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THE SYNTHESIS OF 7-(ADENOSINE-5'-PYROPHOSPHORYL)-D-SEDOHEPTULOSE BY AN ENZYME SYSTEM FROM THE GREEN ALGA *CHLORELLA PYRENOIDOSA*

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SUMMARY

A transketolase-like enzyme that catalyzes the formation of a compound characterized as 7-(adenosine-5'-pyrophosphoryl)-D-sedoheptulose was extracted from autotrophically cultured cells of the green alga *Chlorella pyrenoidosa*. The system required the substrates 5-(adenosine 5'-pyrophosphoryl)-D-ribose and D-fructose 6-phosphate and was stimulated by thiamine pyrophosphate and $MgCl_2$. Apparent K_m and V values were determined for 5-(adenosine 5'-pyrophosphoryl)-D-ribose and D-ribose 5-phosphate as ketol acceptors. The algal enzyme exhibits a greater V with the nucleotide acceptor than with D-ribose 5-phosphate.

INTRODUCTION

We have been attempting recently to study the synthesis of cell wall polysaccharides and their precursors by the unicellular green alga *Chlorella pyrenoidosa*. In the course of our experiments, a cell-free enzyme preparation from that organism was incubated with α-D-[^{14}C]glucose 1-phosphate and NAD; we were surprised to discover that a radioactive compound with the properties of an adenosyl nucleotide could then be isolated from the reaction mixture. Since the formation of that compound was quite unanticipated, it seemed worthwhile to try to characterize it further. The results of the experiments reported here indicate that the compound is 7-(adenosine 5'-pyrophosphoryl)-D-sedoheptulose (Fig. 1) and that it is formed in a transketolase-like reaction, presumably by the transfer of a C_2-unit from D-fructose 6-phosphate to the reducing carbon of D-ribose in 5-(adenosine 5'-pyrophosphoryl)-D-ribose (Fig. 1). The latter compound arises by the enzyme-catalyzed cleavage of NAD.

MATERIALS AND METHODS

Chromatography and electrophoresis

Paper chromatography, unless otherwise indicated, was carried out on Schleicher and Schuell No. 589 Blue Ribbon paper in the following solvents: Solvent I, *n*-

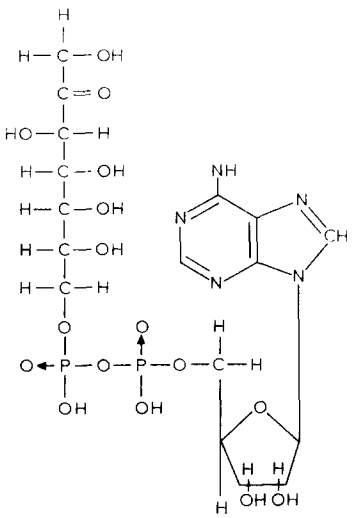

Fig. 1. 7-(Adenosine 5′-pyrophosphoryl)-D-sedoheptulose.

propanol–ethyl acetate–water (7:1:2, by vol.); Solvent II, 95% ethanol–1 M ammonium acetate (7:3, v/v); Solvent III, isobutyric acid–1 M NH_4OH (10:6, v/v); Solvent IV, phenol–water (80:20, v/v).

Paper electrophoresis was performed on Schleicher and Schuell No. 589 Orange Ribbon paper at about 25 V/cm with an apparatus like that described by CRESTFIELD AND ALLEN[1]. The following buffer systems were used: 0.1 M ammonium formate, pH 3.7; 0.05 M sodium tetraborate, pH 9.0; 0.15 M sodium arsenite, pH 9.6.

Chemicals

The principal radioactive substrate, D-[U-^{14}C]fructose 6-phosphate, was prepared from uniformly ^{14}C-labeled D-fructose (100 μC/μmole) by treatment with hexokinase and ATP[2]. It was purified by electrophoresis at pH 3.7.

