BBA 65803

# ENZYMATIC PHOSPHORYLATION OF D-SEDOHEPTULOSE WITH ANIMAL LIVER

HAJIME IWAI, AKIO KIYOMOTO AND MASARU TAKESHITA
*Biological Research Laboratories, Tanabe Seiyaku Co. Ltd., Toda, Saitama (Japan)*

(Received May 21st, 1968)

## SUMMARY

The metabolism of D-sedoheptulose in animals has been assumed to be analogous to that of D-fructose. However, no experimental evidence has been reported.

(1) D-Sedoheptulose was found to be phosphorylated with a partially purified enzyme preparation of liver.

(2) The product of the enzyme reaction was isolated by ion-exchange chromatography, and conclusively identified to be D-sedoheptulose 1-phosphate by a reaction with phenylhydrazine, periodate oxidation and activity with aldolase.

(3) In contrast to the results of other investigators, no other structural requirement for substrate specificity than the common steric configuration in C-1 to C-4 was found.

(4) The enzyme activity was recognized in many species of animals.

(5) Thus, it was assumed that a fructokinase itself acted with D-sedoheptulose.

## INTRODUCTION

D-Sedoheptulose has been reported to lower blood ketone bodies similarly to glucose, while its metabolism as a free sugar has been assumed to be analogous to that of fructose[1]. Recently, it has been observed that the purified fructokinase of rat liver does not seem to act with D-sedoheptulose[2]. L-Galactoheptulose and D-xylulose show activity with the enzyme. These observations suggest the presence of a specific enzyme which phosphorylates D-sedoheptulose to yield D-sedoheptulose 7-phosphate or D-sedoheptulose 1-phosphate in animal liver.

In the present experiment, using a partially purified enzyme preparation, the presence of a kinase which yields D-sedoheptulose 1-phosphate from D-sedoheptulose and ATP was immediately demonstrated. The activity was detected in many species of animals.

## METHODS

### *Materials and determinations*

D-Sedoheptulose was prepared from sedoheptulose anhydride[3] which was

isolated from a culture medium of *Streptomyces naraensis*[4]. D-Xylurose[5] and D-ribulose[6] were prepared from D-xylose and D-ribose, respectively. D-Mannoheptulose was kindly supplied by Professor N. K. RICHTMYER. D-Sedoheptulose 7-phosphate was the gift of Professor B. L. HORECKER. The other materials were commercially purchased.

D-Sedoheptulose[7], D-fructose[8], the other reducing sugars[9], phosphoric acid[10] and protein[11] were chemically determined.

*Paper chromatography and paper electrophoresis*

The method of paper chromatography[12] was modified by using 1 M ammonium acetate (pH 3.8)–ethanol (3:7.5) as solvent for the descending method. The paper electrophoretic method[13] was modified by using a 9.2% *n*-butyric acid (v/v)–0.1% NaOH (w/v) mixture (final concentrations) as the solvent at 3000 V. Toyo filter paper No. 51 was used.

D-Sedoheptulose[14], D-fructose[15], reducing sugars[16] and phosphoric acid[17] were detected on the filter paper. Nucleotides were detected by ultraviolet absorption.

*Enzyme assay*

2 ml of enzyme reaction mixture containing 11.2 $\mu$moles of ATP, 16 $\mu$moles of $MgCl_2$, 50 $\mu$moles of NaF and enzyme in 0.04 M Tris–HCl (pH 7.2) were incubated at 37°. Disappearance of the substrate was measured after deproteinization by SOMOGYI's method[18] by which sugar phosphate is concomitantly removed.

## RESULTS

*Enzyme preparation*

ICR male mice of about 30 g body weight were decapitated, and the liver was quickly homogenized in 2 vol. of cold, 0.154 M KCl in a teflon homogenizer. The homogenate was incubated at 60° for 10 min, and centrifuged for 30 min at 12 000 × *g* in the cold. The supernatant was fractionated by ammonium sulfate precipitation. The fraction between 0.4 and 0.5 saturation was dissolved in a small volume of 0.1 M veronal (pH 7.6) and dialyzed against distilled water.

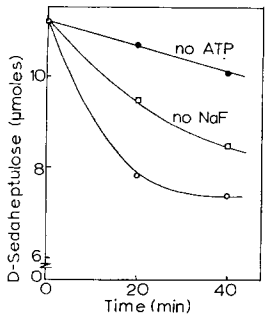
*Phosphorylation of D-sedoheptulose*

Disappearance of D-sedoheptulose, added as substrate in the enzyme reaction mixture, was not appreciable in the crude liver homogenate. It was remarkable, however, in the ammonium sulfate precipitate. The presence of ATP in the reaction mixture was essential, and the disappearance increased with NaF, as shown in Fig. 1.

After incubation, a substance containing sedoheptulose and phosphoric acid together with ADP, AMP and inorganic phosphate was detected by paper chromatography. Thus, D-sedoheptulose phosphate formation was considered to occur during the incubation.

