DIFFERENTIAL TRANSLATION OF RABBIT GLOBIN mRNA. EFFECT OF CONCENTRATIONS OF CREATINE PHOSPHATE, ATP AND GTP

Haruo SUZUKI and Yukimasa HAYASHI

Department of Genetics, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480–03, Japan

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1. Introduction

Rabbit reticulocyte lysate can synthesize the α and β globin chains in equal amounts [1]. There are many works concerning the effect of various factors on the synthetic ratio of α to β globin chains in rabbit reticulocyte lysate systems [1–6]. However, there is no publication that deals with the effect of concentrations of creatine phosphate(CP), ATP and GTP on the ratio of α to β chains synthesized.

The present paper reports the changes in the α and β chains synthesized after incubation of rabbit reticulocyte lysate with a different concentration of CP, ATP and GTP. The possibility that the α/β ratio is controlled by the concentration of ATP through the aminoacylation of tRNA was discussed.

2. Materials and methods

2.1. *Reticulocyte lysate*

Reticulocyte were obtained by the heart puncture of an anemic rabbit [7]. The following procedures were done at 0–4°C. The reticulocytes were washed three times with ice-cold saline solution [8] and lysed by the addition of two volumes of 1 mM magnesium acetate. The resulting suspension was centrifuged at 13 000 rev/min for 15 min in Hitachi RPR 20 rotor and the supernatant (lysate) was used for the experiment.

2.2. *Amino acid incorporation experiment*

Each 50 μl reaction mixture contained the followings: Tris–HCl (pH 7.4), 30 mM; KCl, 70 mM; magnesium acetate, 1.7 mM; creatine kinase, 2.7 μg; mercaptoethanol, 3 mM; L-amino acids without leucine, 0.1 mM; L-[^{14}C]leucine (U), 0.125 μCi (251 mCi/mmol); hemin, 30 μM. A given concentration of ATP, CP and GTP was used in the present experiment. Incubation was carried out at 37°C. The reaction was stopped by cooling in ice-cold water. The product analysis was done for the samples obtained after 80 min incubation at which the maximum incorporation was observed.

2.3. *Product analysis*

The 5 μl reaction mixture was taken into 0.1 ml ice-cold water. Aliquots of 0.2 ml of 9% TCA were added and heated for 10 min at 95°C. The precipitates were collected on a glass fibre disc and washed with 6% TCA and then 95% ethanol. After drying, the incorporation of [^{14}C]leucine was measured in 5 ml scintillation fluid (4 g PPO, 0.1 g POPOP in 1 liter toluene) by Beckman Scintillation Counter, model LS-233, or Aloka Liquid Scintillation Counter, model LSC-650.

The globin was prepared from the rest of the reaction mixture (45 μl) by the acid–acetone treatment [9]. The dried globin was dissolved in 1 ml distilled water. The solution was centrifuged 10 000 rev/min in the Hitachi rotor. The supernatant was used for the separation of α and β chains. The α and β chains were separated by CM-cellulose chromatography [10]. The unlabelled globin of 5 mg was added as the carrier to the ^{14}C-labelled globin solution. The globin mixture was made 0.2 M formic acid, 0.02 M pyridine and applied on Whatman CM-52 column (0.7 × 6.5 cm) which had been previously equilibrated with 0.2 M formic acid, 0.02 M pyridine. The column was eluted with a linear gradient consisting of 30 ml each of 0.2 M formic acid, 0.02 M pyridine and 2 M formic acid, 0.2 M pyridine. Fractions of 1.5 ml were collected

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to measure the absorbance change at 280 nm and the radioactivity. Aliquots of 20% TCA were added to each fraction to the final concentration of 6%. After an hour at room temperature, the precipitates were collected on a glass fibre disc and counted as described. The incorporation ratio of α to β chains was obtained by calculating the total counts in their corresponding peaks. The ratio was corrected for the recovery in the chromatography and for the content of leucine for each globin chain. The chromatographic yields were calculated by using the theoretical absorption coefficient of α and β chain at 280 nm calculated as described in [11]. The value of ϵ_M was calculated to be 9570 for α chain and 15 120 for β chain.

3. Results

3.1. *Effect of concentrations of CP and ATP on the ratio of α to β chains synthesized*

The rabbit reticulocyte lysate was incubated with a different concentration of CP or ATP (Materials and methods). The total volume of reaction mixture was 50 μl. The incorporation of [^{14}C]leucine into hot TCA insoluble fractions was measured for the 5 μl reaction mixture. The maximum incorporation was observed around 10 mM CP for different lysate preparations (fig.1). The very low incorporation was observed without added CP. Fig.2 shows the same kind of experiments for ATP. The concentration of ATP was different from one lysate to another to get a maximum ^{14}C-incorporation. Almost the maximum incorporation was observed without added ATP. These results indicate that the lysate used had a low endogenou CP and a sufficient ATP to synthesize globin chains.

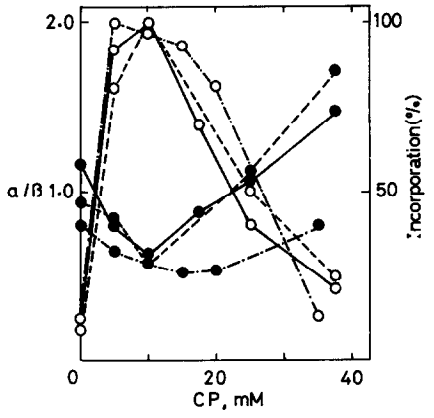

Fig.1. Effect of concentration of CP on the relative incorporation of [^{14}C]leucine into TCA-insoluble materials (○) and the α/β synthetic ratio (●). Concentrations of ATP was 0.35 mM for lysates 1 and 2, and 0 mM for lysate 3. The concentration of GTP was 0.75 mM. Other conditions were in Materials and methods. (– – –), Lysate 1; (——), Lysate 2; (–·–·) Lysate 3.