D-[1-^{14}C]Glucose 6-phosphate (6.2 μC/μmole) and D-[6-^{14}C]glucose 6-phosphate (7.0 μC/μmole) were prepared by the enzyme-catalyzed phosphorylation of the respectively labeled sugars[2]. The radioactive compounds were purified by paper electrophoresis at pH 3.7 followed by chromatography in Solvent II.

a-D-[U-^{14}C]Glucose 1-phosphate (62 μC/μmole) was prepared by the action of sucrose phosphorylase on [U-^{14}C]sucrose[3].

A mixture of sedoheptulosan and D-sedoheptulose was produced by treatment of sedoheptulosan (Sigma) with 0.5 M HCl for 30 min at 100° (ref. 4).

5-(Adenosine 5′-pyrophosphoryl-)D-ribose was cleaved from NAD by the action of NAD nucleosidase[5] (Worthington). The reaction mixture contained 2.5 μmoles NAD, 15 units NAD nucleosidase, 0.75 μmole sodium–potassium phosphate buffer, pH 7.0. It was incubated for 45 min at 37°, and 5-(adenosine 5′-pyrophosphoryl)-D-ribose was isolated by electrophoresis at pH 3.7.

All other reagents were obtained from commercial sources.

Analytical methods

Radioactive compounds on paper were located by exposure to X-ray film (Kodak, no-screen). Quantitative estimations of ^{14}C were made with a Packard Liquid Scintillation Spectrometer, Model 3320.

Non-radioactive sugars were located on paper with the $AgNO_3$ reagent, *p*-anisidine phosphate, or benzidene–periodate[6].

D-Sedoheptulose concentration was estimated by the method of DISCHE[7] and total phosphate by the method of AMES AND DUBIN[8].

Growth of algae

C. pyrenoidosa Chick strain No. 395 was purchased from the Culture Collection of Algae, Department of Botany, University of Indiana, Bloomington. The cells were first cultured aseptically at 25° on 1.5 cm × 12 cm slants of a salts medium[9] containing 1.5% agar 6 inches from the light of two 24-W "Grolux" bulbs (General Electric Co.). The cells from one such slant incubated for 14 days were aseptically introduced into 2 l of the sterile salts medium in a "Microferm" fermentor (New Brunswick Scientific Co.). Illumination was provided by a submerged, water-jacketed quartz lamp (General Electric T-3, λ_{max} 1200 nm) operated at 1000 W. The medium was agitated by an impellor at 180 rev./min and was gassed through a sparger with 1.5% CO_2 in air at about 3 l/min. The fermentor vessel and salts medium were sterilized by autoclaving for 3 h at 120° while the lamp assembly was soaked overnight in a solution of 800 ppm benzalkonium chloride ("Roccal", Winthrop Laboratories). It was washed with sterile water before submersion in the fermentor.

After inoculation, the culture was incubated at 28° with constant illumination for 96 h. 10 l of fresh medium was added to the fermentor, and it was subjected to one cycle of 18 h light and 12 h dark. (Complete darkness was obtained by enclosing the fermentor vessel in a bag made of black felt enclosed in black cotton cloth.) 10 l of the culture were then removed and replaced with fresh medium, and the light–dark cycle was repeated. The dilution and light–dark cycles were repeated a total of three times after which the cells appeared by microscopic examination to be nearly synchronized[9]. After the last dilution, the culture was incubated through 3 more complete light–dark cycles (90 h) without dilution. The cell density had then reached about $3 \cdot 10^7$ cells per ml. Cells were harvested by centrifugation in a Sharples centrifuge and washed two times by suspension in cold water and centrifugation at 7000 × *g* in a Sorvall refrigerated centrifuge. The yield of cells was about 0.6 g wet wt. per l of culture. The centrifugal pellet was frozen and stored at −60°.

Preparation of algal enzyme

In a typical preparation, 8.0 g of the thawed cells were suspended in 30 ml of a buffer consisting of 0.1 M sodium and potassium phosphate, pH 7.0, 0.05 M β-mercaptoethanol, 1 mM EDTA and 5% soluble polyvinylpyrrolidone. Large aggregates of cells were disrupted by briefly homogenizing the chilled suspension in a glass tube with a Teflon pestle. The cells were broken by two passages through a cold French pressure cell (Aminco) at about 15 000 lb/inch^2. All subsequent operations were conducted in the cold. The homogenate was centrifuged at 10 000 × *g* for 10 min, and the residue was discarded. Proteins were precipitated from the supernatant solution with a saturated neutral solution of $(NH_4)_2SO_4$ (Mann, specially purified).

