LOCALIZATION OF RAT LIVER RIBOSOMAL PROTEIN S2 AND ITS INVOLVEMENT IN INITIATION FACTOR eIF-2 BINDING TO THE 40 S RIBOSOMAL SUBUNIT

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1. Introduction

The elucidation of the localization and arrangement of RNA and proteins and their function in active sites of ribosomes, and thus the understanding of the molecular events which occur during protein synthesis, need the application of different experimental techniques [1,2].

In the following the location of ribosomal protein S2 in the small subunit of rat liver ribosomes using immunoelectron microscopy is shown. Furthermore, from inhibition experiments with antibodies against protein S2 it is demonstrated that S2 is involved in binding of the eukaryotic initiation factor IF-2 to the small ribosomal subunit.

From the results it can be concluded that protein S2 is located at or near the ribosomal P-site and that the P-site is organized at least partially in the so-called head region of the small ribosomal subunit.

2. Material and methods

Small ribosomal subunits, initiation factor eIF-2 and [^{3}H]Met-tRNA$_f$ from rat liver were prepared as in [3,4]. Protein S2 was isolated and purified as in [3]. Antibodies against homogeneous S2, checked by two-dimensional polyacrylamide gel electrophoresis, were raised in rabbits [3] and in chicken (unpublished). The antisera obtained did not show any cross-reaction with other single proteins of the 40 S subunit as tested by passive haemagglutination [3].

The experiments for immunoelectron microscopy were performed with the IgG fraction of the antisera. 40 S–IgG–40 S complexes were prepared by incubation of IgG with an excess of ribosomal subunits in buffer A (50 mM Tris, 200 mM KCl, 2 mM $MgCl_2$, pH 7.7) for 5–30 min at 0°C. The complexes were isolated by sucrose gradient centrifugation (15–30% sucrose in buffer A; Spinco rotor SW 40, 22 000 rev/min for 16 h at 2°C) [5].

For electron microscopy, subunits as well as the subunit–IgG complexes were used directly from the sucrose gradients. Negative staining was carried out with uranyl acetate as in [6]. Micrographs were taken with e JEM 100B at a magnification of 60 000 × at 80 kV.

The experiments about the inhibition of eIF-2 binding to 40 S ribosomal subunits by anti-S2 antibodies, purified by affinity chromatography, were performed as follows: Ternary initiation complexes [eIF-2 · GMPPCP · [^{3}H]Met-tRNA$_f$] were preformed in 100 μl samples containing 25 mM Tris, pH 7.5, 80 mM KCl, 2 mM 2-mercaptoethanol, 0.16 mM GMPPCP, about 10 pmol [^{3}H]Met-tRNA$_f$ and 200 μg eIF-2 by incubation for 5 min at 37°C. Simultaneously, 14 μg 40 S subunits were preincubated with different amounts of antibodies in 75 μl buffer (20 mM Tris, 100 mM KCl, 1.5 mM $MgCl_2$, pH 7.5) for 30 min at 0°C. The ternary complex and the 40 S–antibody complexes were mixed, $MgCl_2$ was added to a final concentration of 3.5 mM, and the mixture was incubated for 4 min at 37°C. The binding of [^{3}H]Met-tRNA$_f$ to the 40 S subunits was analyzed by sucrose gradient centrifugation (10–30% sucrose in 20 mM Tris, 80 mM KCl, 5 mM $MgCl_2$, 2 mM β-mercapto-

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ethanol, pH 7.5; Spinco rotor SW 60, 60 000 rev/min for 80 min at 2°C) [4]. Due to the high hydrostatic pressure during centrifugation under these conditions 40 S–IgG–40 S complexes are not stable and therefore are not demonstrable in the gradient.

The designation of ribosomal protein S2 follows the nomenclature in [8].

