OSCILLATIONS IN THE RATE OF HEMOGLOBIN SYNTHESIS IN RABBIT RETICULOCYTES

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1. Introduction

Current concepts of protein synthesis are based on the model, developed by Jacob and Monod [1]. A mathematical treatment of this model by the techniques of statistical mechanics as outlined by Goodwin [2], predicts oscillatory phenomena in the protein synthesizing system. However, relatively few examples of such a phenomenon have been reported. Bernhardt et al. [3] described the oscillatory rate of synthesis of glutamate dehydrogenase in *Saccharomyces cerevisiae* after induction by NH_3^+ and König et al. [4] reported on similar experiments with *Hydrogenomonas* H16 on urease activity after induction by urea.

RNA polymerase was shown by Baker and Yanofsky [5] to react in the transcription of the trp operon of *E. coli* K_{12}. Oscillations outside the protein synthesizing system are also known. Examples of such phenomena have been given by Chance et al. [6] and Hommes [7] for the rate of anaerobic glycolysis in yeast cells, by Pressman [8] for uptake and release of K^+ by rat liver mitochondria and by Schäfer [9] for intermediates of the citric acid cycle in anaerobic rabbit heart mitochondria.

In this communication the results of short-time incubation experiments of rabbit reticulocytes with ^{14}C-labelled amino acids are reported. The rate of hemoglobin synthesis was found to proceed in an oscillatory fashion after synchronization of the cell population by cooling to 0°C and at a limited rate of hemoglobin synthesis. The same phenomenon was observed when human newborn reticulocytes were used, however, with a lower frequency.

2. Materials and methods

Rabbits were made anemic by daily subcutaneous injections of 0.25 ml per kg body weight of a 2% phenylhydrazine solution, for three days. Blood, rich in reticulocytes was obtained on the 4th day by cardiac puncture. The blood was collected in heparin and stored at 0°C until use. On average, the blood contained 40% reticulocytes. The hemoglobin concentration was on average 8%.

The blood was warmed up rapidly to 35°C by pouring through a spiralized glass tube surrounded by a jacket, filled with water of 50°C. Trial experiments indicated that the blood was warmed up to 35°C within 15 sec under these conditions. After addition of 10 μC ^{14}C-leucine per 10 ml of blood, the mixture was incubated in a Dubnoff-shaker at 35°C. Samples of 1.0 ml were taken from the beginning of the experiment at 10 sec intervals and immediately diluted in 20 ml of ice cold distilled water under vigorous stirring. This procedure lysed the cells immediately and stopped the protein synthesis by the drop in temperature. The lysate of each sample was centrifuged at 20.000 × *g* at 0°C for 30 min.

The oxyhemoglobin in the clear supernatant was converted to cyanmethemoglobin as described by Drabkin [10]. The mixture was then dialyzed against 0.01 M phosphate buffer, pH 6.8, with three changes of 15 L of dialyzing medium. The hemoglobin was purified and concentrated according to Huisman and Meyering [11], by means of a short carboxymethylcellulose column. The radioactivity of hemoglobin was assayed by liquid scintillation counting by dis-

solving approximately 150 γ hemoglobin in 5 ml of Bray's solution [12]. The short incubation times resulted in low specific activities. Therefore 10 samples of each hemolysate were counted. Human reticulocytes were obtained from newborn rhesus antagonism exchange babies. This hemolytic condition resulted in this case in 30 reticulocytes per 100 red cells.

3. Results

Fig. 1 shows the results of a representative experiment. After a lag of about 50 sec hemoglobin synthesis, as measured by the incorporation of labelled amino acids into the hemoglobin chains proceeds at an accelerated rate, but levels off after another 50 sec, then increases again.

The periodicity in hemoglobin synthesis is demonstrated in fig. 2, where the rate of hemogolobin synthesis is plotted as a function of time. A period of 50 sec is observed.

