# An amino acid dependent exchange between inorganic pyrophosphate and ATP in microbial extracts

An amino acid dependent exchange of $^{32}PP^*$ with ATP by an enzyme system from rat liver extract has been described recently by HOAGLAND[1]. The exchange is dependent both on the total concentration and the number of amino acids present. No breakdown of ATP occurs unless hydroxylamine is added and then the hydroxamates of the amino acids appear as end products indicating a possible carboxyl activation. It was suggested that the reaction might reflect a mechanism for activation of amino acids for protein synthesis.

We have observed a similar amino acid dependent exchange of $^{32}PP$ with ATP in bacterial extracts[2]. The reaction appears to be widely distributed among microorganisms having been found in the following genera: *Micrococci, Neurospora, Aerobacter, Staphylococcus, Clostridium, Desulfovibrio, Proteus, Streptococcus, Serratia, Rhodospirillum, Azotobacter, Escherichia* and *Saccharomyces*.

Extracts are readily prepared by grinding freshly harvested cells with alumina A 303 and extracting with phosphate buffer pH 6.5. After centrifugation at 100,000 · *g* for 1 hour, the activity is found in the supernatant. A typical experiment is conducted as follows: A reaction mixture consisting of 15 $\mu M$ ATP, 15 $\mu M$ $^{32}PP$, 10 $\mu M$ $MgSO_4$, 10 $\mu M$ KF, 50 $\mu M$ Tris buffer pH 7.4 and 20 $\mu M$ total amino acids is incubated at 37° for 20 min with 0.1 ml of an extract, dialyzed to remove endogenous amino acids. The reaction is terminated by addition of TCA and the ATP separated from $^{32}PP$ by the Norit A method of CRANE AND LIPMANN[3]. The terminal phosphates of ATP are liberated by acid hydrolysis, $P_i$[4], PP[5] and radioactivity are determined. The results obtained with 0.1 ml of *E. coli* extract, when the number of amino acids are varied while keeping the total concentration constant are presented in Table I.

TABLE I

EFFECT OF INCREASING NUMBERS OF AA ON PP-ATP EXCHANGE

| *No. of AA* | *ATP (c.p.m. $\mu M$)* | *% Exchange* |
|---|---|---|
| 0 | 151 | 6 |
| 9 | 600 | 24 |
| 14 | 1000 | 40 |
| 19 | 1820 | 72 |

It can be seen that the per cent exchange between $^{32}PP$ and ATP is dependent upon, although not directly proportional to, the number of amino acids present. Rate studies indicated that the per cent exchange increases exponentially with time. This permitted application of the equation for the exponential law for simple isotope exchange reactions[6] and calculation of the rate of the exchange in terms of $\mu M$ per hour. The disproportionate increase in per cent exchange (Table I) when increasing numbers of amino acids are added suggested that certain amino acids might be more active than others. When each amino acid was tested singly, it was found (Table II) that only 8 amino acids were active in promoting the exchange.

The amino acids were tested at a concentration of 10 $\mu M$ per ml and the complete mixture of 20 amino acids at a concentration of 0.5 $\mu M$ each. The combination of the 8 active amino acids (0.5 $\mu M$ each) can fully replace the complete mixture. A comparison of extracts prepared from *E. coli*, *Streptococcus hemolyticus* and *Neurospora crassa* showed that only these 8 amino acids were active in each extract.

A balance study indicated that no measurable change in concentration of ATP, PP or amino acid occurs during the exchange. PP and ATP apparently exchange in a rapidly reversible manner only in the presence of amino acids and in such a way that no measurable products are formed. Hydroxylamine in high concentration, used with large amounts of enzyme leads to the formation of the corresponding amino acid hydroxamate but at a rate which is less than 0.1 the rate of exchange.

TABLE II

RATE OF PP-ATP EXCHANGE BY INDIVIDUAL AA

| *AA* | *R** | *AA* | *R** |
|---|---|---|---|
| Alanine | 0 | Serine | 0 |
| Arginine | 0 | Threonine | 0 |
| Aspartic | 0 | Tryptophan | 20 |
| Asparagine | 0 | Histidine | 30 |
| Glutamic | 0 | Phenylalanine | 48 |
| Glutamine | 0 | Methionine | 122 |
| Glycine | 0 | Tyrosine | 146 |
| Hydroxproline | 0 | Valine | 146 |
| Lysine | 0 | Leucine | 168 |
| Proline | 0 | Isoleucine | 248 |
| | | Complete mixture | 416 |

* R = $\mu M$ exchange per hour per ml extract (corrected for endogenous activity).

