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Carbohydrates and Nucleotides in the Red Alga *Porphyra perforata*.* II. Separation and Identification of Nucleotides

JONG-CHING SU† AND W. Z. HASSID

From the Department of Biochemistry, University of California, Berkeley, California

Received January 18, 1962

The nucleotides present in the marine red alga *Porphyra perforata* have been investigated. On chromatographic separation on ion-exchange resin and on paper of compounds present in the ethanolic extract of the alga, AMP, UMP, GMP, IMP, ADP, UDP, IDP, DPN, TPN, UDP-D-glucose, UDP-D-galactose, UDP-glucuronic acid, GDP-D-mannose, and GDP-L-galactose were isolated and characterized. A new nucleotide that appears to be adenosine 3′,5′-pyrophosphate was also isolated. It is suggested that (a) GDP-L-galactose is formed from GDP-D-mannose by a mechanism similar to that involved in the GDP-D-mannose–GDP-L-fucose transformation; (b) GDP-L-galactose serves as the glycosyl donor for the L-galactose component in polysaccharide synthesis; and (c) 6-sulfation of the galactosyl residue is the key intermediate step in the modification of the D- and L-galactosyl residues of the polysaccharide by different modes of etherification.

Nucleoside diphosphate sugars are an important and widely distributed class of compounds in nature. Their importance lies in the fact that the sugar moiety of the nucleotide is activated and can undergo a variety of transformations. A most noteworthy metabolic function of the sugar nucleotides is their role in transglycosylation reactions. These reactions include such diverse processes as the synthesis of sucrose, callose, glycosides, and starch in higher plants; chitin in fungi and cell walls of bacteria; trehalose in yeast; and glycogen and glucuronides in animals (Leloir *et al.*, 1960; Hassid and Neufeld, 1962).

While the metabolic functions of the sugar nucleotides have been studied in animal tissues, yeast, and microorganisms, there is practically no information available regarding these compounds in marine algae.

* This investigation was supported in part by a research grant (No. A-1418) from the U. S. Public Health Service, National Institutes of Health, and by a research contract with the U. S. Atomic Energy Commission.

† Present address: Department of Agricultural Chemistry, College of Agriculture, National Taiwan University, Taipei, Taiwan, China.

The identification of the nucleotides guanosine diphosphate L-galactose, guanosine diphosphate D-mannose, and adenosine 3′,5′-pyrophosphate in a red alga, *Porphyra perforata*, has been reported in a previous communication (Su and Hassid, 1960). This paper describes the results of an investigation of these as well as a number of other nucleotides in this alga.

Inasmuch as *Porphyra perforata* contains complex saccharides composed chiefly of galactose moieties of both D- and L-configuration (Su and Hassid, 1962), together with sugar nucleotides containing the two enantiomorphs of galactose, a discussion pertaining to the possibility that these sugar nucleotides are immediate precursors of the complex galactose compounds is presented.

Experimental Procedure

Materials and Methods.—Most of the nucleotides which served as chromatographic and electrophoretic standards were commercial products. Inosinic acid and inosine diphosphate were prepared from the corresponding adenine nucleotides by deamination with nitrous acid (Kaplan, 1957), and the products were purified by paper electrophoresis. α-D-Mannose 1-phosphate, α-D-galactose 1-phosphate, and C^{14}-labeled D-glucose, D-mannose, and D-galactose were provided by Dr. E. W. Putman. Uridine diphosphate (UDP) D-glucuronic acid-C^{14} and UDP-D-galacturonic acid-C^{14} were contributed by Dr. G. Kessler. Potato nucleotide pyrophosphatase was a gift from Dr. H. A. Barker.

Ultraviolet (UV) absorption spectra were determined with a Cary recording spectrophotometer, Model 14. A sensitive Rudolph automatic spectropolarimeter was used to observe the rotation of dilute sugar solutions obtained from the hydrolysates of the sugar nucleotides.