The fraction obtained between 45 and 60% saturation was found to contain the enzyme(s) of interest. That precipitate was dissolved in 0.5 ml of 0.025 M sodium–potassium phosphate buffer, pH 7.0, and desalted on a 1.5 cm × 20 cm column of Sephadex G-25 (fine). The effluent protein solution (3 ml) was frozen and lyophilized immediately. The yield was 34 mg of powder of which 18 mg was protein (estimated by the Biuret reaction[10]). The material was stored at −60°.

Determination of enzyme activity

Reactions were generally carried out in thin-walled glass capillaries. A typical reaction mixture contained: 0.1 μmole 5-(adenosine 5′-pyrophosphoryl)-D-ribose, 0.05 μmole (0.04 μC) D-[U-^{14}C]fructose 6-phosphate, 0.4 mg lyophilized Chlorella 45–60% $(NH_4)_2SO_4$ fraction, 0.02 μmole thiamine pyrophosphate, 0.1 μmole $MgCl_2$ and 2 μmoles Tris–HCl buffer, pH 8.2, in a total volume of 30 μl. The mixture was incubated as described in each experiment, then applied directly to a sheet of paper moistened with ammonium formate buffer, pH 3.7, and electrophoresed. In about 90 min at 25 V/cm 7-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose was separated by about 2 cm from the other principal radioactive component of the mixture, D-[^{14}C]-fructose 6-phosphate. The labeled compounds were located by exposure of the paper to X-ray film. Those areas of the paper were cut out, immersed in 0.01% dimethyl-POPOP, 0.4% PPO in toluene, and the papers were counted in the liquid scintillation spectrometer.

RESULTS

Characterization of the product

A quantity of the compound sufficient for characterization was prepared by incubating the following mixture for 3 h at 37°: 0.5 μmole D-[^{14}C]fructose 6-phosphate, (0.1 μC), 0.6 μmole 5-(adenosine 5′-pyrophosphoryl)-D-ribose, 3 mg Chlorella lyophilized 45–60% $(NH_4)_2SO_4$ fraction, 16 μmoles Tris–HCl, pH 8.2, in a total volume of 0.2 ml. The mixture was applied to a sheet (18 cm × 60 cm) of Schleicher and Schuell No. 589 Orange Ribbon paper moistened with ammonium formate buffer, pH 3.7, and electrophoresed in that buffer. The ultraviolet-absorbing compound that migrated slightly less rapidly than ADP was eluted with water, and the eluate was evaporated to about 50 μl *in vacuo*. (Evaporation to dryness in the presence of residual ammonium formate caused breakdown of the compound.) The eluate was applied to a 9 cm × 45 cm sheet of Schleicher and Schuell Orange Ribbon paper and chromatographed with Solvent III for 50 h. In that time, the 7-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose was separated from 5-(adenosine 5′-pyrophosphoryl)-D-ribose by about 3 cm. The residuum of isobutyric acid from the solvent was removed from the paper by irrigating it in the chromatography tank with 80% ethanol for 8 h. The compound was located by its radioactivity and ultraviolet absorbance and eluted with water. The yield, estimated by ultraviolet absorption at 259 nm (extinction coefficient of ADP-D-glucose[11]) was 0.22 μmole. (In the subsequent microchemical analyses the eluate of a blank chromatogram identically treated was used to correct for the contribution of impurities from the paper and solvent systems.)

The structure of the compound was concluded to be 7-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose on the basis of the following evidence:

(1) Upon electrophoresis at pH 3.7 and chromatography in Solvent II, the ^{14}C-labeled, ultraviolet-absorbing compound migrated like ADP-D-glucose.

