We would like to express our sincere appreciation to Mr. F. WHITE, Miss J. COOKE, and Mr. H. TRITCH, for their expert assistance in much of this work.

ANDREW P. RYLE §
CHRISTIAN B. ANFINSEN

Laboratory of Cellular Physiology and Metabolism, National Heart Institute, Bethesda, Md. (U.S.A.)

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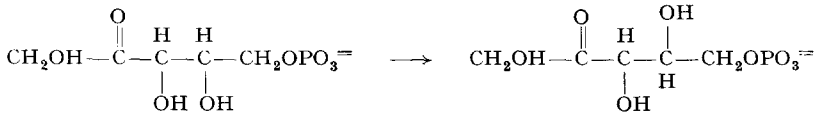
Received March 15th, 1957

§ Commonwealth Fund Fellow. Present address: Medical Research Council, National Institute for Medical Research, The Ridgeway, Mill Hill, London N.W. 7, England.

L-Ribulose-5-phosphate – D-xylulose-5-phosphate stereoisomerase and its role in L-arabinose fermentation*

L-Arabinose fermentation in *Lactobacillus pentosus*[1] and *Aerobacter aerogenes*[2] is initiated by the action of L-arabinose – L-ribulose isomerase. L-Ribulose is phosphorylated by ATP** in the presence of a kinase which has been purified from *A. aerogenes*[3]. L-Ru-5-P has been identified as the phosphorylation product[3]. L-Arabinose, or L-ribulose plus ATP are converted by crude extracts of *A. aerogenes*[2] and *Propionibacterium pentosaceum*[4] to arabinose, ribulose, xylulose, ribose, heptulose, fructose, and glucose phosphates. In addition, D-G-3-P is formed from L-ribulose plus ATP, but not from L-G-3-P[2]. Hence, it has been postulated[2,5] that L-arabinose is transformed into a D-pentose phosphate prior to metabolism via the transaldolase–transketolase and glycolytic pathways[6].

We now wish to report the finding of an enzyme which catalyzes the reaction:



L-Ru-5-P D-Xu-5-P (1)

This enzyme, termed L-Ru-5-P – D-Xu-5-P (C-4) stereoisomerase***, has been purified by treating extracts with protamine, followed by precipitation with ammonium sulfate and by calcium phosphate gel adsorption and elution. An inability to assay crude extracts has prevented an estimate of the purification achieved; however, the L-ribulokinase obtained in another ammonium sulfate fraction in the final step of the same procedure was purified 200-fold[3]. The stereoisomerase was free of phosphoketopentoepimerase (C-3 epimerase)[7,8], L-ribulose – L-arabinose isomerase, transketolase and D-G-3-P dehydrogenase, but contained L-ribulokinase.

* Supported in part by a Grant-in-Aid from the National Science Foundation and from the Rockefeller Foundation. The Authors gratefully acknowledge the generosity of Dr. B. L. HORECKER who made available unpublished manuscripts and experimental materials.

** Abbreviations: ATP = adenosine triphosphate; Ru-5-P = ribulose-5-phosphate; G-3-P = glyceraldehyde-3-phosphate; D-Xu-5-P = D-xylulose-5-phosphate; D-R-5-P = D-ribose-5-phosphate; DPN and DPN·H = oxidized and reduced diphosphopyridine nucleotide; C-3 epimerase = D-phosphoketopentoepimerase; C-4 stereoisomerase = L-ribulose-5-phosphate — D-xylulose-5-phosphate stereoisomerase; TCA = trichloroacetic acid.

*** Until suitable nomenclature can be established, stereoisomerase is provisionally employed to describe the ability to convert the substrate into its stereoisomer.

Stereoisomerase activity was measured as the rate of D-Xu-5-P formation in the following system:

$$\text{D-Xu-5-P} + \text{D-R-5-P} \xrightarrow{\text{Transketolase}^{9}} \text{D-Sedoheptulose-7-P} + \text{D-G-3-P} \quad (2)$$

$$\text{D-G-3-P} + \text{DPN} \xrightarrow[AsO_4^{\equiv}]{\text{D-G-3-P Dehydrogenase}^{10}} \text{D-3-Phosphoglycerate} + \text{DPN}\cdot\text{H} \quad (3)$$