Reducing values of the sugars liberated from the sugar nucleotides after hydrolysis with 0.01 N HCl for 10 minutes at 100° were determined according to Park and Johnson (1949). The pentose content of the nucleotides was determined by the orcinol method of Mejbaum (1939). The 5′-adenylic and uridylic acids served as the standards for the determinations of the pentose in the purine and pyrimidine nucleotides, respectively. Total phosphorus content and phosphorus liberated from the nucleotides after hydrolysis for 10 minutes with 1 N H_2SO_4 at 100° were estimated by the method of Fiske and SubbaRow (1925). For total phosphorus determinations, the organic phosphorus was liberated as inorganic phosphorus by digestion with magnesium nitrate (Association of Official Agricultural Chemists, 1945). Microquantities of D-galactose were estimated by an enzymatic method using the D-galactose dehydrogenase of *Pseudomonas saccharophila* (Su and Hassid, 1962).

Paper electrophoresis was conducted with an apparatus similar to that described by Crestfield and Allen (1955). The following buffers were used: 0.1 M ammonium formate, *p*H 3.6 and 3.8; 0.1 M ammonium acetate, *p*H 5.7; 0.1 M potassium phosphate, *p*H 7.6; 0.05 M sodium tetraborate, *p*H 9.2.

For the isolation of nucleotides present in the fractions obtained by ion-exchange resin chromatography, descending paper chromatography with ethanol–1 M ammonium acetate, *p*H 7.5, 3:7, 0.001 M with respect to ammonium versenate, was used (Paladini and Leloir, 1952). Radial development was employed for qualitative analysis, with a solvent of similar composition but at *p*H 5.1 (Wawszkiewicz, 1961).

Two-dimensional separation of monosaccharides was performed on Whatman No. 1 paper with water-saturated phenol in the first dimension and butanol–acetic acid–water, 52:13:35, in the second (Putman and Hassid, 1954).

The nucleotides were located on the paper by contact printing under ultraviolet light (Markham and Smith, 1949). Phosphate spots were revealed by the method of Bandurski and Axelrod (1951). Reducing sugars were detected with *p*-anisidine phosphate spray reagent (Feingold *et al.*, 1958). Hexitols were detected by periodate-benzidine dipping reagent (Gordon *et al.*, 1956). Radioactive spots were located by autoradiography.

Extraction and Analysis of Nucleotides.—The procedures involving the analysis and separation of the nucleotides were all carried out at 0°. The mercury salts of the nucleotides precipitated from the ethanolic extract of 5.2 kg of the algae (Su and Hassid, 1962) were suspended in water, decomposed with hydrogen sulfide, and aerated to expel the excess H_2S. A small amount of octyl alcohol was added before aeration to prevent excessive foaming. The solution containing the nucleotides was then neutralized to *p*H 7 with concentrated ammonium hydroxide and put on the top of a well-washed Dowex 1 column (chloride form, 4 × 30 cm, 200–400 mesh, ×8). The material that was not absorbed on the column was eliminated by washing with water, and the nucleotides were eluted with 0.01 N HCl, containing increasing concentrations of NaCl (Volkin and Cohn, 1953), at the rate of 7 to 10 ml per minute. The eluate was collected in 0.5-liter fractions by means of an automatic fraction collector (Ginsburg, 1956). Elution of the nucleotides was followed by changes in optical density of the eluate at 260 mμ. The fractions falling under the same peak were pooled and concentrated by adsorption on charcoal (for O.D. of 1 per liter of eluate, 1 g of acid-washed Norite A was found to be sufficient) and subsequent elution with ammonia–ethanol–water (Cabib *et al.*, 1953). The alkaline solution was neutralized with acetic acid to *p*H 7 and concentrated under diminished pressure.

Since analysis by paper chromatography showed that each peak contained more than one compound, the nucleotides were further separated by descending paper chromatography on oxalic

acid-washed Whatman No. 1 paper in the ethanol–ammonium acetate solvent system (Paladini and Leloir, 1952). The separated nucleotides were then eluted from the chromatogram with water.

In every case, the strip of paper containing the separated nucleotide and the eluate from the strip were weighed. A blank strip cut from the same chromatogram was weighed and eluted in the same manner. From these weighing data, it was possible to apply blank corrections on the basis of the equivalent weight of the chromatogram. This method of blank correction was successfully applied for the analysis of the nucleotides.

