laevulinate dehydratase from mammal tissues) are taken as 100% of activity. Tables IIa and IIb summarize the percentages of activity obtained with higher cysteine concentrations for the two molarities of the substrate.

From this study it is possible to consider that 5-aminolaevulinate may react with free –SH groups, especially when added in relatively high concentrations, and thus influence the rate of porphobilinogen formation. Although cysteine molarities needed to reduce enzymic porphobilinogen production seem too high with respect to physiological levels of tissues, the molarity of 5-aminolaevulinate needed to measure the activity *in vitro* must also be high. 5-Aminolaevulinate and free –SH groups (expressed as cysteine) were measured in yeast and liver according to the methods of URATA AND GRANICK[5] and GRUNERT AND PHILLIPS[7], respectively. Values of 90 nmoles of 5-aminolaevulinate and 12 μmoles of cysteine per g of fresh yeast were obtained, while fresh rabbit liver gave contants of 19 nmoles and 2 μmoles per g, respectively; the ratio of the values obtained for cysteine and 5-aminolaevulinate was roughly higher than 100 for both biological sources, so that a chemical interaction would be possible at physiological pH's. Also it would be necessary to consider such interactions in kinetic studies, especially to ascertain the real 5-aminolaevulinate concentration in the incubation mixtures.

The author wishes to express her gratitude to Dr. Luis F. Leloir and Dr. Carlos E. Cardini for allowing her to use the laboratories of the Instituto de Investigaciones Bioquímicas for the performance of this work. She is also indebted to Dr. Israel D. Algranati for helpful advice and to the other members of the Institute for their kind collaboration.

Instituto de Investigaciones Bioquímicas "Fundación Campomar", Obligado 2490, Buenos Aires (28) (R. Argentina)

OFELIA L. CLARA DE BARREIRO

1 O. L. C. DE BARREIRO, *Biochim. Biophys. Acta*, 139 (1967) 479.
2 H. A. ROTSCHILD AND E. S. GUZMÁN BARRON, *J. Biol. Chem.*, 209 (1954) 511.
3 A. DUGAICZIK, M. MALECKI AND J. J. EILER, *J. Biol. Chem.*, 243 (1968) 2236.
4 S. GRANICK AND D. MAUZERALL, *J. Biol. Chem.*, 232 (1958) 1119.
5 G. URATA AND S. GRANICK, *J. Biol. Chem.*, 238 (1963) 811.
6 K. D. GIBSON, A. NEUBERGER AND J. J. SCOTT, *Biochem. J.*, 61 (1955) 618.
7 R. GRUNERT AND P. H. PHILLIPS, *Arch. Biochem.*, 30 (1951) 217.

Received December 12th, 1968

BBA 63376

UDP-D-glucuronate 4-epimerase in blue-green algae

Pectin, a polymer of D-galacturonic acid is a major fraction of higher plant polysaccharides. In algae, D-galacturonic acid-containing polysaccharides are quite rare and appear to be restricted to Cyanophyceae[1] and Rhodophyceae[2,3]. Even in

these algae D-galacturonic acid is only a minor polysaccharide constituent. In the biosynthesis of plant pectin UDPGalUA has been shown to act as D-galacturonosyl donor[4]. Its synthesis in plants from D-galacturonic acid by a kinase and a pyrophosphorylase has been described[5,6]. In addition, plants contain a particle-bound epimerase that mediates the reversible formation of UDPGalUA from UDPGlcUA through epimerization at C-4 of the glycuronosyl moiety (UDPGlcUA 4-epimerase; EC 5.1.3.6)[7]. The same reaction has been demonstrated in Pneumococcus type I, which produces a galacturonic acid-containing capsular polysaccharide[8]. Certain non-capsulated mutants have been shown to also lack UDPGlcUA 4-epimerase[9]. This relationship points to the significance of the epimerase reaction in polysaccharide biosynthesis in these bacteria.

By analogy to the situation in plants and Pneumococci it might be expected that polysaccharide biosynthesis in blue-green and red algae utilizes UDPGalUA as the D-galacturonosyl donor. In support of this hypothesis this communication presents evidence for the existence of UDPGlcUA 4-epimerase in the blue-green alga *Anabaena flos-aquae*, a member of the Nostoc family, for which galacturonic acid-containing polysaccharides are well documented[1].