As shown in Fig. 1, DPN reduction was completely dependent upon added L-Ru-5-P, D-R-5-P, and C-4 stereoisomerase. G-3-P dehydrogenase, and transketolase also were necessary. D-R-5-P did not reduce DPN (Fig. 1, "W/O L-Ru-5-P"). Since the transketolase preparation contained phosphoriboisomerase, the lack of DPN reduction indicates that C-3 epimerase was not present. Substituting a C-3 epimerase from *Lactobacillus pentosus*[8] for C-4 stereoisomerase caused DPN reduction from either L-Ru-5-P or D-R-5-P. L-Ru-5-P did not reduce DPN with a C-3 epimerase purified 50-fold from brewer's yeast[11]. Thus C-4 stereoisomerase is distinct from C-3 epimerase. The C-3 epimerase from *L. pentosus* was presumably contaminated with C-4 stereoisomerase. Triphosphopyridine nucleotide, uridine triphosphate, and inorganic pyrophosphate, did not stimulate the rate of DPN reduction by the complete system. In addition, α-xylose-1-phosphate and xylose-5-phosphate* did not cause DPN reduction. In the same system, L-G-3-P did not yield D-G-3-P as measured by DPN reduction[2]. Therefore a donor of the L-configuration, although possibly a substrate for transketolase, would not yield D-G-3-P and be observed as DPN reduction. Since D-Xu-5-P, rather than D-Ru-5-P, is the glycolaldehyde donor for spinach transketolase[7,9], these results provide substantial evidence that C-4 stereoisomerase catalyzes reaction (1).

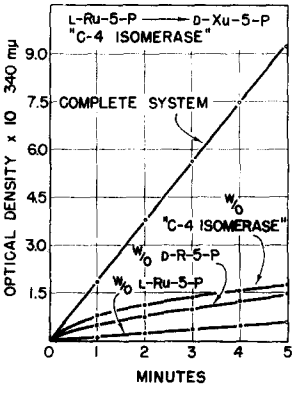


Fig. 1. D-Xu-5-P formation from L-Ru-5-P by a purified enzyme from *A. aerogenes*. The complete system contained 5 μmoles glycylglycine, pH 7.5, 5 μmoles Na glutathione, 8.5 μmoles Na arsenate, 0.2 μmole cocarboxylase, 0.25 μmole DPN, 2 μmoles $MgCl_2$, 2 μmoles D-R-5-P, 0.68 μmole L-Ru-5-P, 0.53 units of spinach transketolase, 153 μg crystalline muscle G-3-P dehydrogenase, 7.8 μg C-4 stereoisomerase, and water to 0.5 ml. The transketolase was free of C-3 epimerase but contained phosphoriboisomerase.

The conversion of L-Ru-5-P to D-Xu-5-P also was measured in the following system:

$$\text{D-Xu-5-P} \xrightarrow{\text{C-3 Epimerase}^{11}} \text{D-Ru-5-P} \quad (4)$$

$$\text{D-Ru-5-P} + \text{ATP} \xrightarrow{\text{Phosphoribulokinase}^{12}} \text{D-Ru-1,5-P} + \text{ADP} \quad (5)$$

ADP[7] or alkali labile phosphate[12] was determined on aliquots removed at intervals. As shown in Table I, C-4 stereoisomerase, C-3 epimerase and phosphoribulokinase were necessary for ADP formation; the addition of DPN to the complete system did not stimulate the rate of ADP formation.

34 μmoles L-Ru-5-P incubated with C-4 stereoisomerase yielded 11 μmoles D-Xu-5-P as measured enzymically. The reaction mixture was treated with acid phosphatase[13], deionized with IR-120(H^+) and IR-45(OH^-) and chromatographed on Dowex-1 borate (1.2 sq. cm × 24.6 cm)[14,15]. Two peaks were located by the cysteine-carbazole[16] and orcinol pentose[17] determinations. The peaks were freed of borate[18], concentrated *in vacuo* and characterized by the cysteine-carbazole test[7], the orcinol spectra[7], and by paper chromatography (Table II).

* We are indebted to Dr. R. W. WATSON for gifts of α-xylose-1-phosphate and xylose-5-phosphate.

TABLE I

THE CONVERSION OF L-RIBULOSE-5-PHOSPHATE TO D-XYLULOSE-5-PHOSPHATE BY A PURIFIED STEREOISOMERASE FROM *A. aerogenes*

The 1 ml reaction mixture contained 40 μmoles triethanolamine buffer, pH 8.3, 2 μmoles Na glutathione, 5 μmoles $MgCl_2$, 3 μmoles ATP, 1.4 μmoles L-Ru-5-P, 8.3 units phosphoribulokinase[12], 0.74 units* epimerase from brewer's yeast[11], and 39 μg purified stereoisomerase as indicated. After 10 min at 37°, the tubes were boiled 5 min and 0.05 ml assayed for ADP with the pyruvate kinase-lactic dehydrogenase system[7].

Enzyme additions	*μmole ADP/10 min*
Complete system	0.684
Complete system minus phosphoribulokinase	0.087
Complete system minus stereoisomerase	0.127
Complete system minus epimerase	0.087

* 1 unit = 1 μmole DPN·H formed per minute under conditions described in Fig. 1. R-5-P was the substrate.

TABLE II

IDENTIFICATION OF PENTULOSES ELUTED FROM DOWEX-1 BORATE

Pentulose	*Cysteine-carbazole development (min)*	*Orcinol spectra* E_{540}/E_{670}	*Paper chromatography**		
			R_F	*Orcinol-TCA***	*Dimethyl** *phenaline*
Peak 1	>60	0.46	0.50	Steel gray	Purple
Peak 2	<15	0.91	0.62	Brownish gray	Rose***
Xylulose standard	>60	0.50	0.48	Steel gray	Purple
Ribulose standard	<15	0.83	0.59	Brownish gray	Rose***

* Whatman No. 1, descending water-saturated phenol at room temperature.
** As modified by M. I. KRICHEVSKY and W. A. WOOD[19].
*** Orange fluorescence under ultraviolet light.