The nucleotides obtained from the paper chromatogram were characterized chiefly by spectrophotometric analysis (acid, neutral, and basic), electrophoretic and chromatographic mobility, and pentose and phosphorus (total and labile) content. The nucleoside monophosphates were identified as the 5′-isomers by electrophoresis in 0.05 M tetraborate, *p*H 9.2, where they can be distinguished from the 2′- and 3′-isomers (Jaenicke and Vollbrechtshausen, 1952).

Results

The chromatographic elution pattern of the nucleotides is shown in Figure 1. The nucleotides

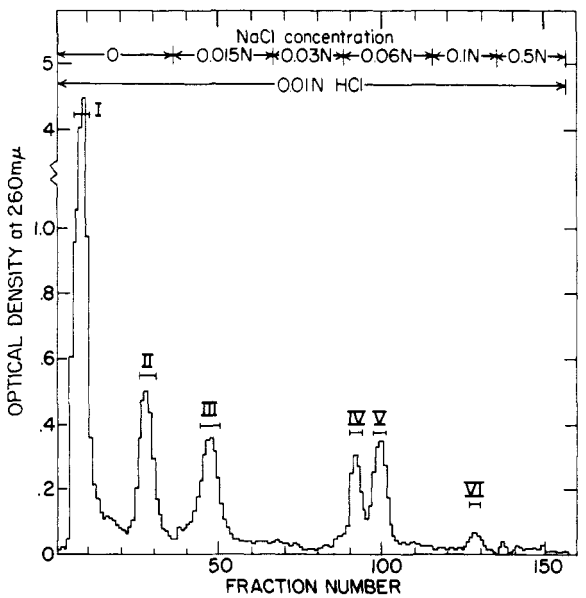


FIG. 1.—Separation of *Porphyra perforata* nucleotides by anion exchange chromatography on a Dowex 1 (Cl^-) column. The nucleotides were eluted with 0.01 N hydrochloric acid containing increasing concentrations of sodium chloride as indicated.

isolated and characterized are summarized in Table I. The components of each peak are named as A, B, C, etc., in the order of migration during the chromatographic separation.

The first half of peak I showed a different type of spectrum from the latter half of the peak. The former substance was attributed to cytidylic acid. However, its identity was not definitely established, because it could not be eluted from the charcoal (Nuchar) used to adsorb the compound.

Prior to emergence of the nucleotides from the column, a compound with a sharp absorption maximum at 335 mμ was eluted with dilute acid (0.01 N HCl). The identity of this compound has not been established.

The nucleotides which were further characterized by the specific tests are described below.

Diphosphopyridine Nucleotide (DPN).—A typical absorption peak at 340 mμ appeared when ethanol and yeast alcohol dehydrogenase were added (Ciotti and Kaplan, 1957). When the nucleotide was reduced with excess substrate and enzyme, the concentration of the nucleotide calculated from the peak at 340 mμ as DPNH was 85% of the value obtained from the optical density at 260 mμ.

Triphosphopyridine Nucleotide (TPN).—An absorption peak at 340 mμ was obtained when D-glucose 6-phosphate and D-glucose 6-phosphate dehydrogenase were added to the nucleotide (Horecker and Kornberg, 1957). The concentration of the reduced coenzyme calculated from the optical density at 260 mμ was equal to that at 340 mμ.

