RELATIONSHIP BETWEEN THE DISCRIMINATING FACTOR AND THE GLOBIN MESSENGER RNA IN DIFFERENTIAL α AND β GLOBIN SYNTHESIS

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1. Introduction

In 1973 an initiation factor with discriminatory effect on the translation of different mRNAs was isolated from 0.5 M KCl extract of reticulocyte polysomes. This discriminating factor, in an ascites cell-extract, stimulates globin mRNA translation [1]. A similar factor has been found also in other cell-lines, including non erythroid ones such as ascites cells [2].

In an ascites cell-free system (S 30) the factor promotes a differential translational effect on different mRNAs. EMC virus RNA is translated with more efficiency than the Mengo virus RNA, α globin synthesis is higher stimulated than β globin synthesis. In any case the translation of β chain mRNA was also stimulated but to a much lower extent than the α chain mRNA [2]. So one single factor seems to stimulate to a different extent the translation of the two globin mRNAs. It was postulated that the rate of stimulation could be related to the ratio of globin mRNA and factor concentrations.

In this work we have shown that the synthesis of both globin chains is a function of the relative concentrations of the discriminating factor and the messenger RNAs.

2. Materials and methods

The discriminating factor was prepared from a 0.5 M KCl extract of reticulocyte polysomes according to Revel [1]. Some modifications were brought to the initial method: after 70% ammonium sulfate precipitation, the factor was eluted from DEAE-cellulose column by a stepwise KCl concentration gradient between 180 and 220 mM KCl and the 'DEAE factor' was purified on a phosphocellulose column. It was eluted with 300 mM KCl (P 300 factor).

At each purification step the factor was characterized by its preferential stimulation on α mRNA translation in an ascites cell-free system.

The ascites cell-free system and conditions of incubation have previously been described [2]. The α and β globins were separated on CMC column according to Dintzis [3].

3. Results

All the experiments reported here were performed with the factor purified on the phosphocellulose column (P 300 factor). The discriminating factor isolated from reticulocyte polysomes presents three bands in SDS–polyacrylamide gel electrophoresis (fig.1). The major band, mol. wt 60 000, was similar to the factor described by Nudel et al. [1]. Two other bands, mol. wt 90 000 and 32 000, were also observed. When added to an ascites cell-free extract the factor promotes a differential effect on α and β globin mRNA translation. A stimulation is observed on α and β globin synthesis, but the rate of stimulation on α globin is much higher (table 1).

Two series of experiments were performed in which the concentrations of either the discriminating factor or globin mRNA were increased.

Increasing amounts of P 300 factor (from 0.6–15

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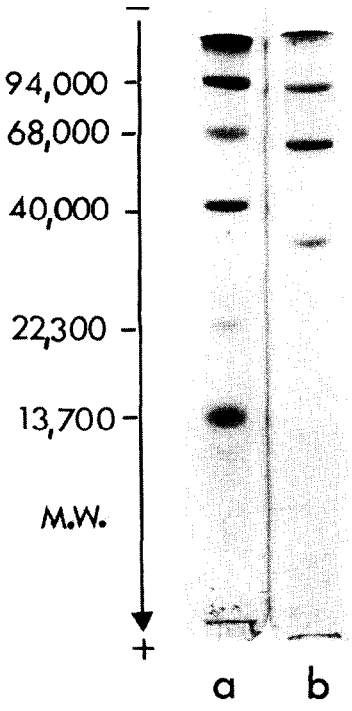

Fig.1. Electrophoresis on SDS–polyacrylamide gel (10%) of (a) control proteins, phosphorylase a 94 000; bovine serum albumin, 68 000; creatine-kinase, 40 000; trypsin, 22 300; and ribonuclease, 13 700. (b) Discriminating factor P 300

μg/ml) were incubated in ascites S 30 in presence of a constant amount of globin mRNA (2.5 μg/ml). In this case the ascites system is not limiting since the system is not saturated even with 40 μg of globin mRNA/ml (fig.2b). It contains a small amount of discriminating factor [2]. Addition of different amounts of this factor promotes a differential response on α and β mRNA translation (fig.2a). With small amounts of the factor β globin synthesis remains higher than α globin. In the presence of higher amounts of discriminating factor the α globin synthesis is more increased than the β globin synthesis, and the α/β ratio increases from 0.6–1.05. These results are also expressed in percentage of stimulation (fig.3a). For each concentration of the factor the percentage of stimulation of α globin is higher than β globin. Increasing the factor concentration has more effect on the rate of α chain stimulation than of β chain. When the ratio of discriminating factor to globin mRNA concentrations increases, α globin synthesis is more stimulated than β globin synthesis.