*Isolation of the sedoheptulose phosphate*

The sedoheptulose phosphate formed in the reaction mixture was isolated by ion-exchange chromatography. 20 ml of the reaction mixture were placed on a Dowex-1 (formate) column, 2 cm × 25 cm, washed with water, and subjected to gradient



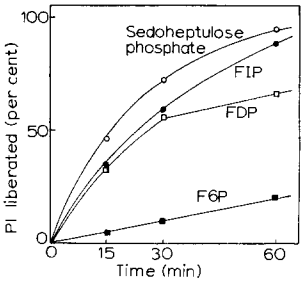




Fig. 1. Disappearance of D-sedoheptulose during incubation. The experimental method is described in *Enzyme assay*. Reaction mixtures were as follows: ○, sedoheptulose, ATP and NaF; ●, sedoheptulose and NaF; □, sedoheptulose and ATP.

Fig. 2. Rate of acid hydrolysis of sugar phosphates. 1 μmole of sugar phosphate per ml was boiled in 1 M HCl.

elution with 400 ml of water and 0.2 M formic acid containing 0.5 M sodium formate. The fractions which were positive for Dische's orcinol reaction were adjusted to pH 6.2 with satd. $Ba(OH)_2$ solution, and precipitated by adding 4 vol. of ethanol. The precipitate was collected by centrifugation, dissolved in a small volume of water, reprecipitated with ethanol, and dried *in vacuo*.

*Identification of the sedoheptulose phosphate*

The sodium salt was a single spot on both paper chromatogram and paper electrophoretogram. The movement more resembled that of fructose monophosphate than fructose diphosphate, and was slightly different from that of sedoheptulose 7-phosphate on the paper electrophoretogram. The lability of the phosphate bond resembles that of fructose 1-phosphate as shown in Fig. 2. The molar ratio of sedoheptulose to phosphoric acid was 1:1.

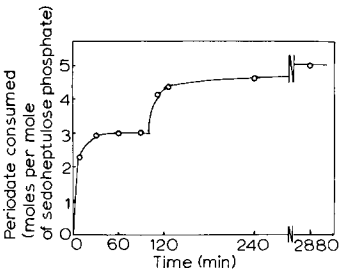
The site of the phosphate bond on C-1 of the sugar moiety was chemically determined by a reaction with phenylhydrazine and by periodate oxidation. The selective liberation of phosphoric acid on C-1 of fructose diphosphate with phenylhydrazine has been described[19].

TABLE I

LIBERATION OF INORGANIC PHOSPHATE BY PHENYLHYDRAZINOLYSIS

Sugar phosphate was boiled for 30 min in 0.75 ml of the reaction mixture (pH 5.0) containing 0.1 ml of 6% phenylhydrazine·HCl, 0.05 ml of satd. sodium acetate and 0.1 ml of satd. $NaHSO_3$.

| *Sample* | *Total phosphate (μmoles/ml)* | *Inorganic phosphate liberated (μmoles/ml)* |
|---|---|---|
| Sedoheptulose phosphate | 1.10 | 1.08 |
| Fructose 1-phosphate | 1.07 | 1.05 |
| Fractose 1,6-diphosphate | 2.68 | 1.33 |
| Fructose 6-phosphate | 1.05 | 0.08 |



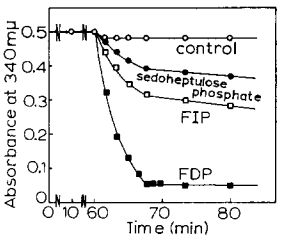




Fig. 3. Consumption of periodate. 8 $\mu$moles of the sedoheptulose phosphate and 0.06 mmole of $NaIO_4$ in 4 ml were kept at 0° in the dark. After 90 min incubation, the reaction mixture was kept at 37°.

Fig. 4. Degradation with aldolase. The mixture of 0.13 $\mu$mole of sugar phosphate, 20 $\mu$g of rabbit muscle aldolase (crystalline) and 0.15 $\mu$mole of NADH in 3 ml of 0.33 M Tris–HCl (pH 7.2) was incubated for 60 min at 37°. Then, 2 $\mu$g of $\alpha$-glycerophosphate dehydrogenase (crystalline) was added. Oxidation of NADH was followed by absorbance readings.

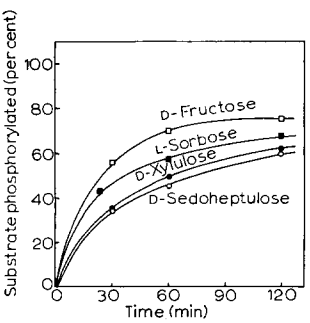
The procedure[20] was modified by use of satd. $NaHSO_3$ because satd. $Na_2SO_3$ was not applicable on fructose 1-phosphate. Almost 100% of the phosphoric acid was liberated from the sedoheptulose phosphate (Table I). Periodate oxidation was done according to literature[21]. Sedoheptulose 1-phosphate and sedoheptulose 7-phosphate theoretically consume 3 moles and 4 moles, respectively, of periodate per molecule. The sedoheptulose phosphate consumed 3 moles of periodate at 0° as shown in Fig. 3.

The activity with muscle aldolase was ascertained to be comparable to that with fructose monophosphate as shown in Fig. 4.