Adenosine 3′,5′-Pyrophosphate.—Fraction IV yielded a new nucleotide in this alga. As seen from Table I, Compound IV_B contained one mole of pentose and two moles of phosphate per mole of adenine, but none of the phosphate was acid-labile. The compound did not produce a reducing sugar after mild acid hydrolysis (0.01 N HCl, 10 minutes). In its chromatographic behavior, the compound resembled AMP rather than ADP. Its electrophoretic mobility at *p*H 3.8 and 5.7 was almost the same as that of ADP, while at *p*H 7.6 it behaved as AMP. Since the secondary *pKa* values of phosphate compounds lie between 6 and 7, this indicated that a secondary phosphate dissociation was not present in the compound. The compound was inert toward a powerful seminal phosphomonoesterase. However, when hydrolyzed with 1 N HCl for 10 minutes at 100° or treated with nucleotide pyrophosphatase in 0.05 M sodium acetate buffer at *p*H 5, compound IV_B was transformed, without any detectable intermediate formation, into another compound, *X*, which behaved as ADP chromatographically and electrophoretically at *p*H 3.8, whereas at *p*H 5.7 or 7.6 it migrated as ATP. Analysis of the derived compound *X* showed the same ratio of constituents (adenine-ribose-phosphate, 1:1.10:1.85) as compound IV_B (see Table I). On prolonged acid hydrolysis of compound IV_B, adenine was liberated without intermediate formation of AMP (1-butanol saturated with 10% urea was used as the chromatographic solvent to separate adenine, adenosine, and adenylic acid [Carter, 1950]). These results indicated that compound IV_B is a cyclic pyrophosphate and that compound *X* could be adenosine 2′,3′-, 2′,5′-, or 3′,5′-

TABLE I

CHEMICAL ANALYSIS OF NUCLEOTIDES ISOLATED FROM *P. perforata*

The samples were isolated by paper chromatography of the nucleotide mixtures contained in the peaks shown in Figure 1. The concentration of the various compounds was calculated from their extinction coefficients at 260 mμ.

Peak No.	Sample	μmoles	Spectral Type	Compound Identified	Composition (moles per mole base)			
					Total P	Labile P	Pentose[a]	Reducing Sugar After Hydrolysis
I	A	200	Adenosine	5′-AMP	0.98	0	0.98	
	B	20	Pyridine nucleotide	DPN	1.80			
II	A	70	Uridine	5′-UMP	0.95	0.02	0.92	
	B	10	Guanosine	5′-GMP	0.95	0.10	0.98	
	C	<1	Pyridine nucleotide	TPN				
III	A	30	Inosine	5′-IMP	0.93	0.13	0.93	
	B	30	Adenosine	ADP	1.91	0.88	0.96	
IV	A	4	Uridine	(= V_A)				
	B	7	Adenosine	Adenosine 3′,5′-pyrophosphate	1.80	0.14	1.07	
	C	26	Guanosine	GDP-D-mannose–GDP-L-galactose, 20:1	1.91	0.82	1.12	0.98
V	A	32	Uridine	UDP-D-glucose–UDP-L-galactose, 5.1:1	1.88	0.81	1.26	0.93
	B	7	Uridine	5′-UMP	1.23	0.19	1.18	
	C	4	Guanosine	(= IV_C)				
	D	2	Uridine	UDP	1.82	0.92	0.98	
VI	A	2	Uridine	UDP-glucuronic acid				
	B	2	Inosine	IDP				

[a] Adenylic and uridylic acids served as the standards for the determinations of the pentose in the purine and pyrimidine nucleotides, respectively.

diphosphate. The potato nucleotide pyrophosphatase used also contained a nonspecific phosphatase, which on prolonged treatment of compound IV_B with the enzyme produced adenosine as the sole UV-absorbing product. However, by choice of appropriate reaction conditions, *e.g.* 0.05 μmole of IV_B and 5 units of enzyme (Kornberg and Pricer, 1950) in 50 μl of 0.05 M acetate buffer, *p*H 5, incubated at 37° for 1 hour, a mixture of two types of adenylic acid was obtained from the enzyme digest. These compounds were first isolated as a single spot from the enzyme digest by paper chromatography (ethanol–ammonium acetate, *p*H 7.5). They were then proved to be adenosine 5′-phosphate (A-5′-P) and adenosine 3′-phosphate (A-3′-P) by electrophoresis in tetraborate and by paper chromatography (0.8 saturated ammonium sulfate containing 2% isopropanol) (Markham and Smith, 1951). The former technique distinguishes A-5′-P from the A-2′-P and A-3′-P isomers and the latter resolves the A-2′-P and A-3′-P isomers. This evidence indicates that compound IV_B is adenosine 3′,-5′-pyrophosphate (Fig. 2).