Increasing amounts of globin mRNA were incubated in an ascites S 30 in absence and in presence of discriminating factor (fig.2b). In the experiments, 15 μg/ml of factor were added, corresponding to the highest concentration of factor added in the previous experiments.

For each concentration of globin mRNA the α and β globin syntheses in presence of the factor are higher than in its absence, and α globin synthesis is always superior to β globin synthesis. With small amounts of globin mRNA, the α/β ratio is above 1 as in the previous experiment, and much higher than in the absence of discriminating factor, but it decreases with increasing amounts of globin mRNA. In the absence of the factor, the α/β ratio increases regularly (fig.2b). The results are also expressed in percentage of stimulation (fig.3b). With very small amounts of globin mRNA the percentage of stimulation increases up to a maximum where α globin is much more stimulated and then decreases more than β globin stimulation, and finally when globin mRNA concentration is high, α and β globin mRNAs seem to become equally translated. When the ratio (factor P 300)/(globin mRNA) increases, the stimulation of α globin synthesis increases till a maximum, then decreases, and finally the β mRNA becomes better stimulated.

Table 1
Differential stimulation of the discriminating factor on α and β globin syntheses in ascites cell-free system in presence of 3 μg/ml of globin mRNA

P 300 Factor	[^{3}H]Leucine incorporation		Ratio α/β	% Stimulation	
	α Globin	β Globin		α	β
–	30 025	39 580	0.76		
+	96 135	70 420	1.37	220	78