The effect of temperature is shown in fig. 3. Reticulocyte rich blood was incubated at 30°, 35° and 37°C. The overall rate of hemoglobin synthesis proved to be highly temperature sensitive. A rise in incubation temperature of 5°C (from 30° to 35°C) resulted in a twofold increase in the rate of hemoglobin synthesis.

The frequency of the oscillation in the rate of hemoglobin synthesis is also temperature dependent.

At a temperature of incubation of 30°C a period of 60 sec was observed, which decreased to 50 sec at 35°C and to 30 sec at 37°C. In order to investigate whether oscillations occur *in vivo* as well or that cooling of the reticulocyte rich blood to 0°C induces oscillation by synchronizing the cell population, the experiment reported in fig. 4 was carried out. One part of the blood of an anemic rabbit was cooled to 0°C and subsequently warmed up to 37°C and incubated with ^{14}C-leucine. Another part of the blood of the same animal was, immediately after blood collection, incubated with the labelled amino acid, at 37°, without precooling. This incubation yielded a straight

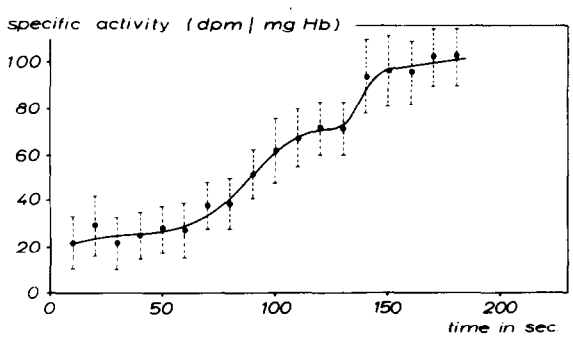

Fig. 1. Specific activity of rabbit hemoglobin as a function of time. The vertical bars give the standard deviation of the average of dpm of 10 independent samples.

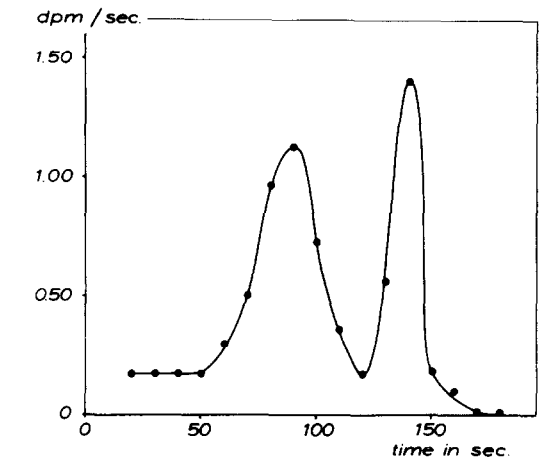

Fig. 2. Rate of hemoglobin synthesis as a function of time. These rates were calculated from the curve of fig. 1.

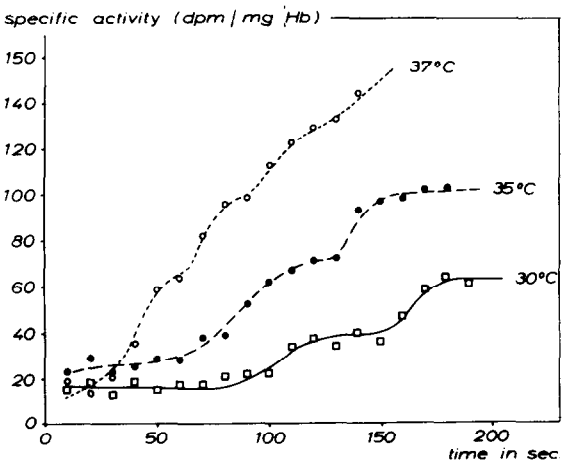

Fig. 3. Specific activities of rabbit hemoglobin as a function of time after incubation of reticulocyte rich blood at various temperatures. For reason of clarity the standard deviations were omitted. They were, however, of the same magnitude as shown in figs. 1 and 5.