When molecular models of the three possible adenosine cyclic pyrophosphates were constructed with Courtauld atomic models, the 3′,5′-pyrophosphate ring was found to be the one with the least stress. Ring closure was impossible for the phosphates on 2′- and 5′-positions, and 2′,3′-pyrophosphate could be formed only with great stress.

That this adenosine cyclic pyrophosphate is not an artifact formed during the isolation of the nucleotides was shown by the fact that its precursor is not ATP or coenzyme A. When both nucleotides were heated in ethanol with an equivalent amount of $MgCl_2$ or with the seaweed material and subjected to paper electrophoresis and chromatographic separation, none of the spots corresponding to compound IV_B could be detected in

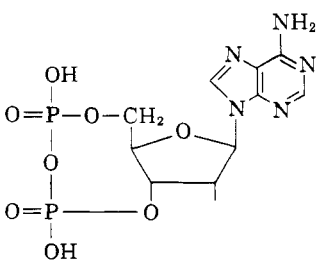


FIG. 2.—Adenosine 3′,5′-pyrophosphate (tentative formula).

any trace. Since the ion-exchange chromatographic technique used in the present study has been one of the standard techniques for the analysis of the above-mentioned two nucleotides, transformation of the nucleotides during this procedure would be improbable. From these considerations, it was concluded that adenosine 3′,5′-pyrophosphate probably occurs as such in the natural state.

GDP-D-Mannose and GDP-L-Galactose.—The analytical data presented in Table I indicated that fraction IV_C could be a GDP-sugar. Both paper chromatography and paper electrophoresis at different *p*H values failed to resolve this fraction into subfractions. The electrophoretic mobility of IV_C was slightly greater than that of ADP but less than that of GDP at either *p*H 3.6 or 5.7. On mild acid hydrolysis, however, IV_C gave two monosaccharides which were identified as D-mannose and L-galactose, shown below. Usually, before analysis, the acid hydrolysate was deionized by the ion-exchange resins Dowex 50 (H^+) and Duolite A-3 (OH^-).

(1) Two-dimensional co-chromatography of the hydrolysate with authentic C^{14}-D-galactose and C^{14}-D-mannose resulted in spots located in corresponding positions.

(2) About 10 μg each of the chromatographically separated mannose and galactose (1-butanol–acetic acid–water, 4:1:1, descending, 2 days' run) were reduced in aqueous medium by sodium borohydride and the products purified according to the procedure of Kessler *et al.* (1961). The hexitols were identified as mannitol and galactitol by electrophoresis in tetraborate, *p*H 9.2.

(3) About 0.2 mg of chromatographically separated mannose was accumulated and its specific phenylhydrazone was prepared (m.p. 190–191°). It did not give a melting point depression when mixed with authentic D-mannose phenylhydrazone. Examination of the optical rotation of a dilute solution of this sugar showed a positive rotation.

(4) The L-configuration of the chromatographically separated galactose was deduced from the fact that it could not be oxidized by the specific D-galactose dehydrogenase from *Pseudomonas saccharophila* (Su and Hassid, 1962; Doudoroff *et al.*, 1958). The total acid hydrolysate of IV_C tested with the enzyme also gave the same negative result. In order to test whether the sugar sample contained enyzme inhibitors that could lead to a negative result, several μg of D-galactose was added to a similar assay mixture. Immediate oxidation of the added sugar by DPN was observed in every case.

Since GDP-D-mannose and GDP-L-galactose could not be separated from each other, their occurrence was further substantiated as follows:

(*a*) Hydrolysis of the sugar nucleotide mixture with nucleotide pyrophosphatase or with 0.1 N acid for 10 minutes at 100° produced only GMP and/or GDP, as revealed by chromatography and electrophoresis.