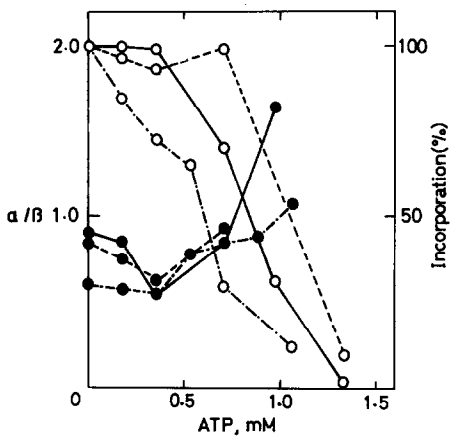

Fig.2. Effect of concentration of ATP on the relative incorporation of [^{14}C]leucine into TCA-insoluble materials (○) and on the α/β synthetic ratio (●). Concentrations of CP and GTP were 10 mM and 0.75 mM, respectively. Other conditions are in Materials and methods. The line indications are the same as in fig.1.

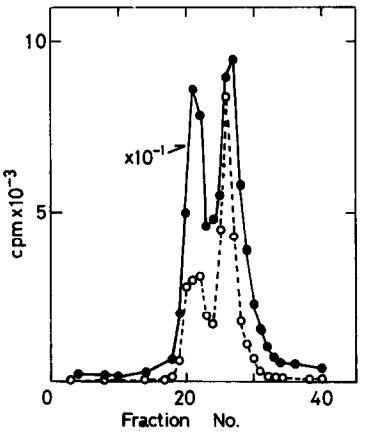

Fig.3. Separation of α and β globin chains. Five mg of unlabelled globin were added as carrier and separated as in the text. The first peak is α chain and the second is β. (●—●), 0 mM CP. (○– –○), 15 mM CP.

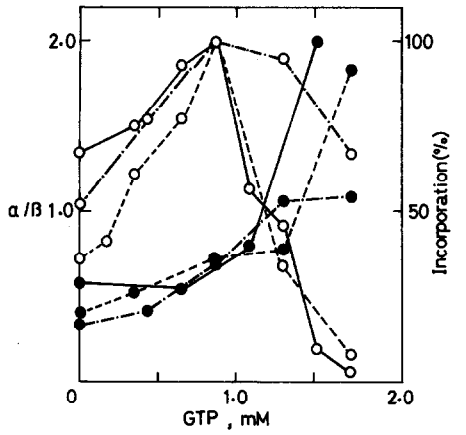

Fig.4. Effect of GTP concentration on the relative incorporation of [^{14}C]leucine (○) and on the α/β ratio (●). Concentration of CP was 10 mM and that of ATP was 0.35 mM for lysates 1 and 2, and 0 mM for lysate 3. Other conditions were in Materials and methods. The line indications are the same as in fig.1.

The rest of the reaction mixture (45 μl) was used to determine the synthetic ratio of α to β chains in each experiment. Fig.3 shows the typical chromatographic pattern of chain separation. The synthetic ratio of α to β chain was very low at 15 mM CP, but almost the same without added CP. Using this method, the α/β ratio was determined at different concentrations of CP (fig.1). The plots were different from one lysate to another, but had a similiar tendency. The α/β ratios changed significantly with an increasing concentration of CP. The ratio was lowest around the optimum concentration of CP to get the maximum ^{14}C-incorporation.

The α/β ratio was measured at a different concentration of ATP. The ratio was lowest around 0.4 mM ATP for different lysate preparations (fig.2).

3.2. *Effect of concentration of GTP on the ratio of α to β chains synthesized*

The incorporation of [^{14}C]leucine into TCA insoluble materials was measured at different concentrations of GTP as described in Materials and methods. 5 μl out of 50 μl reaction mixture were taken to count the radioactivity. The maximum incorporation was observed at 0.75 mM GTP (fig.4). The optimum GTP concentration did not change from one lysate to another. The rest of the reaction mixture was analyzed to determine the α/β ratio. The ratios increased with the increment of concentrations of GTP (fig.4).

4. Discussion

The present study showed that the rabbit reticulocyte lysate contained a sufficient concentration of ATP to synthesize globin chains, but not of CP or ATP. It was also shown that the synthetic ratios of α to β chains were dependent on the concentrations of CP, ATP and GTP.

The α/β ratio changed with increasing concentrations of CP and ATP, respectively. Since it is already known that CP is used to regenerate ATP by the action of creatine kinase, the effect of CP could be attributed to ATP. In the protein synthesis, ATP plays a key role in the aminoacylation of tRNA. As the optimum concentration of ATP in this reaction was different for different kinds of aminoacyl tRNA synthetases [12–14], the degree of aminoacylation of tRNA species can be controlled by the concentration of ATP. The role of tRNA in the regulation of hemoglobin synthesis was suggested [15] and supported by the observation of unequal globin chain synthesis in the absence of a tRNA fraction [2,3]. Considering these with the present results, it can be deduced that ATP controls the synthetic ratio of α to β globin chains by changing the degree of aminoacylation of some tRNA species.

The α/β ratios were increased with the increment of GTP concentration. Since GTP is involved in the various steps of protein synthesis [16], further experiments will be asked to determine which step is responsible for this unequal synthesis of globin chains.

The present observations are very important, since it is suggested that the synthetic ratio of α to β globin chains can be controlled by the fluctuations in the state of energy.

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