A REINVESTIGATION OF THE OXYGEN-EXCHANGE BETWEEN WATER AND PHOSPHATE DURING THE HYDROLYSIS OF UREA BY UREASE

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Slocum, Kouba and Varner (1959) reported experiments on the hydrolysis of urea by urease in phosphate buffer which indicated: a) a transfer of oxygen-18 from ^{18}O-orthophosphate to the product CO_2 and b) a transfer of oxygen-18 from $H_2{}^{18}O$ to orthophosphate. The latter transfer was complex because one atom of oxygen-18 was introduced into each of 50 molecules of orthophosphate per molecule of urea hydrolyzed by urease. This situation is reminiscent of the results of Cohn (1953) whose studies on oxidative phosphorylation showed that oxygen exchange between water and orthophosphate involved more moles of orthophosphate than the number of high energy phosphate bonds formed from orthophosphate.

We considered the urease catalyzed oxygen-18 exchange between water and orthophosphate worthwhile reinvestigating because 1) the oxygen-18 exchange apparently exceeded the rate of hydrolysis of urea, 2) the exchange might be important in understanding urease action and 3) it was possible that a

carbamyl-P[1] intermediate was involved.

Slocum et al. (1959) had considered that urease might catalyze the formation of a carbamyl-P intermediate from urea and phosphate and that "the stability of the carbamyl-P intermediate allows only a slow decomposition of urea, but brings about, through equilibrium with urea, the exchange of oxygen-18 between water and phosphate." These authors also found that free carbamyl-P was apparently not formed because urea incubated with ^{32}P - orthophosphate buffer did not yield carbamyl-^{32}P and urease did not catalyze an exchange of ^{32}P-orthophosphate into carbamyl-P.

Materials and Methods - Crystalline urease was prepared from Jack-bean meal (kindly given to us by Dr. Warren Nelson of Cornell University) by the method of Sumner (1926) and recrystallized by the method of Dounce (1941). The crystals had a specific activity of 105 Sumner units per mg protein. The crystals were dissolved and stored at 0° suspended in 50% glycerol-0.001 M ethylene diamine tetraacetate at a protein concentration of 6 mg protein per ml.

Oxygen-18 water having 1.70 atoms per cent oxygen-18 was obtained from Rehovath, Israel. Reagent grades KH_2PO_4 was used to make the phosphate buffer.

Ammonia was assayed by Nessler's reagent as described by Hawk et al. (1947). Orthophosphate was determined by a modification (Jones and Spector (1960)) of the method of Fiske and Subbarow.

The hydrolysis of urea in $H_2{}^{18}O$ and phosphate buffer was done under the conditions described in Table I. After incuba-

[1] Carbamyl-P is used as an abbreviation for carbamyl phosphate.

tion at 30° for 1 hour, 0.15 ml. of 2 M acetic acid was added and the mixture was heated in a boiling water bath for 3 minutes. The precipitated protein was removed by filtration. The protein residue was washed to remove all water soluble solids. The total volume of the filtrate and washing was 20 ml. The pH of the solution was raised to 8 with KOH and the total volume was brought to 30 ml. A sample, 20 ml., was placed on a Dowex-1 chloride column and orthophosphate was eluted and crystallized as KH_2PO_4 as described by Jones and Spector (1960). The oxygen-18 content of the orthophosphate was determined by the method of Williams and Hager (1958). Oxygen-18 measurements were made on the Consolidated Mass Spectrometer, Type 21-103C, kindly made available to us by the Chemistry Department of Harvard University.

Results and Discussion - Table I shows the results of two experiments, one at pH 6.0 and a second at pH 6.8 where urease was incubated with 10 µmoles of urea and 500 µmoles of phosphate. At pH 6 only 7 µmoles of urea were hydrolyzed after 1 hour at 30°, while at pH 6.8 hydrolysis was complete. In all situations the ^{18}O-content of orthophosphate, expressed as atoms per cent, is only the natural abundance, while if one atom of oxygen-18 were incorporated into each molecule of orthophosphate, the ^{18}O-content observed would have been 0.375 atoms per cent or 0.171 atoms per cent excess. We could have easily detected the incorporation of one atom of oxygen-18 into 50 µmoles of phosphate, but it would have been difficult to detect one atom of oxygen-18 into 10 µmoles of orthophosphate. In this latter case oxygen-18 could have been incorporated into orthophosphate only if urease catalyzed the synthesis of carbamyl-P from urea and orthophosphate and subsequently hydrolyzed this carbamyl-P at the P-O

Table I

Exchange of Oxygen-18 into Orthophosphate Buffer from $H_2{}^{18}O$ during the Hydrolysis of Urea by Crystalline Urease

Omissions	Atom % ^{18}O in KH_2PO_4 when buffer was pH 6.0	pH 6.8
None	0.206	0.204
Urease	0.200	0.201
Urea	0.204	0.204
Urea and Urease	0.201	0.206

The complete incubation mixture contained in a total volume of 3 ml.: 120 units of crystalline urease, 10 µmoles of urea, 500 µmoles of potassium phosphate buffer, pH 6.0 or 6.8 and 2.2 ml. of $H_2{}^{18}O$. The vessels were incubated for 60 minutes at 30°. The subsequent treatment of the samples is given in the Materials and Methods section.

bond.[2] Slocum et al. could not observe the synthesis of carbamyl P, and we have found, in experiments not shown here, that urease does not catalyze the hydrolysis of carbamyl-P. We would conclude, therefore, that under the conditions used here there is no incorporation of oxygen-18 from water into orthophosphate buffer during the hydrolysis of urea by urease.

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[2] Chemical hydrolysis of carbamyl-P at pH 6.0 and 6.8 occurs with 80 and 90% C-O bond cleavage, respectively (Allen and Jones, 1964).

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