(*b*) Hydrolysis catalyzed by nucleotide pyrophosphatase yielded two sugar phosphate spots which migrated on paper as α-D-mannose 1-phosphate and α-D-galactose 1-phosphate (ethanol–1 M ammonium acetate, *p*H 5.1, 85:15). In the solvent system used, the traveling distances for α-D-mannose 1-phosphate and α-D-galactose 1-phosphate were 14.5 and 12.6 cm, respectively, when the chromatogram was developed by descending run for 70 hours.

The approximate ratio of GDP-L-galactose to GDP-D-mannose in the mixture was estimated from the corresponding hexoses recovered from the hydrolysate. The acid hydrolysate was deionized by passing through columns of Dowex 50 (H^+) and Duolite A-3 (OH^-), and chromatographed (1-butanol–acetic acid–water, 4:1:1); the sugars were then eluted from the chromatogram, filtered through sintered glass to avoid any possible contamination with cellulose from the filter paper, and estimated by the anthrone method (Koehler, 1952). Assuming that the recovery for both sugars was identical, the ratio of L-galactose to D-mannose was estimated as 1:20.

UDP-D-Glucose and UDP-D-Galactose.—The analytical data for fraction V_A indicated that it was a UDP-sugar. This fraction also could not be resolved into subfractions by either electrophoresis or chromatography. The sugar components obtained by mild acid hydrolysis (0.01 N HCl, 100°, 10 minutes) were identified as D-glucose and D-galactose essentially in the same manner as described for the identification of D-mannose and L-galactose. For separation of the two sugars, the deionized hydrolysate of the nucleotides was chromatographed in phenol-water by descending chromatography for 2 days. The glucose obtained was further characterized by the dextro-rotation of its dilute solution and the melting point (212–213°) of its phenylosazone.

The D-configuration of galactose obtained from UDP-galactose was deduced from the enzymatic and chemical assays of the chromatographically pure galactose eluate. The same aliquot of the galactose solution gave 13.7 μg by the anthrone method (Koehler, 1952) and 14.1 μg by the enzymatic technique using the specific D-galactose dehydrogenase. The agreement of the results by these two methods indicates that the galactosyl residue of the sugar nucleotide is entirely of the D-configuration.

The ratio of UDP-D-glucose and UDP-D-galactose was also determined enzymatically. An aliquot of the nucleotide solution was hydrolyzed with 0.025 N HCl for 10 minutes at 100°. After cooling, an equivalent amount of NaOH was added to neutralize the acid. To this, the D-galactose dehydrogenase assay components were added. The solution was made up to a definite volume and assayed for D-galactose. The amount of D-glucose was determined by subtracting the D-galactose from the total nucleotides before hy-

drolysis, determined by UV spectrophotometry. The ratio of UDP-D-galactose to UDP-D-glucose was found to be 1:5.1.

UDP-Glucuronic Acid.—Because of the small quantity of the compound isolated, analyses of phosphate and pentose were not carried out on this nucleotide. However, this compound was identified as UDP-glucuronic acid by the following criteria: The carbazole test of Dische (1950) gave 0.93 mole of uronic acid per mole of uridine. The absorption spectrum in the visual region of the color produced by the compound was identical with that of the authentic UDP-D-glucuronic acid specimen. It could not be separated from C^{14}-labeled UDP-glucuronic acid by electrophoresis at *p*H 3.6 or 5.7, or by chromatography with ethanol–1 M ammonium acetate of various *p*H levels and various proportions.

Discussion

The alga *Porphyra perforata* has been found to contain complex saccharides consisting of D- and L-galactose units together with sugar nucleotides containing galactose moieties of both the D- and L-configuration. Since in a number of cases sugar nucleotides proved to be precursors of complex saccharides (Hassid *et al.*, 1959), a discussion pertaining to the mechanism of the formation of the latter is in order.

