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.[1,2] 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.[3]

$$\text{Xu-5-P} + P_i \rightarrow \text{acetyl phosphate} + \text{triose phosphate} \quad (1)$$

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 P_i, reaction (1) requires Mg^{++} and a sulfhydryl compound (GSH, mercaptoethanol, cysteine, or thioglycolate). R-5-P will replace Xu-5-P as substrate only when PRI[4] and PKPE[5] 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 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 P_i utilized (Table II). With 1-C^{14}-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

$$\text{ATP} + \text{pentose} + 2\ P_i + 2\ \text{ADP} \rightarrow \text{acetate} + \text{lactate} + 3\ \text{ATP} \quad (2)$$

TABLE I

DETERMINATION OF ACETYL PHOSPHATE

The incubation mixture contained, per ml., 10 μmoles of R-5-P, 6.6 μmoles of Mg^{++}, 33 μmoles of P_i, 80 μmoles of succinate buffer *p*H 6.0, 5 μmoles of GSH, 0.17 μmole of ThPP, 20 μg of PRI, 17 μg of PKPE, and 0.53 mg. of an ammonium sulfate fraction from *L. pentosus*. Incubation was for 25 minutes at 38°.

Compound measured	μmoles per ml. formed
Triose phosphate[a]	4.30
Acetyl phosphate	
1. Hydroxamic test[b]	4.16
2. Transacetylase + condensing enzyme[c]	3.80
3. Acetokinase[d]	4.42
4. Pigeon liver acetylating enzyme[e]	4.30

[a] Assayed with α-glycerophosphate dehydrogenase.[6] [b] Acethydroxamate was measured as described by Lipmann and Tuttle.[7] The hydroxymate formed had identical chromatographic properties with authentic acethydroxamate.[8] [c] Determined as described by Stern, *et al.*[9] Condensing enzyme was kindly furnished by Dr. S. Ochoa and transacetylase by Dr. E. R. Stadtman. [d] Determined with acetokinase[10] and ADP, coupled to hexokinase and Zwischenferment.[11] [e] Determined by the procedure described by Tabor, *et al.*[12]

TABLE II

STOICHIOMETRY OF PENTOSE PHOSPHATE CLEAVAGE

The incubation mixture contained, per ml., 20 μmoles of R-5-P, 23 μmoles of P_i, 3.3 μmoles of Mg^{++}, 0.17 μmole of ThPP, 5 μmoles of GSH, 67 μmoles of succinate buffer, *p*H 6.0, 20 μg. of PRI, 17 μg. of PKPE, 170 μg. of hexokinase,[a] 3.3 μmoles of ADP, 67 μmoles of glucose, 206 μg. of acetokinase, 1.05 mg. of an ammonium sulfate fraction from *L. pentosus*. Incubation was for 30 minutes at 38°.

Compound measured	μmoles per ml.
R-5-P[b]	−11.9
P_i[c]	−12.0
Acetate[d]	+12.4
Triose phosphate[e]	+12.0

[a] Kindly supplied by Dr. S. Colowick and Mr. Robert Darrow. [b] Determined by the orcinol method,[13] 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. [c] Determined by the procedure of Fiske and SubbaRow.[14] [d] Determined with acetokinase as described by Rose *et al.*[10] [e] Determined with α-glycerophosphate dehydrogenase.[6]

(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, phosphoketopentoepimerase; 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).

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

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

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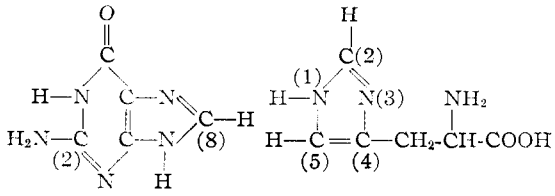
GUANINE AS A SOURCE OF THE NITROGEN 1–CARBON 2 PORTION OF THE IMIDAZOLE RING OF HISTIDINE[1]

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.[2]

(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.

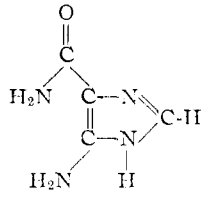
(2) B. Magasanik, H. S. Moyed and D. Karibian, THIS JOURNAL, **78**, 1510 (1956).



Guanine Histidine

The source of the nitrogen of histidine was investigated by growing the organism on glucose in a medium whose only nitrogenous constituents were 12 mg. of unlabelled guanine and 400 mg. of $(N^{15}H_4)_2SO_4$ (60 atom % excess) per liter. The N^{15} content of the histidine isolated from the bacterial protein (determined[3] after dilution with a known amount of carrier) was found to be 38.5 atom % excess, 64% that of the exogenous ammonium sulfate, indicating that only two of its three nitrogen atoms had been derived from the latter. The histidine was hydrolyzed enzymatically with dried cells of histidine-grown *Aerobacter aerogenes* to a mixture of ammonia, glutamic acid, and formamide.[4] The three nitrogenous compounds were separated by consecutive passage of the mixture over columns of Permutite (retaining ammonia) and Dowex-2-chloride (retaining glutamic acid) and analyzed for N^{15} with the following results (expressed in per cent. of the atom % excess of the ammonium sulfate): ammonia (amino group of histidine) 94%, glutamic acid (imidazole-nitrogen 3) 97%, formamide (imidazole-nitrogen 1) 0.8%. It appears therefore that guanine is not only the source of carbon 2,[2] but also of the adjacent nitrogen 1 of the imidazole ring of histidine.