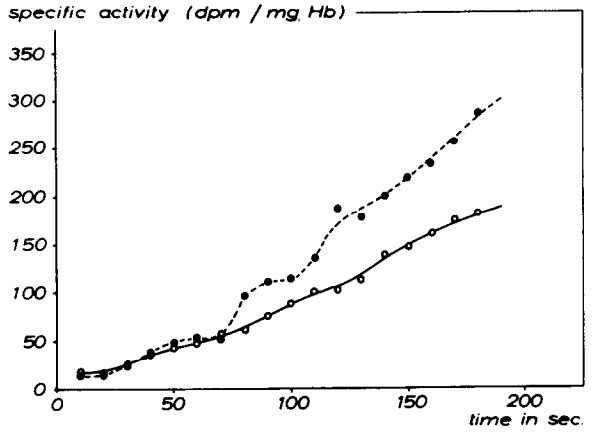

Fig. 4. Specific activities of rabbit hemoglobin as a function of time. ●—● blood precooled to 0°C and then rapidly warmed to 37°C. ○—○ blood from the donor incubated immediately with ^{14}C-leucine.

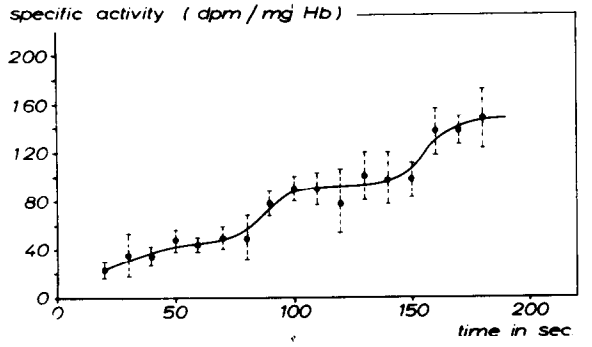

Fig. 5. Specific activities of human hemoglobin as a function of time. The blood for incubation was obtained from an exchange transfusion baby and contained 30% reticulocytes.

line when the specific activity of hemoglobin was plotted as a function of time (fig. 4). Oscillations in the rate of hemoglobin synthesis were, however, observed when the same reticulocyte rich blood was precooled to 0°C.

Oscillations were also observed when the human newborn reticulocyte rich blood was incubated with ^{14}C-leucine (fig. 5). The period was found to be 70 sec at 37°C. The phenomenon of oscillations seems therefore not to be restricted to one species, although the frequency may be species specific (cf. ref. [13]).

4. Discussion

Oscillations in a biochemical pathway offer an unique opportunity to study the control of that pathway. The mechanism of the feedback reactions, which are responsible for the oscillations in the rate of hemoglobin synthesis are unknown, although several feedback systems have been implicated in the hemoglobin synthesizing system of reticulocytes, f.i., control by the presence of heme [14] amd control by one of the chains of the hemoglobin molecule [15]. Oscillations in the rate of hemoglobin synthesis do apparently take place only after synchronization of the cells by cooling to 0°C. Blood incubated directly from the blood donor without precooling did not show the oscillations. Such a synchronizing effect is not uncommon in oscillating systems. The oscillating glycolysis in yeast can only be observed after synchronization by anaerobiosis [6,7]. It is therefore necessary to bring the reticulocyte rich blood as fast as possible to the temperature at which the experiment is carried out. A warming up time of 15 sec is apparently not too long to loose the synchronization.

The oscillations in the rate of hemoglobin synthesis are only observed when the activity with respect to incorporation of labelled amino acids of the reticulocyte rich blood is not too high (a reticulocyte count around 30 per 100 red cells). Highly active rabbit reticulocyte populations, obtained by stronger anemic stimuli (a reticulocyte count of 50 per 100 red cells, or more) do not show oscillations. This again is not uncommon in oscillating systems as the phenomenon of oscillation can only be observed at limited fluxes [7,16].

The frequency of oscillation is of the same order of magnitude as the time necessary for the synthesis of one hemoglobin molecule [17]. The possible relationship between this time and the frequency of oscillation is currently under investigation.

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