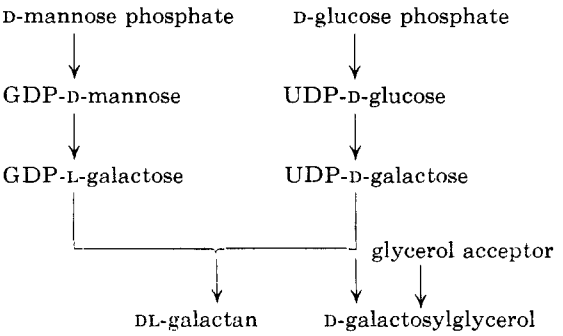
It was assumed for a long time that the biosynthesis of L-galactose is achieved through an enzymatic reduction of D-galactose at C-1 to $-CH_2OH$ with simultaneous oxidation at C-6 to $-CHO$. Inversion of the carbon skeleton of the original D-galactose would then result in the L-enantiomorph.

This suggestion was rendered unlikely by the demonstration that certain bacteria were able to convert specifically labeled D-glucose-C^{14} to L-fucose-C^{14} without inversion or cleavage of the carbon chain (Wilkinson, 1957; Segal and Topper, 1957; Heath and Roseman, 1958). Such a hypothesis is also not in accord with Ginsburg's (1958, 1960) finding that GDP-D-mannose is transformed to GDP-L-fucose by catalytic action of the cell-free extract of *Aerobacter aerogenes* in the presence of TPNH. By analogy, the transformation of D-mannose to L-galactose may occur by a similar mechanism in living systems.

Another GDP-sugar nucleotide, the terminal sugar residue of which is L-colitose (3,6-dideoxy-L-galactose) and has a close relationship to L-galactose, has been isolated by Heath (1960) from *E. coli*.

The discovery of GDP-L-galactose, which is found together with GDP-D-mannose in *P. perforata*, in the present study, strengthens the hypothesis that formation of L-galactose also takes place in the manner in which other sugar transformations occur. The distribution of D- and L-galactose in the different types of nucleotides, *i.e.*, UDP-D-galactose and GDP-L-galactose, and in a galactan containing D- and L-galactose, suggests that GDP-L-galactose participates in the transglycosylation reaction for the formation of L-galactosyl residues in the polymer.

From the above considerations, and on the basis of the view presented by Bean and Hassid (1955) concerning galactoside synthesis in *Iridophycus flaccidum*, a probable pathway of the synthesis of galactosylglycerols and the galactan in *P. perforata* could be depicted (Scheme I).



Scheme I

The DL-galactan present in *P. perforata* can be considered as consisting of modifications of D- and L-galactose isomers. Methylation takes place solely on the hydroxyl group on C-6 of D-galactose, while L-galactose undergoes dehydration to form a 3,6 intramolecular ether linkage. The point of attachment of the ester sulfate group is not certain. However, from the ease with which hexose 6-sulfate undergoes desulfation to form 3,6-anhydrohexose in alkaline solution, provided that C-3 hydroxyl is free (Duff and Percival, 1941), and from the well-known fact that alkyl hydrogen sulfate is the intermediate in the synthesis of an ether from alcohol when sulfuric acid is used as a catalyst, it is suggested that the intact D- and L-galactose residues in the polysaccharide are probably esterified with sulfuric acid at C-6. From these considerations, a pathway for the biosynthesis of *Porphyra* galactan is suggested as follows:

After the galactan backbone is synthesized from the activated D- and L-galactose (UDP-D-galactose and GDP-L-galactose), their C-6 hydroxyl groups are esterified with sulfate, and then part of the D-isomer undergoes etherification to give 6-*O*-methyl-D-galactose while part of the L-galactose undergoes dehydration to form a 3,6 inner molecular ether linkage. These reactions may result in the formation of the complex galactan found in *Porphyra*.

Regarding the origin of the sulfuric acid group in the galactan, it is of interest to compare the structure of the so-called "active sulfate" (adenosine 3′-phosphate-5′-phosphosulfate) with that of adenosine 3′,5′-pyrophosphate discovered in *P. perforata*. It is conceivable that, in *Porphyra*, adenosine 3′,5′-pyrophosphate is the compound that activates inorganic sulfate to form the "active sulfate," which engages in the enzymatic

sulfation of the alga polysaccharide. Along the same line of speculation, this cyclic adenosine pyrophosphate might be considered as a phosphagen, or a precursor of coenzyme A. However, assignment of a definite function to this novel nucleotide must await further investigation.

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