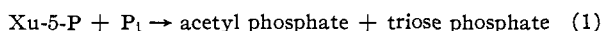


### ACETYL PHOSPHATE FORMATION IN THE PHOSPHOROLYTIC CLEAVAGE OF PENTOSE PHOSPHATE

Sir:

In the fermentation of pentoses by *Lactobacillus* species it has been established that the methyl and carboxyl groups of acetate arise from C-1 and C-2 of pentose, respectively.<sup>1,2</sup> With an enzyme purified from *Lactobacillus pentosus*, obtained from cells grown on *L*-arabinose or *D*-xylose, a phosphorolytic cleavage of xylulose 5-phosphate has now been observed.<sup>3</sup>



ThPP is required, and the reaction appears to represent a new type of ketolase reaction in which an active form of glycolaldehyde is converted to acetyl phosphate. In addition to ThPP and  $\text{P}_i$ , reaction (1) requires  $\text{Mg}^{++}$  and a sulfhydryl compound (GSH, mercaptoethanol, cysteine, or thioglycolate). R-5-P will replace Xu-5-P as substrate only when PRI<sup>4</sup> and PKPE<sup>5</sup> are added; in the presence of these enzymes R-5-P is converted to Xu-5-P. No acetyl phosphate is formed with S-7-P or F-6-P as substrates, and tests for transketolase in the purified enzyme preparations were

TABLE I

#### DETERMINATION OF ACETYL PHOSPHATE

The incubation mixture contained, per ml., 10  $\mu\text{moles}$  of R-5-P, 6.6  $\mu\text{moles}$  of  $\text{Mg}^{++}$ , 33  $\mu\text{moles}$  of  $\text{P}_i$ , 80  $\mu\text{moles}$  of succinate buffer pH 6.0, 5  $\mu\text{moles}$  of GSH, 0.17  $\mu\text{mole}$  of ThPP, 20  $\mu\text{g}$  of PRI, 17  $\mu\text{g}$  of PKPE, and 0.53 mg. of an ammonium sulfate fraction from *L. pentosus*. Incubation was for 25 minutes at 38°.

Compound measured	$\mu\text{moles per ml. formed}$
Triose phosphate <sup>a</sup>	4.30
Acetyl phosphate	
1. Hydroxamic test <sup>b</sup>	4.16
2. Transacetylase + condensing enzyme <sup>c</sup>	3.80
3. Acetokinase <sup>d</sup>	4.42
4. Pigeon liver acetylating enzyme <sup>e</sup>	4.30

<sup>a</sup> Assayed with  $\alpha$ -glycerophosphate dehydrogenase.<sup>6</sup>  
<sup>b</sup> Acetylhydroxamate was measured as described by Lipmann and Tuttle.<sup>7</sup> The hydroxamate formed had identical chromatographic properties with authentic acetylhydroxamate.<sup>8</sup>  
<sup>c</sup> Determined as described by Stern, *et al.*<sup>9</sup> Condensing enzyme was kindly furnished by Dr. S. Ochoa and transacetylase by Dr. E. R. Stadtman.  
<sup>d</sup> Determined with acetokinase<sup>10</sup> and ADP, coupled to hexokinase and Zwischenferment.<sup>11</sup>  
<sup>e</sup> Determined by the procedure described by Tabor, *et al.*<sup>12</sup>

(1) J. O. Lampen, H. Gest and J. C. Sowden, *J. Bact.*, **61**, 97 (1951).

(2) D. A. Rappaport, J. A. Barker and W. Z. Hassid, *Arch. Biochem. Biophys.*, **31**, 326 (1951).

(3) The following abbreviations have been used: Xu-5-P, *D*-xylulose 5-phosphate; Ru-5-P, *D*-ribulose 5-phosphate; R-5-P, *D*-ribose 5-phosphate; S-7-P, sedoheptulose 7-phosphate; F-6-P, fructose 6-phosphate; ThPP, thiamin pyrophosphate; GSH, glutathione; PRI, phosphoriboisomerase; PKPE, phosphoketopentosepimerase; HDP, fructose-1,6-diphosphate.

(4) J. Hurwitz, A. Weissbach, B. L. Horecker and P. Z. Smyrniotis, *J. Biol. Chem.*, **218**, 726 (1956).

(5) J. Hurwitz and B. L. Horecker, *ibid.*, in press.

(6) E. Racker, *J. Biol. Chem.*, **167**, 843 (1947).

(7) F. Lipmann and L. C. Tuttle, *ibid.*, **159**, 21 (1945).