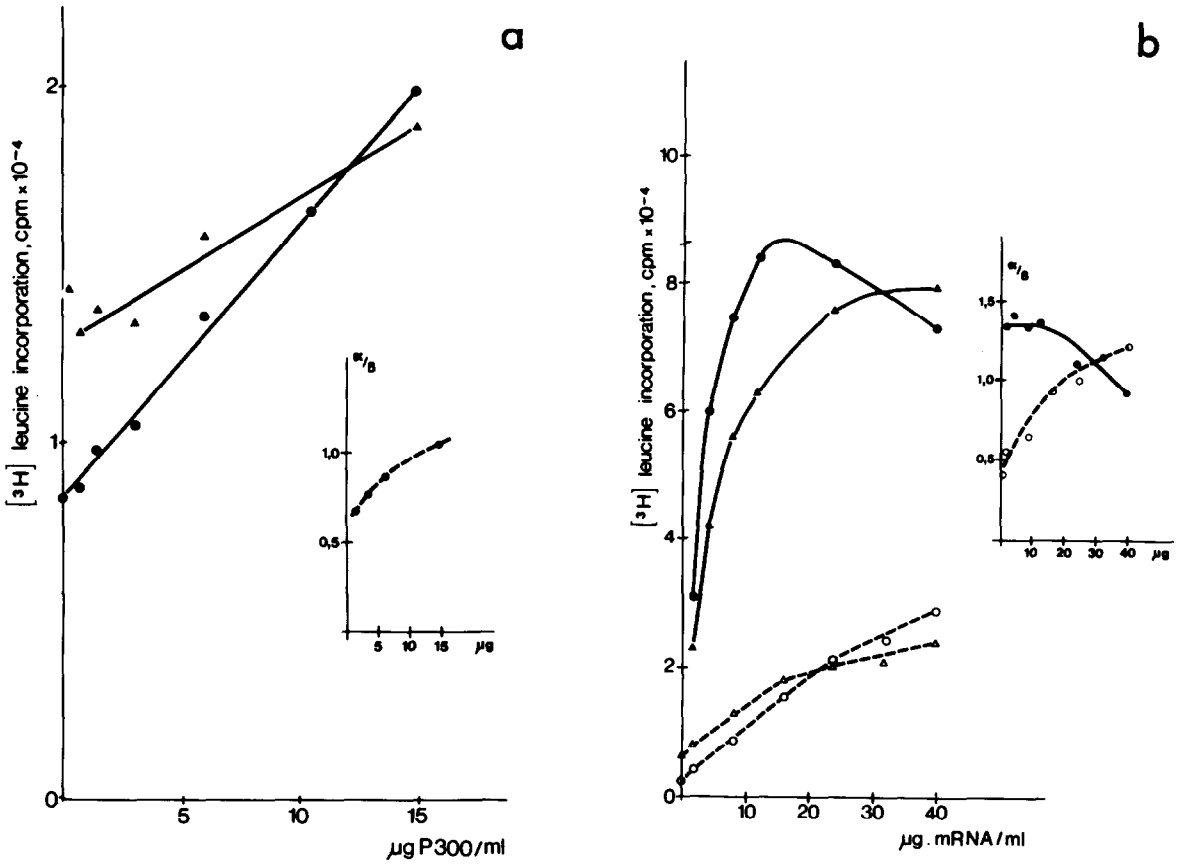

Fig.2. Effect of discriminating factor (P 300) on α and β globins mRNA translation in ascites S 30 system. (a) Increasing amounts of discriminating factor P 300 were incubated in the presence of 2.5 µg/ml rabbit globin mRNA. Rabbit hemoglobin was added and globin chains isolated on CM_{52} column. (●——●)[^{3}H]leucine incorporation in α globin, and (▲——▲)[^{3}H]leucine incorporation in β globin. The insert shows the α/β ratio. (b) 15 µg of discriminating factor P 300 were incubated in the presence of increasing amounts of rabbit globin mRNA and analyzed as in fig.2a. Controls without exogenous discriminating factor were performed. [^{3}H]-leucine incorporation in the presence of P 300 factor, α chain (●——●), β chain (▲——▲), and in the absence of P 300 factor α chain (○- - -○), β chain (△- - -△). The insert shows the α/β ratio in the presence (○——○) and in the absence (○- - -○) of P 300 factor.

4. Discussion

A factor which seems to act in the translation of both α and β mRNA was isolated. The data show that α and β globin mRNAs act in competition for the same factor with respect to their translation. The concentration of the factor with regard to the concentration of the mRNAs (and presumably other constituents of protein synthesis) seems to play an important role for determining the rate of protein synthesis.

In these experiments only the concentrations of globin mRNA and of the factor have changed, the polysomes and other constituents of protein synthesis remain unchanged. We observed that either α or β globin mRNA is more translated according to the ratio of the concentration of discriminating factor to globin mRNA.

When there is a strong competition between the two different globin mRNAs towards the factor, that is when the concentration of the factor is very small as compared to the globin mRNA concentration, the β globin mRNA is more translated. On the contrary, when the concentration of the factor is high as compared to mRNA concentration, the competition is weak and α globin mRNA becomes more translated.

