POLYRIBOSOME FORMATION FROM HAEMOGLOBIN RIBONUCLEOPROTEIN IN VITRO

I.B. PRAGNELL*,**, G. MARBAIX‡, H R.V. ARNSTEIN* and B. LEBLEU‡

‡*Laboratory of Biological Chemistry, Faculty of Sciences, University of Brussels, Belgium*

and

**Department of Biochemistry, University of London King's College, Strand, London, W.C.2, England*

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1. Introduction

When reticulocyte polyribosomes are treated with EDTA, an RPN particle containing 9 S RNA is released [1–4]. This species of RNA is the mRNA for globin [5].

One of the first steps in the initiation of protein synthesis involves the binding of mRNA to the small subparticle [6]. This binding has been demonstrated both on native [7] and derived subparticles [8]. In order to demonstrate the subsequent steps in initiation of protein synthesis it is necessary to show formation of an initiation complex from both subparticles with mRNA and subsequent build up of disomes and larger polyribosomes as a result of repeated initiation on the added mRNA.

We have found that this is possible, using a preparation of ribosomes and enzymes obtained from a rabbit reticulocyte lysate by pH 5.4 precipitation, which is capable of efficient initiation [9].

Furthermore, aurine tricarboxylic acid (ATA) and cycloheximide are found to inhibit different steps in this process.

2. Methods

Rabbit reticulocyte polysomes were obtained as previously described [10]. ^{32}P-labelled mRNP was isolated according to the method of Huez et al. [2] with the exception that the gradients were prepared in 10 mM tris pH 7.5 (20°) rather than phosphate buffer.

The pH 5.4 precipitate of polysomes, subparticles and factor was prepared from the post-mitochondrial supernatant of a reticulocyte lysate according to the method of Arnstein et al. [17] as modified by Fuhr et al. [9].

Incubation mixtures contained (per ml) 20 μmoles tris HCl pH 7.5 (20°), 50 μmoles KCl, 5 μmoles $MgCl_2$ 1 μmole ATP, 0.2 μmole GTP, 15 μmoles creatine phosphate, 100 I.U. creatine phosphokinase, 0.125 μmoles of each of 20 L-amino acids, 5 mg (50 A_{260} units) of pH 5.4 fraction, and 20 μg ^{32}P mRNP (approximately 2000 cpm). Where indicated, ATA and cycloheximide were added to a final concentration of 0.2 mM each case.

The reaction mixtures were incubated at 37° for 7 min, rapidly cooled, layered onto sucrose density gradients (15–30% in incubation buffer) and centrifuged for three hours at 26,000 rpm and 5° in the SW27 rotor of a Spinco ultracentrifuge.

Gradient fractionation was performed as previously described [7]. The direction of sedimentation is from left to right in all figures. The radioactivity of each fraction of the gradient was measured by scintillation counting using a toluene–triton mixture.

3. Results

When ^{32}P-labelled haemoglobin mRNP is incubated

** To whom all requests for reprints should be addressed.

with the pH 5.4 fraction as indicated in the Methods section, ^{32}P mRNA appears in the 80 S region (fig. 1, fractions 12–18) and also in the heavier regions of the profile corresponding to disomes and trisomes (fig. 1, fractions 5–11). There is relatively little labelling in the heavier polyribosomes, but the specific radioactivity of the polyribosomes is considerably higher than that of the 80 S fraction.

If, however, the above procedure is repeated at 0° in otherwise identical conditions formation of the 80 S initiation complex is very much reduced (fig. 2). It is interesting to note here that the binding of the mRNA to the 40 S subparticle is not prevented. It appears, therefore, that the binding of mRNA does not require incubation whereas subsequent steps do.

When the pH 5.4 system is incubated in the presence of ATA and ^{32}P mRNP under the same conditions, the binding of mRNA to the small subparticle is prevented as well as the formation of the 80 S initiation complex (fig. 3). ATA used at this concentration has been shown to inhibit both initiation of endogenous protein synthesis in reticulocyte lysates [12, 13] and initiation of translation of added synthetic mRNA [3] without affecting elongation of polypeptide chains. This results shows that the formation of this initiation

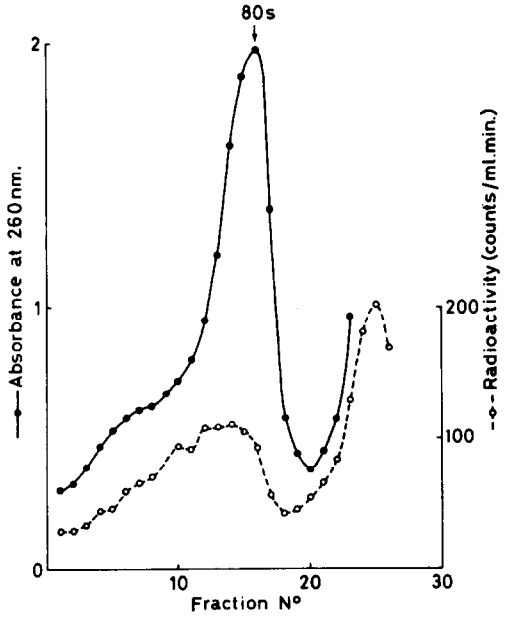

Fig. 1. Incubation of pH 5.4 fraction with ^{32}P mRNP (conditions as described in Methods)

