acid procedure (22). Cysteine content was determined using a performic acid-oxidized sample (17).

About 60 mg of Petalonia cytochrome c-553 was denatured with 90% ethanol and dried in vacuum at 45°C. The denatured protein was digested with chymotrypsin (2 mg) in 5 ml of 0.02 M Tris-HCl buffer, pH 8.0, at 40°C overnight. The hydrolyzate was fractionated on an anion exchange column of AG1-X2 (200–400 mesh, 1.5 × 90 cm) (8). Further purification of peptides was carried out by paper chromatography and electrophoresis as described previously (21).

Four chymotryptic peptides were further digested with thermolysin (20) and trypsin. The peptides thus obtained were purified by paper chromatography as described previously (21). All analytical values of amino acids are expressed in mole per mole of protein in or peptide.

In nomenclatures used for peptides, C- refers to chymotryptic peptides of cytochrome c-553. T- and Th- refer to the peptides derived by the further digestion with trypsin and thermolysin, respectively.

Results

Absorption spectra

The absorption spectra of the present preparations of Petalonia cytochrome c-553 in oxidized and reduced states were all the same as described previously (31). The low temperature absorption spectrum (77°K) of the ferrocytochrome is shown in Fig. 1. The peaks of α-, β- and γ-bands shifted 1 to 2 nm to the shorter wave-

![Absorption spectrum of Petalonia ferrocytochrome c-553.](image-url)
Petalonía fascia cytochrome c-553

Fig. 2. Absorption spectra of Petalonía ferri- and ferro-cytochrome c-553. The cytochrome (0.5 mM) was in 2.5 ml of 50 mM K-phosphate buffer, pH 7.0, and the spectra were taken at 25°C. Ferri-cytochrome (—) was prepared by adding 0.3 mg of solid K-ferricyanide and ferro-cytochrome (----) by adding 0.5 mg of sodium ascorbate.

length, compared with those observed at room temperature. The a-band split into two peaks at 551.5 (major band) and 546.5 nm (minor band). The β-band showed a small peak and three shoulders at 526.5, 512, 510 and 504 nm, respectively.

The absorption spectrum of the ferricytochrome recorded at room temperature is shown in Fig. 2 with an enlargement of the region at 695 nm. Two shoulders were found at around 640 and 695 nm, and the latter has been suggested to be due to the hememethionine coordination (29). The millimolar extinction coefficient (ε mm) at the 695-nm shoulder was calculated to be 1.12 mm⁻¹ cm⁻¹ based on ε mm at 553 nm of ferro-cytochrome of 28.5 mm⁻¹ cm⁻¹ (31). Upon reduction with ascorbate, this shoulder disappeared but the 640-nm shoulder was not affected very much.

Molecular weight

The elution position of the cytochrome from gel filtration was plotted against the logarithm of molecular weight of various marker proteins as shown in Fig. 3. A molecular weight of 10.2×10³ was estimated for the Petalonía cytochrome. The minimum molecular weight was previously reported to be 10.5×10³ on the basis of the iron content (31). Another value of the molecular weight was calculated to be 9.95×10³ from the amino acid composition shown in Table 1. The polyacrylamide gel electrophoresis in the presence of SDS showed a single band at the position corresponding to the molecular weight of 11×10³ (Fig. 4). These results showed that the cytochrome was composed of a single polypeptide with a molecular weight of about 10,000 containing one heme c.
Fig. 3. Estimation of the molecular weight of Petalonia cytochrome c-553 by gel filtration. Gel filtration was carried out with a Sephadex G-75 column (2.64 × 45 cm) using 0.125 M NaCl in 0.02 M K-phosphate buffer, pH 7.0, at 5°C. The flow rate was 35 ml/hr and the eluate was collected in 3.2-ml fractions.

Isoelectric points

Fig. 5 shows the elution profile of Petalonia ferri- and ferro-cytochromes c-553 using a pH gradient of isoelectric focusing. The ferri- (brownish band) and ferrocytochrome (fresh pink band) were clearly separated on the column, and their isoelectric points were at pH 4.3 and 4.1, respectively at 0°C.