Peak 1 (after 476 ml of 0.02 *M* borate) contained 2.66 μmoles xylulose and peak 2 (after 413 ml of 0.03 *M* borate) contained 5.1 μmoles ribulose. Xylulose was not formed in a control containing L-ribulose which was treated in the same manner. 112 μmoles xylulose isolated by the same procedure was of the D-configuration ($[\alpha]_D^{25} = -36.5°$; authentic D-xylulose[8] $[\alpha]_D^{25} = -36.8°$), and was devoid of L-xylulose as measured by the TPN-linked L-xylulose-xylitol dehydrogenase of guinea pig liver mitochondria[20]*. Thus the change from L- to D-configuration is accomplished by the conversion of L-Ru-5-P to D-Xu-5-P, a reaction catalyzed by C-4 stereoisomerase.

Laboratory of Bacteriology, Department of Dairy Science, University of Illinois, Urbana, Ill. (U.S.A.)

M. J. WOLIN**
F. J. SIMPSON***
W. A. WOOD

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* We wish to thank Dr. OSCAR TOUSTER for making this determination.
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Received March 15th, 1957

On the mechanism of the lipolytic action of the lipaemia-clearing factor

In a study of the composition of the mixture of glycerides formed during the heparin-induced lipaemia-clearing reaction[1] it was found that the reactions triglycerides → diglycerides and diglycerides → monoglycerides proceeded at rather similar rates. *In vitro* as well as *in vivo* there was during the earlier phase of the reaction a considerable accumulation of monoglycerides, *i.e.* the reaction monoglyceride → glycerol proceeded relatively slowly. This suggested that the clearing factor might have a specific action on the α-ester bond in the glyceride molecule. If that was so the resulting β-monoglyceride would not be so readily attacked and thus the monoglyceride concentration would increase. A further investigation of this possibility is presented in this paper.

Rat chylomicrons, obtained after cannulation of the thoracic duct[2], were flotated in a Spinco ultracentrifuge and washed twice in slightly buffered saline. The purified chylomicrons were incubated with post heparin clearing factor, prepared from human plasma according to NIKKILÄ[3], in the presence of 5% human serum albumin. 10 mg 1-^{14}C-oleic acid dissolved in 5% serum albumin solution were added to the reaction mixture that had a final volume of 2,000 ml, the final concentration of glyceride being 2 mg/ml. The reaction was carried out at 37° in a phosphate buffer, pH 7.5, and ionic strength 0.05.

The optical density at 700 mμ was registered and at different time intervals 100 ml of the incubation mixture were extracted with 2 l alcohol-ether (3:1). After filtration, the extracts were concentrated to a small volume under reduced pressure at 60° C. The residue was repeatedly extracted with light petroleum ether, the combined extracts dried with sodium sulphate and taken to dryness *in vacuo*. The phospholipids were separated off on a silicic acid column[4], and to obtain complete separation the phospholipids were afterwards precipitated with acetone and $MgCl_2$ in the cold. The glycerides and the free fatty acids obtained from the silicic acid columns were separated on columns of the ion-exchanger IRA-400[5]. Tri-, di- and monoglycerides were separated chromatographically on a silicic acid column[6], using 10 g silicic acid for about 100 mg glyceride mixture. The glycerides were saponified and the fatty acids separated from the unsaponifiable matter by extraction[5].

Radioactivity was determined after mounting 1 mg of fatty acid on aluminium planchettes. At least 1,000 counts were counted.

The specific activities of the phospholipid fatty acids were in all fractions less than 1% of the specific activities of the free fatty acids. The traces of activity found in these fractions were probably contaminants from the other lipid fractions.

In Fig. 1 (upper part) the course of the hydrolysis can be followed from the decrease in optical density and the release of fatty acids. After about 6 h, the reaction had reached a state of equilibrium where the optical density remained practically constant and no additional amounts of fatty acids were released. The specific activity curves for the free fatty acids and glyceride fatty acids are seen in the lower part of the same figure. As is apparent, there was a rapid incorporation of the labelled free acid into the fatty acids of the glycerides simultaneous with a dilution of the labelled free acid with acids released from the glycerides. After a few hours the specific activity curves for the free fatty acids and the glyceride fatty acids are roughly parallel, indicating that an equilibrium has been reached. Here, however, there is still a difference in the specific activities of the two fractions, the specific activity of the glyceride fatty acids only reaching around 60% of that of the free fatty acids. The specific activity of the glyceride fatty acids relative to that of the free fatty acids is seen in the small figure included in the lower part of Fig. 1. This difference in the specific activity of the glyceride fatty acids and the free fatty acids indicates that only part of the glyceride fatty acids are exchangeable.