is also being studied. Some ostensible discrepancies have already been established as experimental errors, but all other reactions and other previously described formations of this compound seem to be compatible with the triazine formula I of $C_3H_3N_3$.

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ON THE MECHANISM OF THE ENZYMATIC SYNTHESIS OF GLUTAMINE[1]

Sir:

It has now been established[2,3] that the synthesis of glutamine by plant enzymes proceeds according to the over-all reaction[4]

$$\text{glutamic acid} + NH_3 + ATP \rightleftharpoons \text{glutamine} + ADP + P_i$$

The detailed mechanism by which this reaction proceeds, however, has hitherto remained obscure. Data bearing on this mechanism have been obtained by measuring the exchange of phosphate residues between P_i and ATP using the glutamine synthetase obtained from peas and purified 270-fold.[2]

The absolute amount of such exchange is low as shown in Table I. The way in which the exchange varies with the components of the reaction mixture is however highly significant and in accord with the reaction mechanism

$$E + ATP \rightleftharpoons E\text{-}P + ADP \quad (1)$$

$$E\text{-}P + Glu \rightleftharpoons E\text{-}Glu + P_i \quad (2)$$

$$E\text{-}Glu + NH_3 \rightleftharpoons E + Glu\text{-}NH_2 \quad (3)$$

This formulation of reactions accounts for the large increase in exchange which occurs in the presence of glutamate (line 3, Table I) since glutamate is an absolute prerequisite for exchange by this mechanism. The fact that exchange is obtained at all in the presence of only the enzyme, ATP and P_i is probably due to a small amount of ATPase known to be present in the enzyme preparation. The presence of ammonia or hydroxylamine (lines 4 and 5, Table I) results in the formation of glutamine or glutamylhydroxamic acid, thereby decreasing the amount of exchange of P_i by keeping E-Glu at a lower concentration.

TABLE I

PHOSPHATE EXCHANGE BETWEEN RADIOACTIVE INORGANIC ORTHOPHOSPHATE AND ATP CATALYZED BY GLUTAMINE SYNTHETASE

Reaction systems were incubated for 60 min. at 23°. All systems contained: 2.5 μ moles $P^{32}O_4$, 2.0 μ moles ATP, 20 μ moles $MgSO_4$, 10 μ moles cysteine, and 45 μ moles tris-(hydroxymethyl)-aminomethane–HCl (*p*H 7.4). Additions to this basal mixture as noted above were 50 μ moles glutamate, 40 μ moles NH_3 (or NH_2OH), and 0.5 mg. of enzyme protein. The ATP and P_i were separated chromatographically by the procedure of Bandurski and Axelrod.[5]

Reaction system	C.p.m./ml. ATP	P_i
1 No enzyme	120	900,000
2 Enzyme alone	1140	900,000
3 Enzyme plus glutamate	9800	900,000
4 Enzyme plus glutamate plus NH_3	2600	900,000
5 Enzyme plus glutamate plus NH_2OH	740	900,000

The results obtained would seem to preclude the possibility of a glutamyl phosphate acting as an intermediate in glutamine synthesis, since such a mechanism

$$\text{Glutamate} + ATP \rightleftharpoons \text{Glutamyl phosphate}$$

$$\text{Glutamyl phosphate} + NH_3 \rightleftharpoons \text{Glutamine} + P_i$$

would require that ammonia be present in order for exchange of P_i into ATP to take place. Similarly, the formation of an adenyl enzyme as recently implicated[6] in the synthesis of acetyl-CoA is not consistent with the present results.

The proposed mechanism for the synthesis of glutamine is also consistent with the suggestion of Elliott[2] that glutamyl transferase activity and glutamine synthetase activity may both reside in a single enzyme. Transferase activity would consist simply of reaction no. 3 above.

Phosphorylation of the enzyme by ATP also has been indicated as the initial step in the synthesis of glutathione from γ-glutamylcysteine and glycine,[7] and in the synthesis of γ-glutamylcysteine from glutamic acid and cysteine.[8] The formation of a phosphorylated enzyme, therefore, may represent one general mechanism for the participation of ATP in biological syntheses.

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(2) W. H. Elliott, *J. Biol. Chem.*, **201**, 661 (1953).

(3) George C. Webster, *Plant Physiol.*, **28**, 724 (1953).

(4) Abbreviations used: ATP (adenosinetriphosphate), ADP (adenosinediphosphate), P_i (orthophosphate), E (enzyme), Glu (glutamate), Glu-NH_2 (glutamine), CoA (coenzyme A).

(5) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(6) Mary Ellen Jones, Fritz Lipmann, Helmut Hilz and Feodor Lynen, THIS JOURNAL, **75**, 3285 (1953).

(7) John E. Snoke, *ibid.*, **75**, 4872 (1953).

(8) George C. Webster and J. E. Varner, unpublished results.