3. Results and discussion

3.1. *Localization of ribosomal protein S2 in 40 S subunits by immunoelectron microscopy*

Figure 1 shows selected electron micrographs of complexes of 40 S subunits with anti-S2 antibodies obtained by incubation of subunits with antibodies and subsequent sucrose gradient centrifugation. The

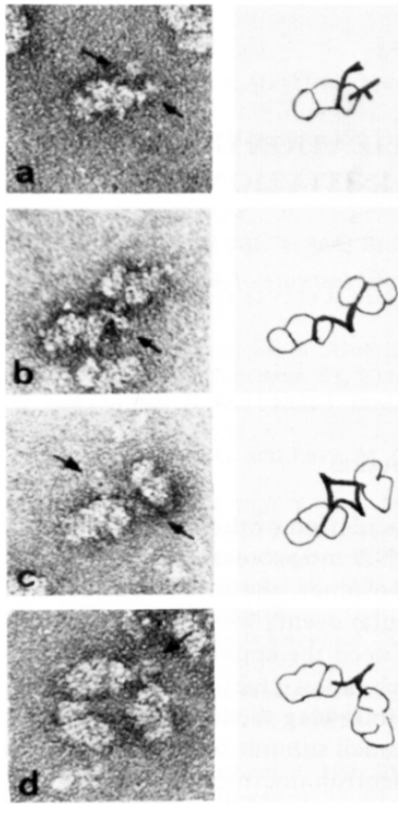

Fig.1. Electron migrographs and interpretative drawings of 40 S ribosomal subunits reacted with chicken anti-S2 antibodies. (a) 40 S subunit to which two antibodies are bound. (b) 40 S subunits joined by one antibody at the contrast line between the head and the body of the subunit. (c) 40 S subunits joined by two antibodies in the same region as shown in (b). (d) 40 S subunit joined by one antibody with the attachment point in the middle of the head of the subunit. Antibodies are marked by arrows. Magnification 300 000 fold.

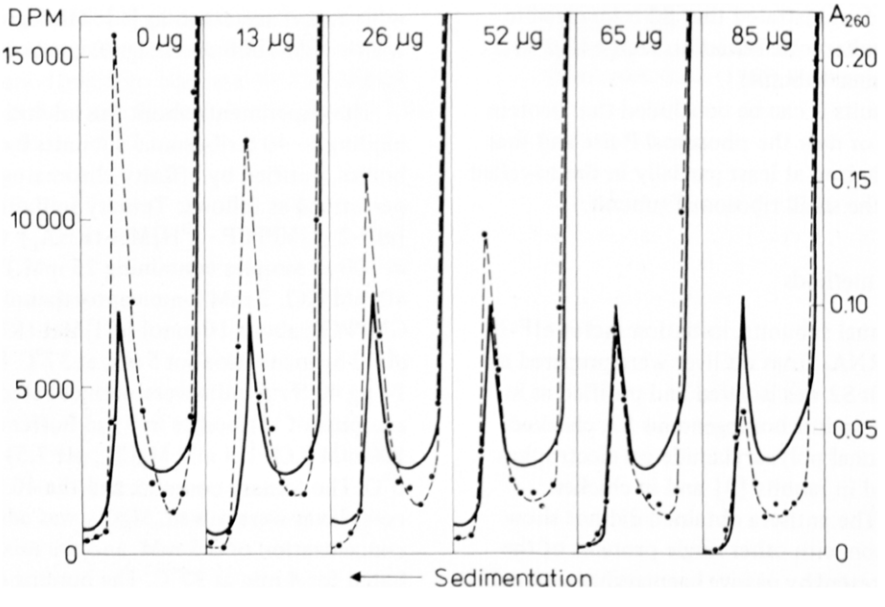

Fig.2. Sucrose gradient analysis of the quaternary complex [eIF-2 · GMPPCP · [^{3}H]Met-tRNA$_f$ · 40 S] formed without or in presence of different amounts of anti-S2 antibodies. (• - - - •) ^{3}H radioactivity (24 000 dpm correspond to 1 pmol Met-tRNA$_f$); (——) A_{260}. The amount of anti-S2 antibodies, present in the assays, is given at the top of each panel.

micrographs point to three different attachment sites of anti-S2 antibodies in the head region of the small subunit. Micrograph (a) represents a 40 S subunit with two antibodies bound at two different positions, one in the middel of the head and the other at the contrast line dividing the head and the body of the subunit. Micrograph (b) shows two subunits joined by one in the middle of the head and the other at the both subunits at the contrast line (cf. micrograph (a)) in corresponding positions. A third attachment site for anti-S2 antibodies is demonstrated in micrograph (c), where two subunits are joined by two antibodies closely neighboured in the neck region of the subunit. This finding indicates that two antigenic determinants of protein S2 are in very close neighbourhood on the surface of 40 S subunits. Micrograph (d) shows a dimeric complex with an antibody bound to a region in the middle of the head (cf. micrograph (a)).