All the chemicals used were commercial products, with the exception of the following: D-mannuronic acid and L-guluronic acid were isolated from hydrolysates of commercial alginic acid[10,11]. UDPGalUA pyrophosphorylase was obtained from mung bean seedlings[5]. UDPGlcUA carboxy-lyase (EC 4.1.1.35) was prepared from *Cryptococcus laurentii*[12]. Ascending paper chromatography was carried out on Whatman 1 paper using the following solvent systems: (1) 95% ethanol–1 M ammonium acetate (pH 7.5)–0.1 M EDTA (70:30:1, v/v/v); (2) *n*-propanol–ethyl acetate–water (7:1:2, v/v/v); (3) *n*-propanol–acetic acid–water (6:1:2, v/v/v); (4) pyridine–ethyl acetate–acetic acid–water (5:5:1:3, by vol.) in a tank saturated with pyridine–ethyl acetate–water (11:40:6, v/v/v). Paper electrophoresis was carried out on Whatman 1 paper at 15–20 V/cm for 2–4 h in the following systems: (A) 0.2 M ammonium acetate (pH 5.8); (B) 0.1 M ammonium formate (pH 3.6); (C) 0.01 M sodium borate, containing 0.005 M calcium chloride (pH 9.2); (D) 0.1 M sodium hydroxide. Oxalic acid-washed paper was used for the electrophoresis of nucleotides and sugar nucleotides. Carbohydrates were detected with *p*-anisidine phthalate; ultraviolet-absorbing compounds, by visual inspection under ultraviolet light; and radioactive compounds, by radioautography. Radioactivity was measured in a gas-flow counter. Reducing sugar was determined with ferricyanide after hydrolysis at pH 1 for 15 min at 100° (ref. 13), organic phosphate, with ammonium molybdate–ascorbic acid after ashing with nitric acid[14], and protein was determined according to LOWRY *et al.*[15].

Cultures of *A. flos-aquae* A-37* were grown on modified Knop's medium[16,17]. They were free of bacterial contamination after examination of 1-ml aliquots in 10 ml nutrient broth and incubation for 48 h. The preparation of crude extracts and ammonium sulfate fractionations were performed as previously described[18] starting with 5 g of algae (wet wt.) suspended in 16 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.5 g/l of EDTA and 0.5 ml/l of 2-mercaptoethanol. UDPGlcUA 4-epimerase precipitates between 1.7 and 3.3 M ammonium sulfate. The precipitated

Abbreviations: UDPGalUA, uridine 5′-(α-D-galactopyranosyluronic acid pyrophosphate); UDPGlcUA, uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate); UDPXyl, uridine 5′-(α-D-xylopyranosyl pyrophosphate); UDPAra, uridine 5′-(β-L-arabinopyranosyl pyrophosphate).

protein was dissolved in 4 ml of the above buffer and dialyzed overnight against 1 l of the same buffer. The dialyzed material (28 mg protein/ml) retained UDPGlcUA 4-epimerase activity after several months of storage at −20°.

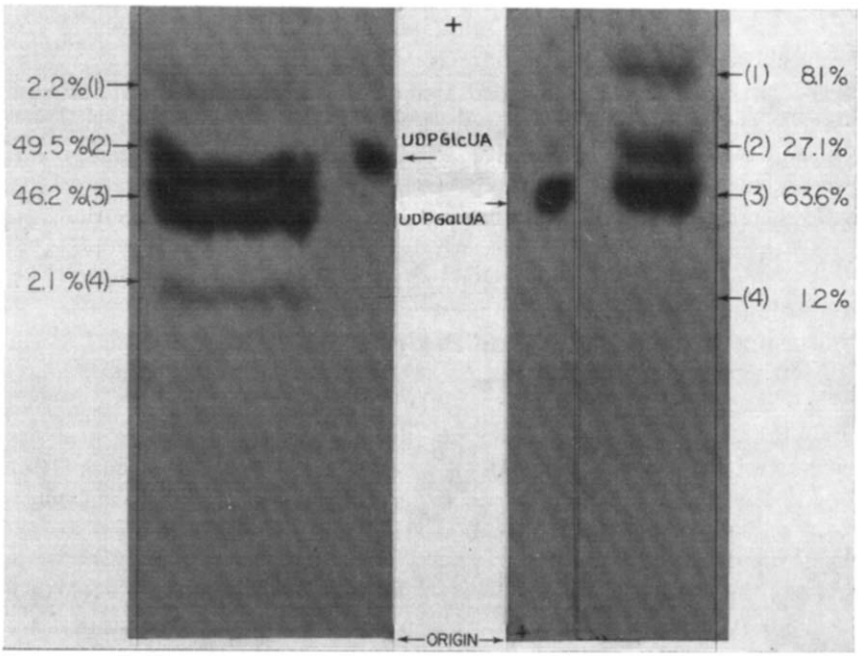

Fig. 1. Paper-electrophoretic separation of reaction mixtures containing 1 μC of UDP[^{14}C]GlcUA and 2.8 mg of enzyme (left) or 0.05 μC of UDP[^{14}C]GalUA and 1.4 mg of enzyme (right) in total volumes of 0.2 and 0.1 ml, respectively (pH 7.0), after 2 h of incubation at 25°, as revealed by radioautography. Also shown is the distribution of label that was determined after elution of each band.