(2) The ultraviolet absorption spectrum of the compound was that of an adenine nucleotide (λ_{max} = 259 nm; absorbance ratios, 280 nm/260 nm = 0.21 and 250 nm/260 nm = 0.77 at pH 7 (ref. 11).

(3) The ratio adenosine/phosphate/sugar in the compound was determined to be 1:2:1 (Table I).

TABLE I

CHEMICAL ANALYSIS OF ENZYMICALLY FORMED 7-(ADENOSINE 5'-PYROPHOSPHORYL)-D-SEDOHEPTULOSE

7-(Adenosine 5'-pyrophosphoryl)-D-sedoheptulose was prepared and isolated as described in the text. The concentration of adenine was determined by its ultraviolet absorption at 259 nm[11], D-sedoheptulose by the cysteine–H_2SO_4 method[7], and total phosphate by the method of AMES AND DUBIN[8].

Analysis	*Total μmole*	*Quantity relative to adenine*
Adenine	0.22	1.0
Sedoheptulose	0.26	1.1
Total phosphate	0.48	2.1

(4) Mild acid hydrolysis (0.05 M trifluoracetic acid, 10 min at 100°) or treatment with a nucleotide pyrophosphatase (0.2 unit Sigma nucleotide pyrophosphatase, 0.7 μmole Tris–HCl, pH 7.5, and 1.5 μmoles $MgCl_2$ in a total volume of 30 μl, incubated 60 min at 37°) produced a ^{14}C-labeled compound that migrated upon electrophoresis at pH 3.7 like a sugar phosphate and an ultraviolet absorbing compound indistinguishable from AMP.

(5) Further acid hydrolysis of the sugar phosphate described above (1 M HCl, 15 min at 100°) was without effect, whereas a new radioactive compound which no longer moved upon electrophoresis at pH 3.7 resulted upon its treatment with alkaline phosphatase (0.3 unit of Sigma calf intestinal mucosa phosphatase, 2 μmoles Tris–HCl, pH 8.2, 1.5 μmoles $MgCl_2$, incubated 60 min at 37°). This suggested that the sugar was linked to a phosphate of the nucleotide at a position resistant to acid hydrolysis and hence not through a hemiacetal bond.

(6) The neutral radioactive compound was not oxidized by bromine (2% bromine in 0.1 M $BaCO_3$, pH 5.4, 60 min at room temperature), but it was apparently reduced by $NaBH_4$ (1 μmole $NaBH_4$, 3 μmoles Tris–HCl, pH 8.2, in a volume of 30 μl, 60 min at room temperature). The reduction was indicated by the change in its chromatographic mobility after treatment with $NaBH_4$. These results suggested a ketose rather than aldose structure in the sugar.

(7) Upon electrophoresis in sodium borate or sodium arsenite buffers, or upon chromatography in Solvent IV the neutral radioactive compound was indistinguishable from authentic D-sedoheptulose.

(8) When mixed with authentic sedoheptulosan and treated with 0.5 M HCl for 30 min at 100°, the neutral radioactive compound yielded two radioactive compounds which separated upon electrophoresis in sodium borate or sodium arsenite or upon chromatography in Solvent IV and which were indistinguishable from authentic

sedoheptulosan and D-sedoheptulose. They were present in the ratio 5.5:1 which is in close agreement with the ratio determined by LAFORGE AND HUDSON[12].

(9) When the intact ^{14}C-labeled nucleotide was reduced with $NaBH_4$ and subsequently hydrolyzed with nucleotide pyrophosphatase and alkaline phosphatase as described previously, the radioactive compound produced was indistinguishable by chromatography and electrophoresis from the compound synthesized by the $NaBH_4$ reduction of authentic D-sedoheptulose. This is considered additional evidence that D-sedoheptulose in the nucleotide has an uncombined reducing group.

Nature of the reaction system

The synthesis of the product was proportional to time and to enzyme concentration. Optimal activity occurred around pH 8.2, with 50% of that activity at pH 7 and 53% at pH 9.

The reaction was stimulated by thiamine pyrophosphate and by $MgCl_2$ with optimal concentrations of $3.3 \cdot 10^{-4}$ M and $3.3 \cdot 10^{-2}$ M, respectively (Table II).