3.2. *Inhibition of eIF-2 binding to 40 S ribosomal subunits by anti-S2 antibodies*

Preincubation of 40 S subunits in the presence of antibodies against ribosomal protein S2 inhibits their ability to bind the ternary initiation complex [eIF-2 · GMPPCP · [^{3}H]Met-tRNA$_f$] (fig.2). The degree of inhibition depends on the amount of antibodies (fig.3).

No inhibition was observed when non-immune IgG or antibodies against serum albumin of the rat were used instead of anti-S2 antibodies (fig.3).

This clearly demonstrates that ribosomal protein S2 is involved in binding of the ternary complex [eIF-2 · GTP · Met-tRNA$_f$] to 40 S ribosomal subunits during initiation complex formation. Therefore, it can be assumed that this protein is located at or near the P-site region of the small ribosomal subunit, because the initiator tRNA is bound to the P-site of the ribosome as generally accepted [7]. Furthermore, since protein S2 is located mainly in the head region, the P-site should be organized at least partially in this region of the small subunit.

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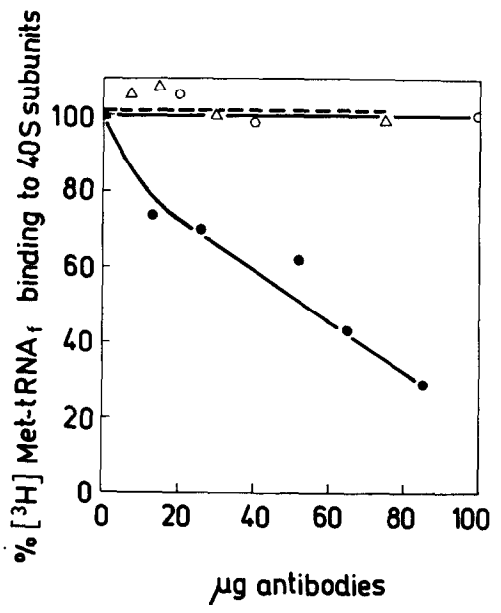

Fig.3. Dependence of the quaternary complex formation on the amount of antibodies. Each point reflects the [^{3}H]Met-tRNA$_f$ binding to the 40 S subunit calculated from the summarized radioactivity of the 40 S fractions of one sucrose gradient analysis in relation to the control experiment without antibodies. 100% corresponds to about 1.5 pmol [^{3}H]Met-tRNA$_f$ bound to 14 µg 40 S subunits. (o——o) Nonimmune IgG; (△- - -△) antibodies against rat serum albumin; (•——•) anti-S2 antibodies.

References

[1] Pongs, O., Nierhaus, K. H., Erdmann, V. A. and Wittmann, H. G. (1974) FEBS Lett. 40, 28–37.
[2] Wittmann, H. G. (1976) Eur. J. Biochem. 61, 1–13.
[3] Noll, F., Theise, H. and Bielka, H. (1974) Acta Biol. Med. Germ. 33, 547–553.
[4] Bommer, U.-A., Henske, A. and Bielka, H. (1978) Acta Biol. Med. Germ. in press.
[5] Lutsch, G., Noll, F., Theise, H. and Bielka, H. (1977) Acta Biol. Med. Germ. 36, 287–290.
[6] Valentine, R. C., Shapiro, B. M. and Stadtman, E. R. (1968) Biochemistry 7, 2143–2152.
[7] Weissbach, H. and Ochoa, S. (1976) Ann. Rev. Biochem. 45, 191–216.
[8] Welfle, H., Stahl, J. and Bielka, H. (1972) FEBS Lett. 26, 228–232.