After incubation of UDP[^{14}C]GlcUA (173 mC/mmole) with the algal enzyme preparation, electrophoresis (System A) resolves the reaction mixture into several radioactive bands (Fig. 1). One of the major bands (band 2) is unreacted UDPGlcUA, the other (Band 3) has the mobility of UDPGalUA. The minor band 1 was not investigated further. Band 4 is a mixture of UDPXyl and UDPAra[18]. Band 3 was eluted and purified further by electrophoresis (System B) and chromatography (Solvent 2). Similarly, unlabeled "band 3" was isolated and purified from a reaction mixture containing 4 μmoles of unlabeled UDPGlcUA and 28 mg of enzyme in a total volume of 1 ml after incubation for 8 h at 25°. This material had an ultraviolet spectrum characteristic of uridine compounds. The ratios of uridine:total phosphate:reducing sugar were 0.9:2:1.1. Mild acid hydrolysis (pH 1, 100°, 15 min) released an ultraviolet-absorbing compound with electrophoretic and chromatographic mobilities of authentic UMP (System B, Solvent 1). The carbohydrate released by this treatment migrated like galacturonic acid upon electrophoresis (System B). Similarly, the

* Obtainable through the Culture Collection of Algae at Indiana University, Bloomington, Ind.

radioactive material released from the radioactive band 3 by mild acid hydrolysis migrated identically to authentic galacturonic acid upon co-electrophoresis (Systems B and C) and co-chromatography (Solvent 4). As expected for galacturonic acid, no lactone was observed. The carbohydrate moiety could be clearly separated from authentic guluronic, glucuronic, and mannuronic acids in the systems employed.

Further proof that the material obtained after mild acid hydrolysis was galacturonic acid was sought using radioactive band 3. An aliquot (0.15 μC) was mixed with 500 mg of authentic unlabeled D-galacturonic acid and oxidized with bromine water[19]. The mucic acid thus obtained was washed several times with cold water, dried, and the specific radioactivity determined. The material was then dissolved in the calculated amount of 1.5 M NaOH and reprecipitated with 4 M HCl. After several washings of the precipitate with water the specific radioactivity of the dried material was again determined. Recrystallization and washing were repeated 2 more times. The observed specific activities were as follows: first crystals, 35; second, 33; third, 32; fourth, 35 counts/min per mg.

Since bromine oxidation of both D- and L-galacturonic acids results in the formation of mucic acid, the D configuration of the carbohydrate portion of radioactive band 3 was shown by reduction to D-galactose. An aliquot (0.1 μC) was converted to methyl (methyl galactopyranoside) uronate by heating it in a sealed capillary tube at 120° for 1 h with 20 μl of 5% methanolic HCl. The reaction mixture was dried and reduced with 40 μl of 0.5 M aq. sodium borohydride at 25° for 15 min[20]. After removal of Na^+ with cation exchanger (H^+) and of boric acid by repeated evaporation in the presence of methanol, the methyl galactoside was hydrolysed in 1 M HCl at 100° for 1 h. The resulting radioactive product had the mobility of authentic galactose upon chromatography (Solvents 2, 3) and electrophoresis (System D). A portion of the radioactive reduction product (0.02 μC) was mixed with unlabeled D-galactose (0.05 mg) and incubated with 1 unit of galactose oxidase (EC 1.1.3.9) and 0.13 mg of catalase (EC 1.11.1.6) at pH 7.0 for 2 h at 37°, followed by chromatography in Solvent 3. Authentic D-[^{14}C]galactose was treated in an identical fashion. Both reaction mixtures revealed conversion of the radioactive material to D-galacto-hexodialdose[21]. Since L-galactose does not react with this enzyme[21], the uronic acid moiety of the original sugar nucleotide must have the D configuration.

Incubation of an aliquot of band 3 (0.03 μC) with UDPGalUA pyrophosphorylase (0.1 mg) in the presence of 0.5 μmole of sodium pyrophosphate, 0.5 μmole of $MgCl_2$, 5 μmoles of NaF and 0.5 μmole of 2-mercaptoethanol at 25° for 1 h (pH 7) resulted in its conversion to a compound with the electrophoretic mobility of galactopyranosyluronic acid 1-phosphate (System A). Such a product was not observed when pyrophosphate was left out of the reaction mixture, indicating that the reaction was indeed due to pyrophosphorolysis. Since the β anomer does not react with this enzyme[5], the sugar moiety of the algal reaction product must have the α configuration.

Epimerization of UDPGalUA was demonstrated by incubating radioactive UDPGalUA (173 mC/mmole) with Anabaena enzyme, followed by electrophoresis (System A) as shown in Fig. 1. The newly-formed major band has the mobility of authentic UDPGlcUA. That UDPGlcUA was the major product of the back reaction was confirmed by its conversion to a compound with the chromatographic and electrophoretic mobilities of authentic UDPXyl upon incubation with UDPGlcUA carboxy-lyase from *Cryptococcus laurentii* (Solvent 1, System A).