TABLE II

EFFECT OF Mg^{2+} AND THIAMINE PYROPHOSPHATE CONCENTRATIONS ON THE ENZYMATIC PRODUCTION OF 7-(ADENOSINE 5'-PYROPHOSPHORYL)-D-SEDOHEPTULOSE

The reaction mixture contained (in addition to those components listed above); 0.1 μmole 5-(adenosine 5'-pyrophosphoryl)-D-ribose, 0.05 μmole (0.04 μC) D-[U-^{14}C]fructose 6-phosphate, 0.4 mg lyophilized Chlorella 45–60% $(NH_4)_2SO_4$ fraction, and 2 μmoles Tris–HCl buffer, pH 8.2, in a total of 30 μl. The mixture was incubated 15 min at 37° and assayed as described in the text.

Addition	*Concentration (M)*	*%^{14}C incorporated into product*
None	—	12.1
$MgCl_2$	$3.3 \cdot 10^{-2}$	13.4
	$3.3 \cdot 10^{-3}$	11.2
	$3.3 \cdot 10^{-4}$	11.5
Thiamine pyrophosphate	$3.3 \cdot 10^{-3}$	13.0
	$3.3 \cdot 10^{-4}$	13.2
	$3.3 \cdot 10^{-5}$	12.9
$MgCl_2$ and thiamine pyrophosphate	$3.3 \cdot 10^{-2}$ and $3.3 \cdot 10^{-3}$	19.7
	$3.3 \cdot 10^{-3}$ and $3.3 \cdot 10^{-4}$	18.3
	$3.3 \cdot 10^{-4}$ and $3.3 \cdot 10^{-5}$	17.0

The nature of the substrates, products, and cofactors suggested that this is a transketolase-like reaction[13], hence several experiments were performed to test that hypothesis.

It was first demonstrated that the Chlorella enzyme preparation appears to contain an ordinary transketolase. A mixture containing the following materials was incubated for 60 min at 37°; $6 \cdot 10^{-4}$ μmole (0.06 μC) D-[^{14}C]fructose 6-phosphate, 0.2 μmole D-ribose 5-phosphate, 0.6 mg lyophilized Chlorella 45–69% $(NH_4)_2SO_4$ precipitate, 2.5 μmoles Tris–HCl, pH 8.2, in a total volume of 30 μl. After incubation, alkaline phosphatase (0.3 unit) was added to the mixture which was incubated for an additional 45 min at 37°. Radioactive D-sedoheptulose was isolated from the mixture by electrophoresis in 0.1 M sodium arsenite, pH 9.6. About 20% of the total recovered radioactivity was in a compound indistinguishable from D-sedoheptulose.

In another experiment, yeast transketolase (Sigma) was tested for its ability to synthesize 7-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose. The reaction mixture contained $6 \cdot 10^{-4}$ μmole (0.06 μC) D-[^{14}C]fructose 6-phosphate, 0.06 μmole 5-(adenosine 5′-pyrophosphoryl)-D-ribose, 0.15 μmole $MgCl_2$, 0.03 μmole thiamine pyrophosphate, 1.5 μmole Tris–HCl, pH 7.6, and 3 μl (0.02 unit) Sigma yeast transketolase. The mixture was incubated at 25° for 60 min, then inactivated in a 100° bath for 2 min. A similar reaction mixture was incubated with 0.06 μmole of D-ribose 5-phosphate in place of 5-(adenosine 5′-pyrophosphoryl)-D-ribose to measure the normal activity of the enzyme. The mixtures were treated with nucleotide pyrophosphatase and alkaline phosphatase (0.2 and 0.3 unit of each for 45 min at 37°), 0.2 μmole authentic D-sedoheptulose was added to each, and they were electrophoresed on paper in 0.15 M sodium arsenite buffer, pH 9.6. The papers, after drying, were left at room temperature overnight, after which authentic D-sedoheptulose appeared as a brown spot. This area of the paper was cut out and its radioactivity determined in the liquid scintillation counter. When 5-(adenosine pyrophosphoryl)-D-ribose served as ketol acceptor, the D-sedoheptulose area contained only 3.9% of the total radioactivity on the paper. In the control with D-ribose 5-phosphate, 35% of the total radioactivity was found in D-sedoheptulose. In similar experiments, Sigma yeast transaldolase was found unable to catalyze the transfer of carbons to 5-(adenosine 5′-pyrophosphoryl)-D-ribose from D-fructose 6-phosphate.