(8) E. R. Stadtman and H. A. Barker, *ibid.*, **184**, 769 (1950).

(9) R. J. Stern, B. Shapiro, E. R. Stadtman and S. Ochoa, *ibid.*, **193**, 703 (1951).

(10) I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954).

(11) A. Kornberg, *ibid.*, **182**, 805 (1950).

(12) H. Tabor, A. H. Mehler and E. R. Stadtman, *ibid.*, **204**, 127 (1953).

negative. Arsenate will replace phosphate, yielding acetate rather than acetyl phosphate.

Acetyl phosphate was identified by the reactions summarized in Table I. Equivalent amounts of acetyl phosphate and triose phosphate were formed for each mole of pentose phosphate and  $\text{P}_i$  utilized (Table II). With 1-C<sup>14</sup>-R-5-P the resulting acetyl phosphate was labeled exclusively in the methyl position.

When ADP is included in the incubation mixture, acetate rather than acetyl phosphate accumulates. Acetokinase present in pentose-grown cells may play an important role in the generation of ATP during the conversion of pentose to acetate and lactate. During the formation of the latter compound from triose phosphate by the Embden-Meyerhof pathway two moles of ATP would be produced. The over-all reaction involved in the fermentation of pentoses by *L. pentosus* would be summarized as

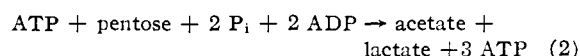


TABLE II

#### STOICHIOMETRY OF PENTOSE PHOSPHATE CLEAVAGE

The incubation mixture contained, per ml., 20  $\mu\text{moles}$  of R-5-P, 23  $\mu\text{moles}$  of  $\text{P}_i$ , 3.3  $\mu\text{moles}$  of  $\text{Mg}^{++}$ , 0.17  $\mu\text{mole}$  of ThPP, 5  $\mu\text{moles}$  of GSH, 67  $\mu\text{moles}$  of succinate buffer, pH 6.0, 20  $\mu\text{g}$ . of PRI, 17  $\mu\text{g}$ . of PKPE, 170  $\mu\text{g}$ . of hexokinase,<sup>a</sup> 3.3  $\mu\text{moles}$  of ADP, 67  $\mu\text{moles}$  of glucose, 206  $\mu\text{g}$ . of acetokinase, 1.05 mg. of an ammonium sulfate fraction from *L. pentosus*. Incubation was for 30 minutes at 38°.

Compound measured	$\mu\text{moles per ml.}$
R-5-P <sup>b</sup>	-11.9
$\text{P}_i^c$	-12.0
Acetate <sup>d</sup>	+12.4
Triose phosphate <sup>e</sup>	+12.0

<sup>a</sup> Kindly supplied by Dr. S. Colowick and Mr. Robert Darrow.

<sup>b</sup> Determined by the orcinol method,<sup>13</sup> corrected for the equilibrium mixture of R-5-P, Ru-5-P and Xu-5-P formed at 38° in the presence of PRI and PKPE.

<sup>c</sup> Determined by the procedure of Fiske and SubbaRow.<sup>14</sup>

<sup>d</sup> Determined with acetokinase as described by Rose *et al.*<sup>10</sup>

<sup>e</sup> Determined with  $\alpha$ -glycerophosphate dehydrogenase.<sup>6</sup>

(13) W. Z. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).

(14) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(15) Research Fellow of the American Heart Association.

(16) Fellow in Cancer Research of the American Cancer Society.

NATIONAL INSTITUTE OF ARTHRITIS  
AND METABOLIC DISEASES

E. C. HEATH<sup>16</sup>

NATIONAL INSTITUTES OF HEALTH

J. HURWITZ<sup>16</sup>

UNITED STATES PUBLIC HEALTH SERVICE  
BETHESDA, MARYLAND

B. L. HORECKER

RECEIVED JULY 30, 1956

### GUANINE AS A SOURCE OF THE NITROGEN 1-CARBON 2 PORTION OF THE IMIDAZOLE RING OF HISTIDINE<sup>1</sup>

Sir:

A mutant, strain HP-1, of *Escherichia coli* whose requirement for guanine is spared by histidine has previously been shown to derive carbon 2 of the imidazole ring of histidine exclusively from carbon 2 of guanine.<sup>2</sup>

(1) This work was supported in part by a research grant (NSF-G1295) from the National Science Foundation, and by funds received from the Eugene Higgins Trust.

(2) B. Magasanik, H. S. Moyed and D. Karibian, *THIS JOURNAL*, **78**, 1510 (1956).