* Abbreviations: PP = inorganic pyrophosphate, ATP = adenosinetriphosphate, $P_i$ = orthophosphate, AA = amino acid, Tris = tris(hydroxymethyl)aminomethane.

Amino acid derivatives such as acetylleucine and leucylhydroxamic acid do not promote the exchange. The simple dipeptides leucylglycine and glycylleucine are inactive. Chloramphenicol at a concentration of 100 $\mu$g/ml did not affect the reaction. The amino acid analogues, norleucine, tryptozan and O-F-phenylalanine, did not inhibit the exchange promoted by their corresponding amino acids, leucine, tryptophan and phenylalanine. The analogues could not substitute for the amino acids in promoting the exchange.

The rapid rate, as well as the widespread distribution of the exchange reaction, suggests that it may reflect a system of major metabolic importance. The relationship of this reaction to protein synthesis must await the demonstration of its requirement in a system where amino acids are being actively incorporated into protein. The significance of the fact that the reaction is apparently specific for only 8 amino acids is not readily apparent. It is possible that these 8 AA represent a primary activation mechanism and the apparently inactive amino acids could be secondarily activated through some transfer reaction with one of the 8 active amino acids. Work now in progress is directed toward the purification of this enzyme system and to the determination of the active form of the amino acid.

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## The preparation of a water soluble dinitrophenyl-gliadin

The detailed examination of the chemistry and structure of the gliadin of wheat is made especially difficult by the lack of methods for fractionating it. Consequently, although the protein has been shown by physico-chemical methods to comprise several components it has not been possible to prove whether these are distinct individuals or the products of association from a single monomer. It is the purpose of this communication to draw attention to the fact that by treatment with fluorodinitrobenzene gliadin may be divided into two fractions, one of which is water soluble and is a more suitable subject for certain studies than the native protein.

Gliadin was prepared from Manitoba No. 2 flour as previously described (MILLS[1]). A solution of the dried gliadin in 30 ml 50% ethanol-water was passed through a column of Amberlite IRA 400 resin which had been equilibrated against 50% ethanol. To this solution were added 50 mg $NaHCO_3$ and 0.2 ml fluorodinitrobenzene. The mixture was shaken for 30 min and then allowed to stand for 18 hours. After removing the precipitate, the supernatant liquid was poured into 100 ml of deionised water. The soluble DNP-gliadin was precipitated by the addition of 15 ml of saturated NaCl solution, was washed rapidly with a small amount of water and dried over $P_2O_5$ *in vacuo*. The final yield was 27%.

A typical sample of this product, referred to as S-gliadin, contained 16.2% total N, 0.56% S and amide N equal to 28.1% of the total N, all on an ash and moisture free basis. It was readily soluble in water or alkaline buffers of low ionic strength but was insoluble below pH 6. The precipitate formed by acidifying aqueous solutions could only be dissolved in alkaline buffers, *e.g.* 0.005 *M* $NaHCO_3$. In alcoholic solutions (50–70%) it was soluble at all pH values.

In contrast to the findings of KOROC[2] and DEUTSCH[3] a complex mixture of dinitrophenyl derivatives was liberated on hydrolysis (18 h in equal parts of 5 *N* HCl and formic acid). Of the fifteen chromatographically distinct derivatives isolated only three have so far been identified, namely: DNP-aspartic acid, DNP-serine and DPN-valine. In each case only traces were present.

Characterisation in the ultracentrifuge indicated that the results were dependent on the nature of the solvent. In aqueous glycine buffer of pH 9.4 the behaviour was that of a system of molecules capable of forming a number of association complexes; while in 0.005 *M* $NaHCO_3$ the S-gliadin was present mainly as a single component of very high sedimentation rate. In alcoholic solutions a single well defined, though usually asymmetrical peak was always observed. Some of the results obtained in these solvents are summarized in Table I. $S_{20,w}$ fell in the range 2.15–2.35 Svedbergs except when the solution was buffered with acetate or citric acid-phosphate