Evidence was obtained by the use of differentially labeled substrates that the carbons transferred to 5-(adenosine 5′-pyrophosphoryl)-D-ribose from D-fructose 6-phosphate arise from the carbonyl end of the molecule. That would be the mechanism predicted for a transketolase-like reaction. Differentially labeled D-fructose 6-phosphate was not available to us, so D-[1-^{14}C]glucose 6-phosphate and D-[6-^{14}C]glucose 6-phosphate were used instead. (Previous observations had indicated that radioactive D-glucose 6-phosphate rapidly gave rise to radioactive D-fructose 6-phosphate when incubated with the Chlorella enzyme fraction.) Details of the experiment and quantitative results are shown in Table III. The small incorporation of ^{14}C from D-[6-^{14}C]-glucose 6-phosphate is presumably due to the activities of various glycolytic enzymes in the preparation.

TABLE III

FORMATION OF 7-(ADENOSINE-5′-PYROPHOSPHORYL)-D-SEDOHEPTULOSE FROM SPECIFICALLY ^{14}C-LABELED SUBSTRATES BY CHLORELLA ENZYMES

The reaction mixture contained (including the above reactants): 0.1 μmole 5-(adenosine 5′-pyrophosphoryl)-D-ribose, 0.2 mg lyophilized Chlorella 45–60% $(NH_4)_2SO_4$ fraction, 2 μmoles Tris–HCl, 0.1 μmole $MgCl_2$, and 0.02 μmole thiamine pyrophosphate, in a total of 30 μl. The reaction was incubated 1 h at 37° and assayed as described in the text.

Substrate	*Total radioactivity (counts/min)*	*Radioactivity recovered in 7-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose (counts/min)*	*% Radioactivity incorporated*
α-D-[1-^{14}C]Glucose 6-phosphate	89 364	41 107	46
α-D-[6-^{14}C]Glucose 6-phosphate	125 000	7 500	6

TABLE IV

DATA FROM LINEWEAVER–BURK PLOTS

Components of the reaction mixtures, estimation of product formation and methods used in determining those constants are given in the text.

Substrate	*App. K_M (M)*	*(Counts/min incorporated into sedoheptulose 7-phosphate per min)*
Ribose 5-phosphate	$1.1\ (\pm 0.4)\cdot 10^{-4}$	900 ±60
5-(Adenosine 5′-pyrophosphoryl)-D-ribose	$4.8\ (\pm 1.1)\cdot 10^{-4}$	3333 ±300

We were unable to demonstrate unequivocally that the reaction catalyzed by the Chlorella enzyme is reversible because of the presence of other enzymes in the preparation. Thus, when enzymically synthesized 7-(adenosine 5′-pyrophosphoryl)-D-[^{14}C]sedoheptulose and the ketol acceptor, D-ribose 5-phosphate, were incubated with the $(NH_4)_2SO_4$ fraction and the appropriate cofactors, radioactivity was found not only in D-fructose 6-phosphate but in a number of other unidentified compounds.

