Enzymatic Addition of Lysine to a Uridine Nucleotide

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In the preceding note (1), the enzymatic reactions which lead to the stepwise synthesis of the peptide in UDP-GNAc-lactyl-L-Ala-d-Glu-L-Lys-n-Ala-n-Ala have been described. The lysine-adding enzyme, which catalyzes Equation 1, has been purified 460-fold from an extract of Staphylococcus aureus.

\[
\text{UDP-GNAc-lactyl-L-Ala-d-Glu} + \text{C}^{14}\text{lysine} + \text{ATP} \rightarrow \text{ADP} + \text{Pi} + \text{UDP-GNAc-lactyl-L-Ala-d-Glu-L-Lys} + \text{Lysine}
\]  

(1)

With purified enzyme, no reaction was observed in the absence of uridine nucleotide, ATP, or divalent cation (Table I). When the amount of uridine nucleotide substrate added was limited, 1 mole each of ADP, inorganic phosphate, and UDP-GNAc-lactyl-L-Ala-d-Glu-L-Lys were formed per mole of added UDP-GNAc-lactyl-L-Ala-d-Glu (Table II). No AMP formation was detected, although added AMP could be quantitatively recovered.

ADP formation, absolutely dependent on added lysine, was measured fluorometrically. D- or L-Alanine, D- or L-glutamic acid, glycine, and L- or diaminopimelic acid (a mixture of the meso- and Ll-isomers) could not substitute for L-lysine. The failure of diaminopimelic acid to act as a substrate is of special interest since the specificity of this enzyme may be the basis for the occurrence of L-lysine in the basal structure of some bacterial cell walls and of (meso)diaminopimelic acid in others. With the use of radioactive amino acids, the preparation could also be shown to be free of the enzymes which add L-alanine or d-Ala-d-Ala to the appropriate uridine nucleotide substrate and of the enzyme which catalyzes the synthesis of d-Ala-d-Ala (1). Although this preparation was contaminated with the D-glutamic acid adding enzyme, a preparation free of this activity was obtained by another procedure.

The enzyme was able to catalyze an exchange of P32-inorganic phosphate with ATP, dependent upon the addition of UDP-GNAc-lactyl-L-Ala-d-Glu only. The rate of the exchange reaction was approximately the same as the rate of the peptide bond synthesis. Lysine did not catalyze an exchange of P32-inorganic phosphate with ATP, and, when both lysine and

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1 GNAc-lactyl is 3-O-lactic acid ether of N-acetylglucosamine.
2 O. H. Lowry, personal communication.

tamic acid is activated before addition of lysine. One possible interpretation of the occurrence of an inorganic phosphate exchange in the absence of added lysine is that the activation may be in the form of a derivative of ADP as has also been suggested for the intermediate in formation of carbonyl biotin (3). However, other interpretations are equally plausible.

Thus, the mechanism of synthesis of this peptide bond is similar to the mechanism of synthesis of glutamine (4) or of the two peptide bonds in glutathione (5). A most important feature of the reaction in the light of other current work on peptide bond synthesis (6) is the fact that the free amino acid does not catalyze an exchange reaction with ATP as one of the substrates and that presumably no activated form of the free amino acid occurs as an intermediate.

REFERENCES

A Soluble Protein Required in Mitochondrial Contraction; "Leakage" of Active Factors from Mitochondria

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Rat liver mitochondria swollen in vitro by exposure to glutathione (1) do not contract when adenosine triphosphate + Mg++ + bovine serum albumin (BSA) are added (2), whereas mitochondrial swelling caused by thyroxine and other substances (cf. (3)) is readily reversed by these agents with extrusion of water (2-4). This report demonstrates that with addition of a soluble, heat-labile, nondialyzable factor obtained from mitochondria ("C-factor") glutathione-swollen mitochondria also contract with adenosine triphosphate + Mg++ + bovine serum albumin.

Experiments in Fig. 1A show that a factor necessary for contraction "leaks" into the medium from mitochondria exposed to GSH and apparently becomes sufficiently diluted so as to limit contraction. A relatively heavy suspension of rat liver mitochondria (equivalent to 20 mg of liver per ml) was first allowed to swell in a medium of 0.125 M KC1 + 0.02 M Tris, pH 7.4, at 25° and the swollen mitochondria then carefully removed by centrifugation. To the clear supernatant fluid (designated "used medium") was added a small inoculum of fresh mitochondria (equivalent to 4.0 mg per ml), which rapidly underwent swelling induced by the GSH present in the medium. The addition of ATP + Mg++ + BSA to the mitochondria swollen in the "used medium" caused immediate and nearly complete contraction. On the other hand, an identical concentration of mitochondria swollen by GSH in a fresh, "unused" medium failed to contract in the presence of ATP + Mg++ + BSA, as observed before (2). The contraction-promoting activity ("C-factor") which "leaks" into the "used medium" from GSH-treated mitochondria is destroyed by 10 minutes in a water bath at 100° (Fig. 14). Dialysis of the "used medium" against fresh 0.125 M KC1 + 0.02 M Tris, pH 7.4, causes no loss of activity (Fig. 14). Nonspecific proteins such as serum albumin, and so forth, cannot replace C-factor; ATP, Mg++, and BSA are each essential for its activity. The C-factor-stimulated contraction...
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