of ACPC by bacterial cells compared with the animal where ACPC is readily incorporated[8,9].

Berlinguet *et al.*[10] have presented evidence that ACPC antagonizes the *in vitro* and *in vivo* incorporation of valine into proteins of the rat and indicate that the site of its action is prevention of the attachment of valine to transfer RNA.

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L. J. Machlin
R. S. Gordon
F. Puchal

Laboratory of Biochemistry and Nutrition,
Monsanto Chemical Co.,
St. Louis, Mo.

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Photochemical Reduction of Triphosphopyridine Nucleotide by Cell-free Extracts of Blue-green Algae

The photochemical reduction of triphosphopyridine nucleotide (TPN) by extracts of photosynthetic organisms has been shown to be catalysed by a protein factor, photosynthetic pyridine nucleotide reductase (PPNR)[1]. PPNR has been detected in a variety of higher plants, algae and photosynthetic bacteria[1,2]. It has been shown[2] to be similar to, but not identical with, the electron transport factor ferredoxin which has been isolated from several non-photosynthetic bacteria[3]. Thus far, however, PPNR has not been demonstrated in blue-green algae. Indeed, it has not proved possible to obtain cell-free TPN reduction by these organisms[4] although spectroscopic studies on whole cells[5] have shown that endogenous pyridine nucleotides undergo oxidation-reduction changes under illumination. In this communication, we report the isolation and purification of PPNR from a blue-green alga and also a method of obtaining photochemical TPN reduction in a cell-free system.

TPN and ADP were purchased from the Sigma Chemical Co., and inorganic phosphorus-32 from the Squibb Laboratory. *Nostoc muscorum*, *Anacystis nidulans*, *Anabaena variabilis* and *Tolypothrix tenuis* were cultured as described previously[6]. Cell-free preparations were prepared by vacuum-drying, essentially as described by Schwartz for *Chlorella*[7]. The drying could be performed at up to 40° C and the preparations stored at 4° C or −15° C for several months without appreciable loss of activity. These preparations contain all the pigments present in the original cells. The preparation was suspended in 0·1 M *tris*–hydrochloric acid buffer, *p*H 7·7, and ground vigorously in a Ten-Broeck homogenizer just prior to use.

The photochemical activity of the cell-free preparation of *Anacystis* is illustrated in Table 1. 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) completely inhibited the reduction of TPN, but the activity could be restored by the addition of 2,6-dichlorophenol-indophenol (DCIP) and ascorbate, as described by Vernon and Zaugg[8] for spinach chloroplasts. The reaction was linear for 30 min, proportional to chlorophyll concentration up to 15 μg/ml., and was saturated at about 1,000 ft.-candles of white light. Attempts to demonstrate photophosphorylation by these extracts in the presence of TPN, phenazine methosulphate, flavin mononucleotide or vitamin K_3 were all unsuccessful. We were, however, able to confirm the phenazine methosulphate phosphorylation reported by Petrack and Lipmann[4] when particles were prepared in the presence of a high molecular weight polymer.

Table 1. Photochemical Reduction of TPN by Dried Cell-free Preparations from *Anacystis nidulans*

Addition to reaction mixture	μMoles TPNH/mg chlorophyll/h
Control	19·8
+ CMU (2×10^{-5} M)	0
+ DCIP (0·2 μmole), ascorbate (14 μmoles)	22·7
+ CMU, DCIP, ascorbate	22·2

The complete reaction mixture contained the following components: *tris*-HCl buffer, *p*H 7·7, 40 μmoles; $MgCl_2$, 4 μmoles; ADP, 2 μmoles; KH_2PO_4–K_2HPO_4, *p*H 7·5, 4 μmoles; TPN, 1·5 μmoles; extract of blue-green alga; total volume, 3 ml. Dark controls were used throughout. The assays for ATP^{32} and TPNH formation were carriedo ut as previously described (ref. 9). Illumination was at 3,000 ft.-candles of white light at room temperature (22°–24° C). Chlorophyll concentration was determined by van Baalen's method (ref. 10).

Using the assay described by San Pietro[1] we were able to detect PPNR activity in *Anacystis*, *Anabaena*, *Nostoc* and *Tolypothrix* in the presence of spinach chloroplasts. The enzyme from *Nostoc* was purified through the 'Dowex–Bentonite' step[1] and the final preparation had a specific activity of 30 units/mg protein[1]. The absorption spectrum of the purified protein showed peaks at 320 mμ and 420 mμ, a shoulder from 450 to 470 mμ and a trough at 396 mμ, thus resembling the absorption spectra of PPNR preparations from other sources[1,2].

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Clanton C. Black*
Charles A. Fewson
Martin Gibbs

Department of Biochemistry,
Cornell University,
Ithaca, New York.

* Present address: Charles F. Kettering Research Laboratory, Yellow Springs, Ohio.

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Structure of Bilirubin

Conjugated bilirubins (esters) are formed within the liver cells from bilirubin and glucuronic or sulphuric acid. These compounds are very unstable because of the highly reactive central methylene bridge. They are oxidized rapidly by the air and react immediately with diazotized sulphanilic acid. Contrary to the esters, pure bilirubin is relatively stable and reacts with the diazoreagent only when an accelerator is added.

This characteristic difference between bilirubin and its esters may be explained by the presence in pure bilirubin of intramolecular hydrogen bonds. Two possible structures with hydrogen bonds between the carboxyl-groups and the pyrrol nitrogens are shown in Fig. 1.

The activating stimulus from the pyrrol-rings affecting the central methylene bridge is nearly eliminated in these structures. Steric hindrance may also be an inhibiting factor in structure 2.

Infra-red spectra are shown of bilirubin, mesobilirubin (with ethyl instead of vinyl) together with the dimethyl-ester of mesobilirubin.