Amino acid composition, terminal sequences and peptide sequences

The amino acid composition of Petalonia cytochrome c-553 is shown in Table 1. The acidic amino acids are prominent, and one residue each of histidine, arginine, tyrosine and tryptophan are present. The total number of amino acid residues was 86.

Fig. 4. Estimation of the molecular weight of Petalonia cytochrome c-553 by SDS gel electrophoresis. The polyacrylamide gel electrophoresis was carried out in 15% gel in the presence of 0.1% SDS and 0.1 M phosphate buffer, pH 7.2, at 7 mA per gel (0.5 × 10 cm) for 6 hr.
### Table 1  Amino acid compositions of Petalonia fassia cytochrome c-553 and several other cytochromes c

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Values after hydrolysis</th>
<th>Average</th>
<th>Nearest integer</th>
<th>Sp</th>
<th>Sm</th>
<th>Pt</th>
<th>MI</th>
<th>BF</th>
<th>Ae</th>
<th>Eg</th>
<th>Cyt. e</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>24 hr</td>
<td></td>
<td></td>
<td>5.38</td>
<td>5.70</td>
<td>6.01</td>
<td>1.03</td>
<td>1.04</td>
<td>1.01</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>8</td>
<td>48 hr</td>
<td></td>
<td></td>
<td>5.98</td>
<td>6.35</td>
<td>6.70</td>
<td>1.07</td>
<td>1.09</td>
<td>1.06</td>
<td>1.06</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Table 1 Amino acid compositions of Petalonia fassia cytochrome c-553 and several other cytochromes c

- Sp: Spinnalis planata (39); Sm: S. maxima (1); Pt: P. longipalpus (1); MI: Monopodia tuberi (4); BF: Monopodia tuberi (4); Ae: Algae cellulosa (4); Eg: Algae cellulosa (4).  
- The average of two determinations per one mole of hemoglobin.  
- Value extrapolated to zero time of hydrolysis.  
- Material: 30%; 25% hydrolysis.  
- Value based on acid hydrolysis with thioglycolic acid.
Y. Sugimura, K. Wada and H. Matsubara

Fig. 5. Determination of the isoelectric points of Petalonia cytochrome c-553 in its oxidized and reduced forms. The isoelectric points were measured by the density gradient (49.1-4.54% glycerol) isoelectric focusing method using Pharmalyte (2.55%) of the pH range of 2.5-5.0 in a 110-cm column at 800 v and 0°C for 30 hr. The solution was collected in 1-ml fractions. The absorbances at 410 nm (△) for ferri-cytochrome and 415.5 nm (○) for ferro-cytochrome were measured at 20°C, and the pH (×) of each fraction was measured at 0°C.

The N-terminal sequence of the cytochrome was identified as Val-Asp-Ile- by Edman degradation procedure. The C-terminal residue was determined to be tryptophan by the digestion of the ethanol-denatured cytochrome with carboxypeptidase (CPase) A.

The chymotryptic peptides were separated on an anion exchange column, AG1-X2, and the elution pattern is shown in Fig. 6. Five fractions were purified by paper chromatography and electrophoresis and only four peptides, C-9, C-14, C-15 and C-17, were studied for the sequence determination. Purification methods, compositions, and properties of these peptides are summarized in Table 2.

Peptide C-9 was subjected to six steps of Edman degradation to reveal the N-terminal sequence, Gly-Lys-Asx-Ala-Met-Pro, and the remainder was digested with CPase A to release phenylalanine and alanine successively. Therefore, the peptide was concluded to have the sequence, Gly-Lys-Asx-Ala-Met-Pro-Ala-Phe.

