Table 1. Ammonia content of oil sardine preserved with ammonia, of oil sardine preserved with ammonia and washed repeatedly with water, and of fish flour prepared from preserved, washed fish.

Moisture (%)	Ammonia (mg/100 g)	*p*H
Fish preserved 2 months		
76	458	9–10
Fish preserved 2 months and then washed		
76	55	7.5–8.0
Fish flour made from preserved, washed fish		
3	Trace	6.9

at ordinary temperatures for later processing into fish flour.

We have explored the possibility of treating fish with a safe and easily obtainable agent that has a good preservative action and can, at the same time, be removed easily during drying or other processing. Ammonia in liquid or gaseous form has proved to be promising. Our experience has so far been confined only to a few varieties of marine and fresh-water fish, such as, oil sardine, "Bombil" (*Harpodon nehereus*), *Barbus carnaticus,* and *Labeo* sp. We immersed the eviscerated fish (10 kg) in an equal volume of 1*N* ammonia solution for about 1 to 2 hours and then transferred it to an air-tight vessel (capacity, 20 kg) fitted with a perforated disk about 5 cm above the bottom. In the space below the perforation was some ammonia in solution (2*N*), or paper pulp or another medium soaked in ammonia. The device maintains a concentration of ammonia vapor in the space but does not add appreciably to the moisture content of the fish. The procedure helps to minimize the dissolution of proteins by the ammonia. The fish we have preserved have a *p*H of about 10, and have already kept for over 2 months at a temperature of 25° to 30°C. The excellent condition of the fish suggests that a much longer storage life may be expected.

The treatment is comparatively easy. We have found (Table 1) that, after drying and processing (ethanol extraction for fish flour production), there is no measurable residue of ammonia in the final product as estimated by the Conway diffusion method (*1*). The preserved fish is free from pathogens and has a low bacterial count.

Most of the ammonia from treated fish was leached into the medium by repeated washings with potable water or seawater. Treated fish became soft and continued to remain soft even after repeated washings. Treatment of the washed fish with 0.1*N* citric acid or 0.2-percent calcium chloride improved its texture and made it even firmer than the freshly caught fish.

Further studies have shown that the available lysine, methionine, cystine, and tryptophan and the protein efficiency ratio of fish flour prepared from the preserved fish are not appreciably different from those of flour prepared from fresh, untreated fish (Table 2).

The condition of oil sardines is not changed by the treatment, but "Bombil," which has a much higher water content (90 percent) has a tendency to drip. This does not, however, affect the keeping quality.

Comparative studies with other methods of preservation have shown that ammonia treatment has several advantages over the others. Strong bases (*2*) such as caustic alkali (*3*) and various chemical preservatives (*4*) tend to affect the quality or to leave residues.

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Table 2. Protein efficiency ratios (at 4 weeks) and available lysine, methionine, cystine, and tryptophan (in grams per 100 g of protein) in fish flour prepared from fresh oil sardines and from ammonia-preserved oil sardines.

Protein efficiency ratio	Lysine	Methionine	Cystine	Tryptophan
Flour from untreated fish				
3.5	8.96	4.0	1.2	0.94
Flour from treated fish				
3.2	8.74	3.7	1.2	0.94

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Cytochromes of a Blue-Green Alga: Extraction of a c-Type with a Strongly Negative Redox Potential

Abstract. *Aqueous extraction of lyophilized* Anacystis nidulans *cells followed by chromatography on diethylaminoethyl cellulose separates three different c-type cytochromes. Of the two present in highest concentration, cytochrome-554 has a +0.35-volt redox potential and resembles the cytochrome* f *of other photosynthetic tissues, while cytochrome-549 has a −0.26-volt potential. The possible participation of cytochrome-549 in electron transport in photosynthesis in this alga at a more negative oxidation-reduction potential than previously postulated for any cytochrome is inferred from the similarities in its spectra to the light-induced spectral changes in vivo observed by others.*

The participation of cytochromes in photosynthesis has been implicated by the isolation of *c*- or *f*- and *b*-type cytochromes from chloroplasts and from the light-induced absorbancy changes in vivo at wavelengths where cytochromes are known to absorb strongly. In the blue-green alga, *Anacystis nidulans,* light-induced absorbancy changes at 425, about 550, and 556 mμ have been ascribed to oxidation and reduction of *c*- and *f*-type cytochromes (*1*). In the blue-green algae isolation of only a *c*-type cytochrome from *Tolypothrix tenuis* has been reported (*2*). We have isolated from *Anacystis* three water-soluble cytochromes whose properties may have important implications for the interpretation of data obtained in vivo.

Water-soluble cytochromes, along with other pigments, are readily extracted from lyophilized algal cells (*3*). Chromatography of such an extract on diethyl aminoethyl (DEAE)-cellulose permits separation of three cytochrome-containing fractions: (i) cytochrome-552 (*4*) which is not adsorbed on the cellulose and is isolated from that portion of the extract passing through the column; (ii) cytochrome-554 which is adsorbed to the cellulose and is eluted in reduced form at concentrations of about 0.03*M* phosphate buffer, *p*H 7.0; and (iii) cytochrome-549, which is eluted in oxidized form with about 0.09*M* buffer. Cytochrome-552 was partially purified by chromatography on Amberlite XE-64. Cytochromes-549 and -554 were freed of contaminating proteins by chromatography on DEAE-cellulose with elution by *p*H 6.0 and 7.0 phosphate buffers and fractionation with ammonium sulfate, and, in the case of cytochrome-554, an electrophoresis on polyacrylamide gel.

Properties of these cytochromes are summarized in Tables 1 and 2. The

Table 1. Absorption maxima (in millimicrons) of water-soluble cytochromes isolated from the blue-green alga, *Anacystis nidulans*.

