The General Peptide Synthetase

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Studies of the last steps in the biosynthesis of collagen have raised interest in the enzyme performing the synthesis of peptide bonds during protein biosynthesis. Nathans and Lipmann [1] and Takanami [2] have studied this enzyme, using as a test reaction the transfer of amino acids to ribosomes. This reaction system was rather ill-defined, so that they did not detect that the protein needs ascorbic acid as a cofactor.

The apoenzyme has been prepared from calf liver by homogenizing 500 g liver in 2 l of a 0.02 M phosphate buffer, pH 7.6, containing 10 g sodium deoxycholate. The pH has been lowered to 5.0 by adding 1 M HAc. The precipitate obtained was discarded. The pH of the solution was raised again to 7.6 and the solution was precipitated by ammonium sulphate. The precipitate between 38 and 50 % has been collected and dissolved in water. The solution was put on a DEAE-cellulose column and washed with 0.15 M KCl 0.02 M phosphate buffer, pH 7.0, and the apoenzyme was released with 0.25 M KCl 0.02 M phosphate buffer, pH 7.0. The solution containing the enzyme was dialysed and then freeze dried. The freeze dried material has been dissolved to a 2 % solution in 70 % ethanol containing 0.001 M $HgCl_2$. The clear solution was put into an ice box for a day. An easily collectable precipitate was obtained. It was soluble in water and an active apoenzyme could be obtained by removing the mercury ion. The apoenzyme together with ascorbic acid gives the general peptide synthetase.

Methyl esters of the amino acids have been used as substrate for the peptide synthesising reaction. To obtain a reaction the following conditions must be held. An equivalent amount of guanosine triphosphate must be added. Magnesium ions must be present in amounts between 3 – 5 mM. In the experiments a 0.1 M tris buffer containing 0.05 M KCl has been used. The pH range in which a reaction occurs is narrow, being 7.4 – 7.8. Without these conditions an initiator is necessary to get any reaction. It seems that no reaction can occur unless the pK of the reacting α-amino group is 8 or less. Thus no reaction occurs if a dipeptide such as GlyGly with pK 8.4 is added, but the tripeptide GlyGlyGly with pK 7.9 gives an immediate reaction, as shown by the strong decrease in the ninhydrin value of the solution. Diiodotyrosine and thyroxine are excellent starters of the synthesis. They are exceptions among the amino acids in that the pK values of their amino groups are not in the range 9 – 10.

Under the same conditions (*i.e.* including an initiator) amino acids esterified to s-RNA can be used as substrate, giving peptides without the presence of ribosomes.

1. Nathans, D. and Lipmann, F. *Biochim. Biophys. Acta* **43** (1960) 126.
2. Takanami, M. *Biochim. Biophys. Acta* **51** (1961) 85.

A Rapid Semicontinuous Method for Purification of Ceruloplasmin from Human Serum

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A three-step method for ceruloplasmin purification has been devised, which operates throughout under mild conditions (4°, potassium phosphate buffer, pH 6,8, with potassium chloride to stabilize ceruloplasmin). The first and third step utilizes protein-protein displacement on DEAE-Sephadex (Pharmacia, Uppsala) and hydroxylapatite, respectively, while the second step consists of gel filtration on Sephadex G-100. Insufficiently purified ceruloplasmin fractions obtained at various stages are diluted to obtain the ionic strength of the original material (for instance retroplacental serum) and are returned to the starting point for reutilization.

The degree of purification after step 2 corresponds to a value of the ratio of the absorbancies at 610 mμ and 280 mμ of 0.040. After the third step this value increases to 0.042. The net recovery for all three steps is about 85 %, when recycling of insufficiently pure material is included. One complete cycle, starting with 700 ml of serum, takes 2.5 days and gives 200 – 250 mg of pure material. A new cycle can be started once a day.