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Identification of proteins of the 40 S ribosomal subunit involved in interaction with initiation factor eIF-2 in the quaternary initiation complex by means of monospecific antibodies

Ulrich-Axel Bommer, Joachim Stahl, Annemarie Henske, Gudrun Lutsch* and Heinz Bielka

Central Institute of Molecular Biology, Academy of Sciences of the GDR, Department of Cell Physiology and **Department of Electron Microscopy, Robert-Roessle-Strasse 10, DDR-1115 Berlin-Buch, GDR*

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Monospecific polyclonal antibodies against seven proteins of the 40 S subunit of rat liver ribosomes were used to identify ribosomal proteins involved in interaction with initiation factor eIF-2 in the quaternary initiation complex [eIF-2 × GMPPCP × [^{3}H]Met-tRNA$_f$ × 40 S ribosomal subunit]. Dimeric immune complexes of 40 S subunits mediated by antibodies against ribosomal proteins S3a, S13/16, S19 and S24 were found to be unable to bind the ternary initiation complex [eIF-2 × GMPPCP × [^{3}H]Met-tRNA$_f$]. In contrast, 40 S dimers mediated by antibodies against proteins S2, S3 and S17 were found to bind the ternary complex. Therefore, from the ribosomal proteins tested, only proteins S3a, S13/16, S19 and S24 are concluded to be involved in eIF-2 binding to the 40 S subunit.

40 S ribosomal subunit; eIF-2 binding; Inhibitory antibody; Ribosomal protein

1. INTRODUCTION

The interaction of proteins of the 40 S ribosomal subunit with initiation factor eIF-2 in the quaternary initiation complex [eIF-2 × GTP × Met-tRNA$_f$ × 40 S subunit] has been investigated by crosslinking experiments [1,2] as well as by application of antibodies against ribosomal proteins [3,4] (review [5]). Most of the proteins shown to be involved in eIF-2 binding have been located at the surface of the 40 S ribosomal subunit by immunoelectron microscopy [6–8]. However, the location of some of these proteins deviates considerably from the location of eIF-2 within quaternary initiation complexes observed recently by the same method [9]. Therefore, we reinvestigated the involvement of ribosomal proteins in the interaction between eIF-2 and the 40 S ribosomal subunit by using polyclonal antibodies against ribosomal proteins, which were shown to be monospecific by immunoblotting and were able to form dimeric immune complexes with 40 S ribosomal subunits. The results demonstrate that ribosomal proteins S3a, S13/16, S19 and S24 are involved in eIF-2 binding, which is in agreement with the observed location of these proteins [7,8] at or near the eIF-2 binding area [9] of the 40 S subunit.

Correspondence address: U.-A. Bommer, Central Institute of Molecular Biology, Academy of Sciences of the GDR, Department of Cell Physiology, Robert-Roessle-Strasse 10, DDR-1115 Berlin-Buch, GDR

2. MATERIALS AND METHODS

2.1. *Materials*

Guanylyl (β,γ-methylene)diphosphonate (GMPPCP) and total tRNA from yeast was purchased from Boehringer (Mannheim), and [^{3}H]methionine (555 GBq per mmol) was obtained from Amersham (England).

2.2. *Preparations*

The preparation of 40 S ribosomal subunits from rat liver [10] and of [^{3}H]Met-tRNA$_f$ from total yeast tRNA [11] was described earlier. Initiation factor eIF-2 from rat liver was prepared according to Nygård et al. [12] with some modifications of the procedure described recently [9]. Ribosomal proteins from 40 S subunits were prepared by a procedure

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including chromatography on CM-cellulose and reversed-phase liquid chromatography [13]. Immunization of rabbits as well as the purification of antibodies against ribosomal proteins and the test for monospecificity by immunoblotting will be reported separately [8].

2.3. *Assay*

Inhibition of eIF-2 binding to 40 S ribosomal subunits by antibodies against 40 S proteins was tested by the following procedure: 40 S ribosomal subunit × antibody complexes were preformed in reaction mixtures of a total volume of 200 μl containing 20 mM Tris-HCl, pH 7.5, 200 mM KCl, 2 mM $MgCl_2$, 25 μg 40 S subunits and amounts of purified antibodies against 40 S proteins as given in the legend to fig.1. The relatively high salt concentration was chosen in order to avoid self-dimerisation of 40 S subunits. The reaction mixtures were incubated for 20 min at 37°C and 60 min at 0°C to allow immune complex formation. Prior to the addition of the ternary initiation complex, the samples were diluted with an equal volume of 20 mM Tris-HCl, pH 7.5, in order to adjust the KCl concentration to 100 mM.

