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Preparative synthesis of globin in a continuous cell-free translation system from rabbit reticulocytes

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Effective cell-free translation systems using a continuous flow of feeding solution with amino acids, ATP and GTP through the reaction chamber have recently been developed (1). These systems can function for long periods of time (tens of hours) with high yields of synthesised polypeptides using Escherichia coli and wheat embryo extracts. The question arose whether systems based on animal cell extracts could work in the same way. Here we report the result of globin synthesis in a continuous cell-free translation system from rabbit reticulocytes (Fig.1). This system can work efficiently for an extended period producing about 2 mg of α- and ß- globin from 0.5 ml of extract after 100 hours.

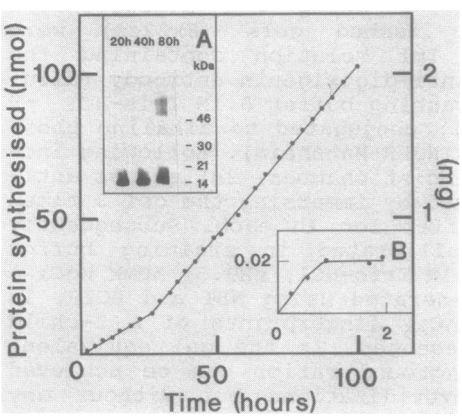

Fig.1. Kinetics of globin synthesis at 30°C. Polypeptide was synthesised as in (1) using ultrafiltration membrane XM-100A. 0.5ml of the incubation mixture contained 250ul of micrococcal nuclease treated rabbit reticulocyte lysate (Amersham), 3ug of globin mRNA obtained as described (2) and 250ul of a feeding solution containing 25mM Hepes.HCl, pH 7.4, 150mM KAc, 0.6mM spermidine, 1mM ATP 0.2mM GTP, 10mM creatine phosphate, 20uM haemin, 40mM [^{35}S] methionine, 40uM each of the other amino acids. Flow rate of feeding solution was 3ml/hr. Insets: (A) electrophoresis (3) and subsequent fluorography of translation products in the filtrate after 20,60 and 80 hours. Some high molecular weight bands are derived from mRNA contaminating the globin mRNA. When no mRNA is added, no synthesis is observed. α- and ß- globin chains are not resolved. (B) Kinetics of globin synthesis in a standard cell-free translation system of the same composition and volume.

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