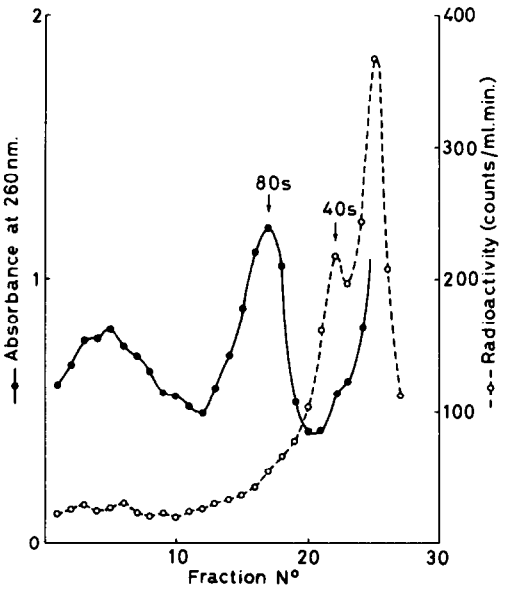

Fig. 2. Addition of ^{32}P mRNP to pH 5.4 fraction at 0° (in otherwise identical conditions to fig. 1).

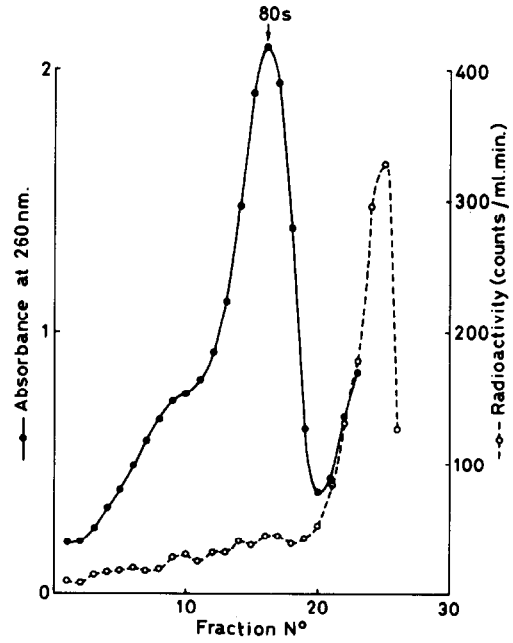

Fig. 3. Incubation of pH 5.4 fraction with ^{32}P mRNP in the presence of aurine tricarboxylic acid (in otherwise identical conditions to fig. 1).

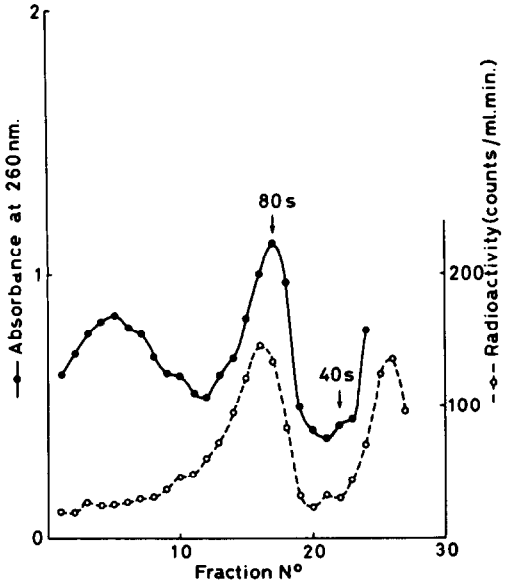

Fig. 4. Incubation of pH 5.4 fraction with ^{32}P mRNP in the presence of cycloheximide.

complex is not artefactual since it requires the previous binding of the mRNA to the 40 S subparticle.

When cycloheximide is added to the mixture and incubated as in fig. 1, incorporation of mRNA into polyribosomes is inhibited (fig. 4); it appears however that formation of the 80 S initiation complex is not prevented, a result which agrees with the accepted mechanism of cycloheximide inhibition [14]. It is also worth noting that the inhibition of the formation of the 80 S initiation complex which occurs in the absence of incubation results in the accumulation of the 40 S initiation complex (fig. 2). Furthermore, inhibition of elongation by cycloheximide induces the accumulation of the 80 S initiation complex (fig. 4).

4. Discussion

The pH 5.4 system used in these experiments is clearly capable of forming an initiation complex in the 80 S region and to carry out subsequent steps in translation such that disomes and trisomes incorporating the added mRNA are formed. However, this system may not be as active over an extended period of time as the unfractionated lysate [5], but it was chosen for practical reasons since a very concentrated ribosomes fraction can be prepared in this way.

The results outlined above are consistent with a model of initiation in which the first step is the binding of mRNA to the small subparticle [6, 11, 15]. This step can be inhibited by ATA but is not cold sensitive. However, the subsequent process of formation of an 80 S initiation complex is cold sensitive [16] and is not prevented by addition of cycloheximide.

Our results are consistent with current ideas concerning initiation of protein synthesis in eukaryotic systems but they do not indicate whether the proteins attached to the mRNA are incorporated into the initiation complex or the polyribosomes. Lebleu et al. [18] have been able to demonstrate that the proteins bound to the haemoglobin mRNA in the mRNP are required for the binding of mRNA to deoxycholate-washed native 40 S subparticles. It would be interesting now to determine the date of these proteins after the initial binding of the mRNA to the small subparticle. This currently under investigation.

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