The N-terminal sequence of Peptide C-14 was determined to be Lys-Lys-Asp-Ala by Edman degradation and CPase A digestion released methionine and glutamic acid successively. The peptide was digested with thermolysin and gave three peptides, C-14-Th-1 to 3. Their compositions were Lys3, Asp1, Ala1 for Th-1, and Asp1, Glu3, Met1, Leu1 for both Th-2 and Th-3. The sequence of Th-3 was Leu-
Fig. 6. Elution pattern of chymotryptic peptides of Petalonia cytochrome c-553. The chymotryptic peptides were chromatographed on a Dowex AG 1-X2 column (1.5×90 cm) and developed with the following buffer systems: (a) 0.124 M pyridine, pH 9.0 (160 ml), (b) a linear gradient from 0.124 M pyridine (200 ml) to 0.124 M pyridine/3 mM acetic acid, pH 6.8 (200 ml), (c) 0.124 M pyridine/3 mM acetic acid, pH 6.8 (200 ml), (d) a linear gradient from 0.124 M pyridine/3 mM acetic acid, pH 6.8 (250 ml) to 1 M acetic acid (250 ml), (e) 1 M acetic acid (100 ml), (f) a linear gradient from 1 M acetic acid (200 ml) to 5 M acetic acid (200 ml) and (g) 5 M acetic acid (100 ml). Each fraction was monitored at 570 nm after the ninhydrin reaction (7).

Glx-Glx by Edman degradation. These results suggested the total sequence of Peptide C-14 to be Lys-Lys-Asp-Ala-Leu-Glx-Glx-Asx-Glu-Met.

Peptide C-15 was positive to the Ehrlich reaction, suggesting the presence of tryptophan. The N-terminal sequence was Val-Ile as revealed by Edman degradation. Trypsin digestion of this peptide gave two peptides, Ehrlich-negative C-15-T-1 and Ehrlich-positive C-15-T-2. The compositions were Lys₁, Ser₂, Glu₂, Val₁, Ile₁ for T-1 and Gly₁, Trp₁ for T-2. Six steps of Edman degradation and trypsin specificity suggested the sequence of T-1 to be Val-Ile-Ser-Gln-Ser-Gln-Lys. The chymotrypsin specificity suggested the sequence of Peptide T-2 to be Gly-Trp. Therefore, the sequence of Peptide C-15 was concluded to be Val-Ile-Ser-Gln-Ser-Gln-Lys-Gly-Trp which was also identified as the C-terminal peptide of this cytochrome because of the presence of the sole tryptophan residue in this peptide.

Peptide C-17 was subjected to seven steps of Edman degradation and digested with CPase A to release phenylalanine and valine in nearly equal amounts. These results and chymotrypsin specificity suggested the sequence of Peptide C-17 to be Val-Asp-Ile-Asn-Asp-Gly-Glu-Ser-Val-Phe.

Peptide C-18 was more anionic than C-17, but both peptides showed the same composition. The N- and C-terminal sequences of this peptide were identical with
Table 2  Amino acid compositions and purification methods of chymotryptic peptides, C-9, C-14, C-15 and C-17 of Petalonia cytochrome c-553

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>C-9</th>
<th>C-14</th>
<th>C-15</th>
<th>C-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>1.10 (1)</td>
<td>1.90 (2)</td>
<td>1.00 (1)</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>1.47 (1)</td>
<td>2.13 (2)</td>
<td></td>
<td>2.81 (3)</td>
</tr>
<tr>
<td>Ser</td>
<td></td>
<td>1.73 (2)</td>
<td>0.90 (1)</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td></td>
<td>3.12 (3)</td>
<td>2.32 (2)</td>
<td>1.16 (1)</td>
</tr>
<tr>
<td>Pro</td>
<td>0.97 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>1.04 (1)</td>
<td>0.98 (1)</td>
<td></td>
<td>1.10 (1)</td>
</tr>
<tr>
<td>Ala</td>
<td>1.97 (2)</td>
<td>0.68 (1)</td>
<td>0.93 (1)</td>
<td>1.82 (2)</td>
</tr>
<tr>
<td>Val</td>
<td>0.73 (1)</td>
<td></td>
<td>0.92 (1)</td>
<td>1.00 (1)</td>
</tr>
<tr>
<td>Met</td>
<td></td>
<td>0.94 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>0.93 (1)</td>
<td></td>
<td>0.94 (1)</td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Purification</td>
<td>PE</td>
<td></td>
<td>PC</td>
<td></td>
</tr>
</tbody>
</table>

* Ehrlich reaction positive, suggesting of the presence of one tryptophan residue.  PE, paper electrophoresis at pH 3.6.  PC, paper chromatography using butanol/pyridine/acetic acid/water = 15:10:3:12, v/v.