In another experiment the mutant was grown in the glucose–ammonium sulfate medium supplemented with 50 mg. of guanine-8-C^{14} per liter. The ribotide and riboside of 4-amino-5-imidazole carboxamide were found to accumulate in the culture



4-Amino-5-imidazole carboxamide

fluid and were isolated by adsorption to charcoal, elution with a mixture of ethanol, ammonia and water, followed by chromatography and electrophoresis on filter paper.[5] The radioactivity (in counts per micromole) of the carboxamide obtained by the acid hydrolysis of its derivatives was equal to that of the exogenous guanine, and the amount accumulated, 7 mg. per liter, was roughly equivalent to the amount of histidine in the cells.

The results suggest that guanine is converted to the ribotide of 4-amino-5-imidazole carboxamide by the loss of a C–N unit which eventually becomes the nitrogen 1–carbon 2 portion of the imidazole ring of histidine.

(3) I am indebted to Dr. D. Elwyn for help with the analysis.

(4) B. Magasanik and H. R. Bowser, *J. Biol. Chem.*, **213**, 571 (1955).

(5) A. R. Greenberg and E. L. Spilman, *ibid.*, **219**, 411 (1956).

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EVIDENCE FOR THE PRESENCE OF COBALT HYDROCARBONYL UNDER CONDITIONS OF THE OXO REACTION

Sir:

Recent kinetic studies of the oxo reaction[1,2] purport to indicate that the cobalt catalyst is present as dicobalt octacarbonyl and that the first step in the reaction consists of olefin attack on this catalyst. Although cobalt hydrocarbonyl has been postulated repeatedly to be present during the reaction,[3] no conclusive evidence for its presence (or absence) has been presented, owing to its instability.[4]

In some early work, not concerned with the oxo reaction, it was reported that treatment of dicobalt octacarbonyl at 165° for 18 hours with H_2:CO, gave a small (unspecified) quantity of cobalt hydrocarbonyl.[5] We have now found that dicobalt octacarbonyl under carbon monoxide pressure is rapidly converted by hydrogen at 110° to the hydrocarbonyl. The hydrocarbonyl was isolated (as the anion) by rapid cooling (−50°) of the pressure vessel. However, if an olefin is present at the time the vessel is cooled, no hydrocarbonyl can be isolated. If the conventional oxo reaction is allowed to proceed until the olefin is consumed, the hydrocarbonyl again appears uncombined. The results listed in Table I also show that the partial pressure of hydrogen affects the carbonyl conversion. These results strongly suggest olefin–hydrocarbonyl rather than olefin–octacarbonyl interaction as the step in the oxo synthesis.

TABLE I

CONVERSION OF $[Co(CO)_4]_2$ TO $HCo(CO)_4$

1-Hexene, mole	$[Co(CO)_4]_2$ mmoles	Synthesis gas, p.s.i. at 112° Start	Finish	Time (min.)[a]	Per cent. Co as $HCo(CO)_4$
0	4.38	3120	3120	10	50[b]
0	4.59	2600	2600	10	27
0.40	4.00	3200	3000	10	0[b]
.04	4.00	3200	3100	10	29
.226	4.52	3400	2300	77	34
.226	4.52	4000	2850	100	62

[a] After addition of hydrogen to twice the carbon monoxide pressure. [b] Duplicate experiments.

An additional experiment using the technique of rapid cooling showed that the standard procedure[6] for the preparation of $[Co(CO)_4]_2$ results in the formation of $HCo(CO)_4$. The former is isolated as

(1) A. R. Martin, *Chem. Ind.*, 1536 (1954).

(2) G. Natta, R. Ercoli, S. Castellano and F. H. Barbieri, THIS JOURNAL, **76**, 4049 (1954).

(3) M. Orchin in "The Chemistry of Petroleum Hydrocarbons," edited by B. T. Brooks, C. E. Boord, S. S. Kuntz and L. Schmerling, Reinhold Publishing Corp., New York, N. Y., 1955, vol. 3, p. 343.

(4) H. Sternberg, I. Wender, R. A. Friedel and M. Orchin, THIS JOURNAL, **75**, 2717 (1953).

(5) W. Hieber, H. Schulten and R. Marin, *Z. anorg. Chem.*, **240**, 261 (1939).

(6) I. Wender, H. Greenfield and M. Orchin, THIS JOURNAL, **73**, 2656 (1951).