It is of interest to note that the Anabaena enzyme catalyzes epimerization of UDPGlcA to the same extent in the absence, as well as in the presence, of 2 mM NAD^+ and is not inhibited by 2 mM NADH. In this respect blue-green algae appear to behave like plants[22] and not like bacteria, for which NAD^+ activation of this enzyme has been reported[23]. If NAD^+ is involved in the epimerization reaction, it must be tightly bound to the algal enzyme, thus resisting removal by dialysis.

This work was supported by a research grant from the U.S. Public Health Service (AM 11411-02). The technical assistance by Miss Kathy Bartol is gratefully acknowledged.

Department of Biochemistry,
Marquette School of Medicine,
Milwaukee, Wisc. (U.S.A.)

HELMUT ANKEL

Department of Microbiology,
Mississippi State University
State College, Miss. (U.S.A.)

ROBERT G. TISCHER

1 J. K. N. JONES, L. HOUGH AND W. H. WADMAN, *J. Chem. Soc.*, (1952) 3393.
2 D. M. BOWKER, Ph. D. Thesis, University of Wales, England, 1966.
3 V. A. EVTUSHENKO, M. A. ANTONOVA AND G. V. MAKHNOVA, *Zh. Prikl. Khim.*, 40 (1967) 1767.
4 C. L. VILLEMEZ, A. L. SWANSON AND W. Z. HASSID, *Arch. Biochem. Biophys.*, 116 (1966) 446.
5 E. F. NEUFELD, D. S. FEINGOLD, S. M. ILVES, G. KESSLER AND W. Z. HASSID, *J. Biol. Chem.*, 236 (1961) 3102.
6 D. S. FEINGOLD, E. F. NEUFELD AND W. Z. HASSID, *Arch. Biochem. Biophys.*, 78 (1958) 401.
7 D. S. FEINGOLD, E. F. NEUFELD AND W. Z. HASSID, *J. Biol. Chem.*, 235 (1960) 910.
8 E. E. B. SMITH, G. T. MILLS, H. P. BERNHEIMER AND R. AUSTRIAN, *Biochim. Biophys. Acta*, 29 (1958) 640.
9 R. AUSTRIAN, H. P BERNHEIMER, E. E. B. SMITH AND G. T. MILLS, *J. Exptl. Med.*, 110 (1959) 585.
10 R. L. WHISTLER AND J. N. BEMILLER, in R. L. WHISTLER AND M. L. WOLFROM, *Methods in Carbohydrate Chemistry*, Vol. 2, Academic Press, New York, 1963, p. 35.
11 A. HAUG AND B. LARSEN, in A. D. DEVIRVILLE AND J. FELDMAN, *Proc. 4th Intern. Seaweed Symp.*, Pergamon Press, Oxford, 1961, p. 237.
12 H. ANKEL AND D. S. FEINGOLD, *Biochemistry*, 5 (1966) 182.
13 J. T. PARK AND M. J. JOHNSON, *J. Biol. Chem.*, 181 (1949) 149.
14 B. AMES, in E. F. NEUFELD AND V. GINSBURG, *Methods in Enzymology*, Vol. 8, Academic Press, New York, 1966, p. 115.
15 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
16 B. G. MOORE AND R. G. TISCHER, *Science*, 145 (1964) 586.
17 B. G. MOORE AND R. G. TISCHER, *Can. J. Microbiol.*, 11 (1965) 877.
18 H. ANKEL, E. ANKEL, D. S. FEINGOLD AND J. S. SCHUTZBACH, *Biochim. Biophys. Acta*, 136 (1967) 172.
19 R. M. MCCREADY, in R. L. WHISTLER AND M. L. WOLFROM, *Methods in Carbohydrate Chemistry* Vol. 2, Academic Press, New York, 1963, p. 27.
20 B. A. LEWIS, F. SMITH AND A. M. STEPHEN, in R. L. WHISTLER AND M. L. WOLFROM, *Methods in Carbohydrate Chemistry*, Vol. 2, Academic Press, New York, 1963, p. 68.
21 G. AVIGAD, D. AMARAL, C. ASENSIO AND B. L. HORECKER, *J. Biol. Chem.*, 237 (1962) 2736.
22 E. F. NEUFELD, in E. F. NEUFELD AND V. GINSBURG, *Methods in Enzymology*, Vol. 8, Academic Press, New York, 1966, p. 276.
23 E. E. B. SMITH, G. T. MILLS, R. AUSTRIAN AND H. P. BERNHEIMER, *J. Gen. Microbiol.*, 22 (1960) 265.

Received December 9th, 1968