Fig. 7.  Comparison of amino acid sequences of four chymotryptic peptides of Petalonia cytochrome c-553 (Pf) with the corresponding regions of other algal cytochromes c-553.  Sm, S. maxima; Pt, P. tenera; Ml, M. lutheri; Ae, A. esculenta and Eg, E. gracilis.  The alignment is based on that of Ambler and Bartsch (1).  The common amino acid residues to the six cytochromes are boxed.  One-letter notation is used for each amino acid residue.  —indicates a deletion.
Discussion

The water-soluble cytochrome c-553 of *P. fascia* was further studied and confirmed to be composed of a single polypeptide chain with a molecular weight of about 10,000 containing one mole of heme c from the results of gel filtration, SDS polyacrylamide gel electrophoresis, and amino acid composition, agreeing with the results obtained by the hemochrome and iron analyses previously reported (31).

The absorption spectrum in the red region of a concentrated solution of *Petalonia* cytochrome c-553 in the oxidized form showed a small shoulder with a maximum at 695 nm. This suggested that the sixth ligand of heme iron of this cytochrome is a methionine sulfur as suggested for the mitochondrial cytochrome c (29). Similar observations were reported for *Euglena* cytochrome c-552 (3) and *Spirulina* cytochrome c-554 (39). The invariant methionine is present only at position 62, corresponding to that of *S. maxima* cytochrome c-554, in the five cytochromes c-553 from *S. maxima*, *P. tenera*, *Monochrysis lutheri*, *Alaria esculenta* and *Euglena gracilis*. One of the three methionine residues in *Petalonia* cytochrome c-553 was found in Peptide C-9 and corresponded to the heme ligand methionine in other cytochromes c.

Isoelectric focusing separated the oxidized and reduced forms of *Petalonia* cytochrome c-553. The more positive isoelectric point of the oxidized form (pH 4.3) than that of the reduced form (pH 4.1) accounts for the observation that the cytochrome moved more rapidly on a DEAE-cellulose column in the oxidized state than in the reduced one (31). The change of the surface charge of the cytochrome found in these results may be due to its conformation change caused by the heme-iron valency change of the protein.

The amino acid compositions of seven algal cytochromes c-553 and one higher plant cytochrome c (25) are listed together with *Petalonia* cytochrome c-553 in Table 1. The *Petalonia* cytochrome most closely resembles the *Alaria* cytochrome; incidentally, these two algae belong to the same division. In general, algal cytochrome c-553 has fewer lysine, proline and leucine residues. The content of aspartic acid in the cytochrome of *S. platensis*, *S. maxima*, *P. tenera*, *P. fascia* and *A. esculenta* is high but that of *E. gracilis*, *M. lutheri* and *B. filiformis* is nearly the same as that of higher plant cytochrome c. The higher content of aspartic acid and the lower content of lysine may contribute to the acidic nature of *Petalonia* cytochrome c-553.

Fig. 6 compares the amino acid sequences of the four chymotryptic peptides studied in this paper with the corresponding sequences in other algal cytochromes (2, 17, 18, 28) on the basis of the alignment of Ambler and Bartsch (1).

Three residues in the N-terminal peptides, Asp-3, Gly-6 and Phe-10, are conserved in five algal cytochromes c-553 as well as in respiratory cytochrome c. There are eight common residues in the six algal cytochromes and Met-62 is assumed to be the sixth heme-iron ligand equivalent to Met-80 in the respiratory cytochrome c. Clearly, from the present sequence comparison, *Petalonia* cytochrome c-553 is a homologous protein with the other five algal cytochromes having a common ancestor and probably also has a common ancestor with the respiratory cytochrome c.

The authors express their thanks to Dr. T. Yamanaka for his interest and encouragement in this work. They also thank Dr. T. Hase, H. Tokunaga and F. Yoshizaki for their support and assistance in the various steps of this work.
References