*Substrate specificity and optimum pH*

At a concentration of 5.6 mM, D-fructose, L-sorbose and D-xylulose also showed activity with the enzyme, as shown in Fig. 5. At the same concentration, no activity could be detected with the following sugars: D-mannoheptulose, D-ribulose, D-glucose, D-mannose, D-xylose, D-ribose, D-erythrose.

The optimum pH for the enzyme reaction with D-sedoheptulose was in the neutral range (Fig. 6).



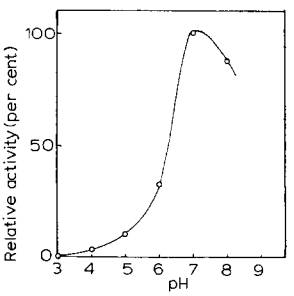




Fig. 5. Substrate specificity. The experimental method is described in *Enzyme assay*.

Fig. 6. Optimum pH of D-sedoheptulose phosphorylation.

TABLE II

D-SEDOHEPTULOSE PHOSPHORYLATING ACTIVITY OF VARIOUS MAMMALIAN LIVER ENZYMES
The experimental method is described in *Enzyme assay*.

| *Species of animal* | *Relative enzyme activity per mg protein* |
|---|---|
| Mouse | 100 |
| Hog | 99.0 |
| Ox | 79.0 |
| Horse | 63.2 |
| Rat | 58.5 |
| Dog | 50.5 |
| Rabbit | 26.0 |
| Guinea-pig | 12.1 |

*Distribution of enzyme activity in species of animal*

The enzyme activity was detected in many species (Table II). The relative activities in the table might not be exactly correct, because heating and ammonium sulfate precipitation were necessary to prepare the active enzyme preparations.

## DISCUSSION

The results of chemical identification, lability to acid hydrolysis, and activity with aldolase, conclusively indicated that the product of the enzyme reaction was D-sedoheptulose 1-phosphate. It was obscure whether the enzyme preparation contained more than the single kinase. Nothing was found, however, to contradict the assumption that a fructokinase itself acts with D-sedoheptulose. No structural requirement other than the common steric configuration in C-1 to C-4 was observed in the present experiment, though D-sedoheptulose anhydride was not active with the enzyme preparation.

The present data do not exclude the possibility of the presence of a kinase which yields D-sedoheptulose 7-phosphate in animal liver, since heating and ammonium sulfate precipitation might inactivate or impair its activity. In the case of D-xylulose, a specific kinase which yields D-xylulose 5-phosphate has been demonstrated[22].

## ACKNOWLEDGEMENT

The authors are very grateful to Dr. KYUJI ABE, Director of our laboratories, for his kind instruction.

## REFERENCES

1 E. SIMON, R. O. SCOW AND S. S. CHERNICK, *Am. J. Physiol.*, 201 (1961) 1073.
2 R. C. ADELMAN, F. J. BALLARD AND S. WEINHOUSE, *J. Biol. Chem.*, 242 (1967) 3360.
3 N. K. RICHTMYER, *Methods in Carbohydrate Chemistry*, Vol. 1, Academic Press, New York, 1962, p. 167.
4 T. OKUDA, M. TAKESHITA, H. NAKAMURA AND Y. ITO, *J. Biochem. Tokyo*, 54 (1963) 107.

5 O. TOUSTER, *Methods in Carbohydrate Chemistry*, Vol. 1, Academic Press, New York, 1962, p. 98.
6 C. GLATTHAAR AND T. REICHSTEIN, *Helv. Chim. Acta*, 18 (1935) 80.
7 Z. DISCHE, *J. Biol. Chem.*, 204 (1953) 983.
8 J. H. ROE, J. H. EPSTEIN AND N. P. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.
9 T. MOMOSE, Y. MUKAI AND S. KAWABE, *J. Med. Technol. (Japan)*, 5 (1961) 107.
10 R. J. L. ALLEN, *Biochem. J.*, 34 (1940) 858.
11 A. L. GORNALL, C. J. BARDAWILL AND M. M. DAVID, *J. Biol. Chem.*, 177 (1949) 751.
12 J. ESPADA, *J. Biol. Chem.*, 237 (1962) 3577.
13 H. E. WADE AND D. M. MORGAN, *Biochem. J.*, 60 (1955) 264.
14 L. HOUGH AND J. K. N. JONES, *Methods in Carbohydrate Chemistry*, Vol. 1, Academic Press, New York, 1962, p. 28.
15 A. BEVENUE AND K. T. WILLIAMS, *Arch. Biochem. Biophys.*, 34 (1951) 225.
16 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.
17 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
18 M. SOMOGYI, *J. Biol. Chem.*, 160 (1945) 69.
19 H. J. DEUTICKE AND S. HOLLMANN, *Z. Physiol. Chem.*, 258 (1939) 160.
20 C. E. CARDINI, A. C. PALADINI, R. CAPUTTO, L. F. LELOIR AND R. E. TRUCCO, *Arch. Biochem., Biophys.*, 22 (1949) 87.
21 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.*, 1957, p. 1216.
22 J. HICKMAN AND G. ASHWELL, *J. Am. Chem. Soc.*, 78 (1956) 6209.