The two compounds, D-ribose 5-phosphate and 5-(adenosine 5′-pyrophosphoryl)-D-ribose, were compared as substrates in the Chlorella enzyme system by obtaining the apparent K_m and V values for each. Conditions were chosen such that each reaction was in its linear stage when the formation of product was measured. The mixtures contained 0.1 μmole D-[^{14}C]fructose 6-phosphate (0.1 μC), 0.1 μmole $MgCl_2$, 0.02 μmole thiamine pyrophosphate, 0.07 mg lyophilized Chlorella 45–60% $(NH_4)_2SO_4$ precipitate and 3 μmoles Tris–HCl, pH 8.2, in a volume of 30 μl. Three of the mixtures contained 0.003, 0.005 and 0.01 μmole, respectively of 5-(adenosine 5′-pyrophosphoryl)-D-ribose and three other mixtures contained 0.003, 0.005 and 0.01 μmole, respectively, of D-ribose 5-phosphate. The mixtures were incubated 5 min at 25° and inactivated by heating in a 100° bath for 2 min. 7-(Adenosine 5′-pyrophosphoryl)-D-[^{14}C]sedoheptulose was isolated by electrophoresis on paper at pH 3.7. It was located by the ultraviolet absorption of standard 5(adenosine 5′-pyrophosphoryl)-D-ribose which migrated at essentially the same rate as the ^{14}C-labeled D-sedoheptulose nucleotide. The ultraviolet-absorbing area of the paper was cut out and its radioactivity determined in the liquid scintillation counter. D-[^{14}C]Sedoheptulose 7-phosphate, formed when D-ribose 5-phosphate, served as substrate, was isolated as the free sugar following the procedure described above in the assay of Sigma transketolase.

Measurements of enzyme activity were made in triplicate for each concentration of the two substrates. Apparent K_m and V values (Table III) were obtained from the averages of those data by the method of LINEWEAVER AND BURK[14]. They indicate that the system with D-ribose 5-phosphate as substrate has a slightly lower apparent K_m but a lower V than the system with 5-(adenosine 5′-pyrophosphoryl)-D-sedoheptulose as substrate.

DISCUSSION

C. pyrenoidosa, unlike most other unicellular organisms, does not reproduce by binary fission[9]. Each spherical cell increases in volume as its grows, exhibiting no other gross morphological change. Then, over a period of a few hours, the cell forms

within itself a number of new cells known as "autospores", the wall of the mother cell ruptures, and the newly formed cells are released into the medium to begin the cycle again. The number of autospores produced is characteristic of the cell variety and of the growth conditions; there may be as few as 2 and as many as 32.

KANAZAWA *et al.*[15] have reported changes in the relative proportions of several metabolites during the growth cycle of Chlorella. These changes presumably reflect regulation of the activities of various enzymes. We have noticed enhanced rates of synthesis of certain sugar nucleotides by enzymes extracted from synchronous Chlorella cells harvested at the stage immediately preceding autospore formation (R. C. White, unpublished data). However, the enzymes involved in the synthesis of 7-(adenosine 5'-pyrophosphoryl)-D-sedoheptulose seem to be equally active at all stages of the life cycle. This perhaps indicates that this transketolase-like enzyme is involved in some general metabolic process which goes on at a more or less uniform rate throughout the life of the cell.

Interpretation of the K_m and V values calculated for the Chlorella system is difficult since they were obtained with a crude enzyme extract. For whatever reason, though, in the presence of D-fructose 6-phosphate, 5-(adenosine 5'-pyrophosphoryl)-D-ribose is converted to the D-sedoheptulose derivative more rapidly than D-ribose 5-phosphate is converted to D-sedoheptulose 7-phosphate. This is in contrast to the substrate specificity of yeast transketolase which much more rapidly catalyzes the conversion of D-ribose 5-phosphate to D-sedoheptulose 7-phosphate. Thus if the reaction in Chlorella is brought about by a transketolase, the characteristics of that enzyme differ considerably from those of the yeast enzyme. Whether there is another more conventional transketolase in that alga cannot be determined without considerably more purification of the enzymes involved. It is of interest that VILLAFRANCA AND AXELROD[16] recently purified a transketolase from spinach leaves that uses a variety of ketol donors and acceptors including a number of non-phosphorylated sugars.

We are unable to suggest any role for 7-(adenosine 5'-pyrophosphoryl)-D-sedoheptulose in the metabolism of Chlorella. Indeed it may be simply an artifact of enzyme action *in vitro*. However, it is indisputable that *C. pyrenoidosa* possesses the enzymes and substrates required to synthesize that compound, and, if it does not, the cell must in some way prevent the process from occurring.

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