State	Cyt-552	Cyt-554	Cyt-549
Oxidized	526	525–30	525–30
Oxidized	410	411	408
Oxidized	352	360	351
Oxidized	279	275	278
Reduced (α)	552	554	549
Reduced (β)	523.5	522.5	521.5
Reduced (γ)	420	416.5	417.5

spectrum of the alkaline pyridine hemochromogen prepared from each of the cytochromes is identical to that derivative of cytochrome *c*, identifying the porphyrin as a mesoporphyrin.

Quantitative data (Table 3) were obtained for two different batches of cell material grown under slightly different conditions of illumination. The cytochrome concentration was obtained from the pyridine-hemochromogen content in each cytochrome fraction after separation (but not purification) on a DEAE-cellulose column of an extract prepared by extracting the cells three times. The heme content of each fraction was calculated from the molar extinction coefficient of the hemochromogen (*5*) after correction for interference due to phycocyanin present in the fractions.

Cytochrome-552—present only in very small amounts (Table 2)—has not been studied in detail and may actually be a mixture. The low concentration of this cytochrome may be related to the relatively weak respiratory rate of this alga.

Cytochrome-554 closely resembles in spectral properties and oxidation-reduction potential (E_o') the cytochrome isolated from *Tolypothrix* (*2*) and cytochrome *f* first isolated and characterized from green plant chloroplasts (*6*) and recently from red and green algae (*7*).

Table 2. Descriptive characteristics of water-soluble cytochromes isolated from the blue-green alga, *Anacystis nidulans*.

Characteristic	Cyt-552	Cyt-554	Cyt-549
Porphyrin	Meso-	Meso-	Meso-
IEP	Basic	Acidic	Acidic
Autoxidation		–	+
CO reaction		–	+
E_o' (pH 7.0)		+0.35 v* ±0.01 v	–0.26 v† ±0.02 v

* Spectrophotometric titration with ferricyanide/ferrocyanide ($E_o' = +0.43$ volt) as a couple (*6*) in air. † Spectrophotometric titration with oxidized and reduced forms of 2-anthroquinone-sulfonate ($E_o' = -0.226$ volt) (*14*) in a system poised with sodium dithionite (*9*) in a hydrogen atmosphere.

Cytochrome-549 appears to have the most negative E_o' of any *c*-type cytochrome isolated. The strongly negative E_o' was first suspected when ferrous oxalate and reduced riboflavin failed to reduce it although reduced benzyl viologen did. Reduction by reduced Janus green suggested an E_o' near –0.23 volt, but overlapping absorption characteristics prevented use of that dye for quantitative determinations. With 2-anthraquinone sulfonate (Table 1), titrations of two samples of different purities gave similar results. In its properties, cytochrome-549 more closely resembles two bacterial cytochromes than any cytochrome thus far isolated from other algae or from higher plants. *Chromatium* cytochrome *c* reacts with carbon monoxide (*8*) while *Desulfovibrio* cytochrome c_3 has an E_o' of –0.205 volt (*9*).

The strongly negative E_o', autoxidizability, and reaction with carbon monoxide suggest the possibility that the cytochrome may be isolated in a denatured form. However, mild conditions of nearly neutral *p*H at 4°C and the constant quantitative relationship of cytochromes-554 and 549 during extractions do not support this point. The same experimental conditions allow isolation of cytochrome-554 in an apparently undenatured form; fractionation on DEAE-cellulose of an extract obtained from fresh cells disrupted by ultrahigh-frequency sound in acetate buffer *p*H 4.7 also separates cytochromes-549 and 554.

The molar ratio of chlorophyll to cytochrome-549 (Table 2) suggests a size for the photosynthetic unit in *Anacystis* similar to that indicated by the 400:1 ratio of chlorophyll to cytochrome *f* found earlier in chloroplasts (*6*).

Photosynthetic pyridine nucleotide reductase is present in blue-green algae (*10*) and is eluted from the cellulose column with 0.5*M* phosphate buffer. The reductase (or chloroplast ferredoxin) isolated from spinach has the most negative E_o' known for a constituent of the photosynthetic apparatus, –0.43 volt (*11*), and on a molar basis is similar in concentration in our extracts to that of cytochrome-549.

Light-induced absorption changes in vivo in *Anacystis* have been ascribed to the *f*-type cytochrome found in higher plant chloroplasts (*1*). It is implied that the cytochrome is operative in photosynthesis at a potential close to that of the special form of chlorophyll, P700 ($E_o' = +0.43$ volt) (*12*). Isolation of cytochrome-549 ($E_o' = -0.26$ volt) as the major cytochrome component in *Anacystis* suggests that in this organism, absorption changes may reflect electron transport at redox levels close to that of photosynthetic pyridine nucleotide reductase as well (*13*).

Table 3. Concentration of photosynthetic pigments, and of water-soluble cytochromes (expressed in micromoles of heme) and photosynthetic pyridine nucleotide reductase (PPNR) in extracts of *Anacystis nidulans*.

Pigment	Concn. (μmole/g dry wt)		Av. Chl*
	Expt. 34	Expt. 35	
Chlorophyll†	20	13	
Phycocyanin‡	1.2	0.94	16
Cyt-552	0.00082	.00069	22,000
Cyt-554	.013	.0080	1,600
Cyt-549	.080	.068	220
PPNR§	.10	.090	190

* Micromoles of chlorophyll per micromole of other assayed pigments. † Determined by acetone extraction (*15*) and calculated with mol. wt. = 893.5. ‡ Determined from material obtained after treatment with ultrahigh-frequency sound (*15*) and calculated with mol. wt. = 138,000 (*16*). § Concentration calculated from the provisional millimolar extinction coefficient of the spectrally similar spinach ferredoxin (*17*).

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