This factor present in different cells has a differen-

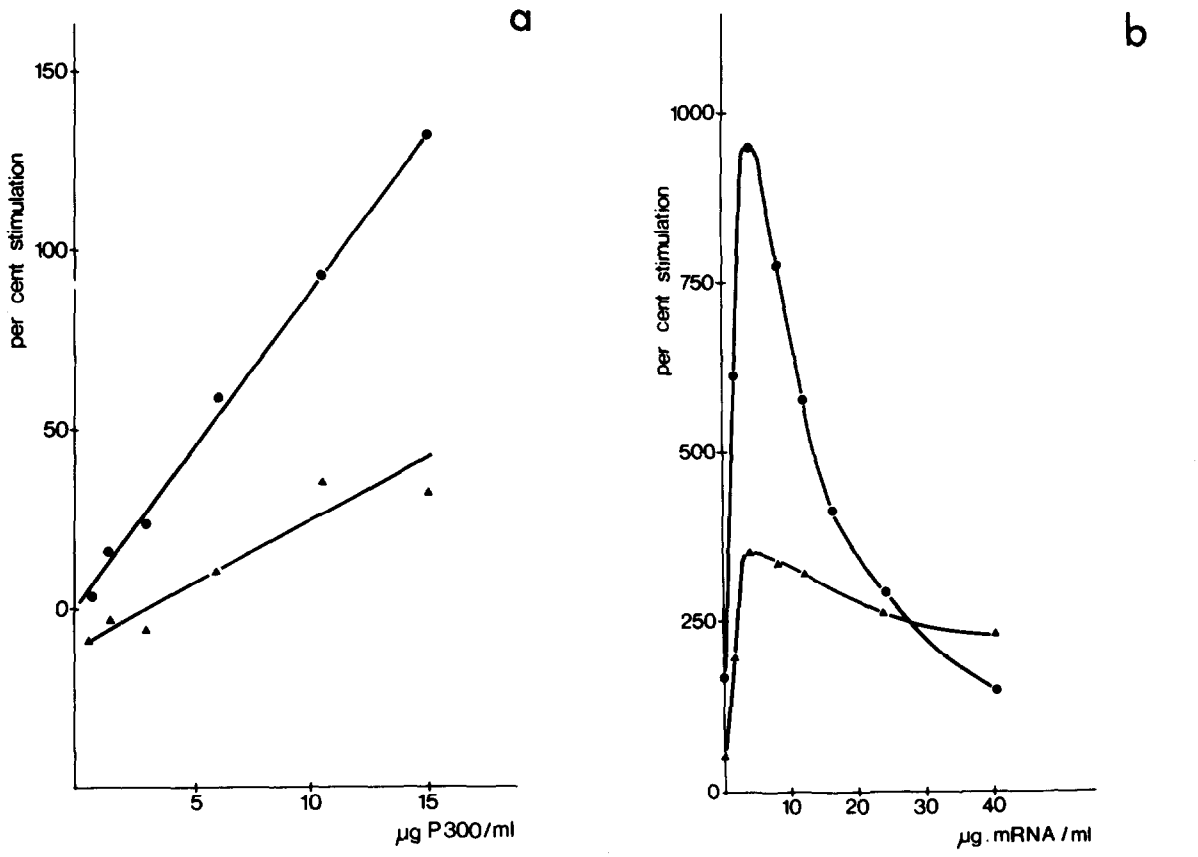

Fig.3. Stimulation of α and β globin synthesis by discriminating factor P 300. (●——●) % Stimulation of α chain, and (▲——▲) % stimulation of β chain. (a) Incubations were performed in the presence of increasing amounts of discriminating factor P 300 as in fig.2a. To determine the percentages of stimulation, the 100% incorporation in each chain in the absence of discriminating factor, were substracted. (b) Incubations were done in the presence of increasing amounts of rabbit globin mRNA as in fig.2b. Percentages of stimulation were determined by substracting the 100% incorporation in each chain in the absence of discriminating factor.

tial translational effect on various messengers and could be a universal factor, the activity of which is to modulate the affinity of ribosomes towards the messengers. At the present time, the discriminating factor was not found to be identical to any initiation factor described by Staehelin's group [4]. Three of the initiation factors IF_{e3}, IF_{e4}, IF_{e6} play an important role for messenger binding on ribosome small units. IF_{e4} was described as a single polypeptide chain of approximately 50 000 daltons, IF_{e6} seems to have one subunit of 80 000 daltons, and at least another subunit of lower molecular weight. IF_{e3} is a complex structure of 9–10 subunits not present in stoichiometric amounts [5]. As the structures of the different initiation factors, especially IF_{e6} and IF_{e3}, are not yet well-defined, it is not possible to exclude that P 300 factor may be constituted by subunits of one of these factors. The other possibility could be that the discriminating factor has no relationship with any of the initiation factors already described, and is responsible for messenger discrimination through its different affinity for various mRNAs.

Acknowledgements

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