Ternary initiation complexes [eIF-2 × GMPPCP × Met-$tRNA_f$] were formed in reaction mixtures of a total volume of 100 μl containing 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 2 mM 2-mercaptoethanol, 0.5 mM GMPPCP, 16 pmol [^{3}H]Met-$tRNA_f$ and 20 μg purified eIF-2 each, by incubation for 10 min at 37°C. Subsequently, the ternary complexes were mixed with the preformed 40 S immune complexes, the $MgCl_2$ concentration was adjusted to 7 mM, and the mixture was incubated for 5 min at 37°C in order to allow formation of quaternary initiation complexes.

The incubation mixtures were then analyzed by centrifugation in 10–30% sucrose gradients containing 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$ in a Spinco rotor SW 40 for 16 h at 23000 rpm at 2°C. The gradients were monitored at 260 nm in a Gilford 2400 spectrophotometer, fractionated and counted for radioactivity in a scintillation mixture containing methanol and toluene scintillator. Under these conditions, 16600 dpm per pmol [^{3}H]methionine were counted.

3. RESULTS AND DISCUSSION

The participation of ribosomal proteins S2, S3, S3a, S13/16, S17, S19 and S24 in the interaction of the 40 S ribosomal subunit with initiation factor eIF-2 was investigated by testing the inhibitory effect of monospecific polyclonal antibodies against these proteins on the binding of ternary initiation complexes [eIF-2 × GMPPCP × [^{3}H]Met-$tRNA_f$] to 40 S subunits. Details of the experimental procedure are given in section 2.

From the results presented in fig.1 it is obvious that all applied antibody preparations are able to form dimeric immune complexes, however, to varying extents. The dimeric 40 S complexes mediated by the antibodies used differ considerably in their ability to bind [^{3}H]Met-$tRNA_f$, which is a direct reflection of the extent of eIF-2 binding. In contrast, the remaining monomeric 40 S particles are in each case able to bind Met-$tRNA_f$. For evaluation of the inhibitory effect of the individual antibodies only the dimeric 40 S complexes are considered, because dimerisation is largely dependent on antibody binding to 40 S subunits. The relative amount of 40 S dimers formed in the absence of antibodies (control: 11% dimers) is significantly lower than that formed in the presence of the antibody preparation with the lowest dimerisation efficiency (anti-S17: 20% dimers). Furthermore, the inability of 40 S dimers formed by anti-S3a, anti-S13/16, anti-S19 and anti-S24 antibodies to bind Met-$tRNA_f$ underlines the antibody dependence of the formation of 40 S dimers.

A quantitative evaluation of the observed Met-$tRNA_f$ binding to the 40 S dimers is given in fig.2. It is obvious that the antibodies anti-S3a, anti-S13/16, anti-S19 and anti-S24 clearly inhibit eIF-2 binding to 40 S subunits. In contrast, 40 S dimers formed by the antibodies anti-S2, anti-S3 and anti-S17 are still able to bind Met-$tRNA_f$ to about the same extent as the 40 S monomers of the control. It is concluded that ribosomal proteins S3a, S13/16, S19 and S24 are involved in eIF-2 binding, whereas S2, S3 and S17 are not.

These results agree only partially with the conclusions drawn from our earlier experiments [3,4], according to which proteins S3, S6, S13 and S19 are involved in eIF-2 binding. There is good agreement of the results concerning proteins S13 and S19. On the other hand, the results about protein S3 were contradictory. Now, we would consider the former results with some reservation, because: (i) some of the antibodies against ribosomal proteins used in those experiments showed a certain unspecificity as revealed now by immunoblotting (Stahl, J., unpublished); (ii) gradient centrifugation analysis was performed under high hydrostatic pressure (60000 rpm, SW 60 rotor), under which disruption of dimeric 40 S immune complexes could not be excluded, and only monomeric 40 S subunits were considered to evaluate inhibition of Met-$tRNA_f$ binding by the different antibodies; (iii) the results reported in this paper are supported by the observed locations of protein S3 and of eIF-2 (see below; fig.3).

Concerning ribosomal protein S6, we were not

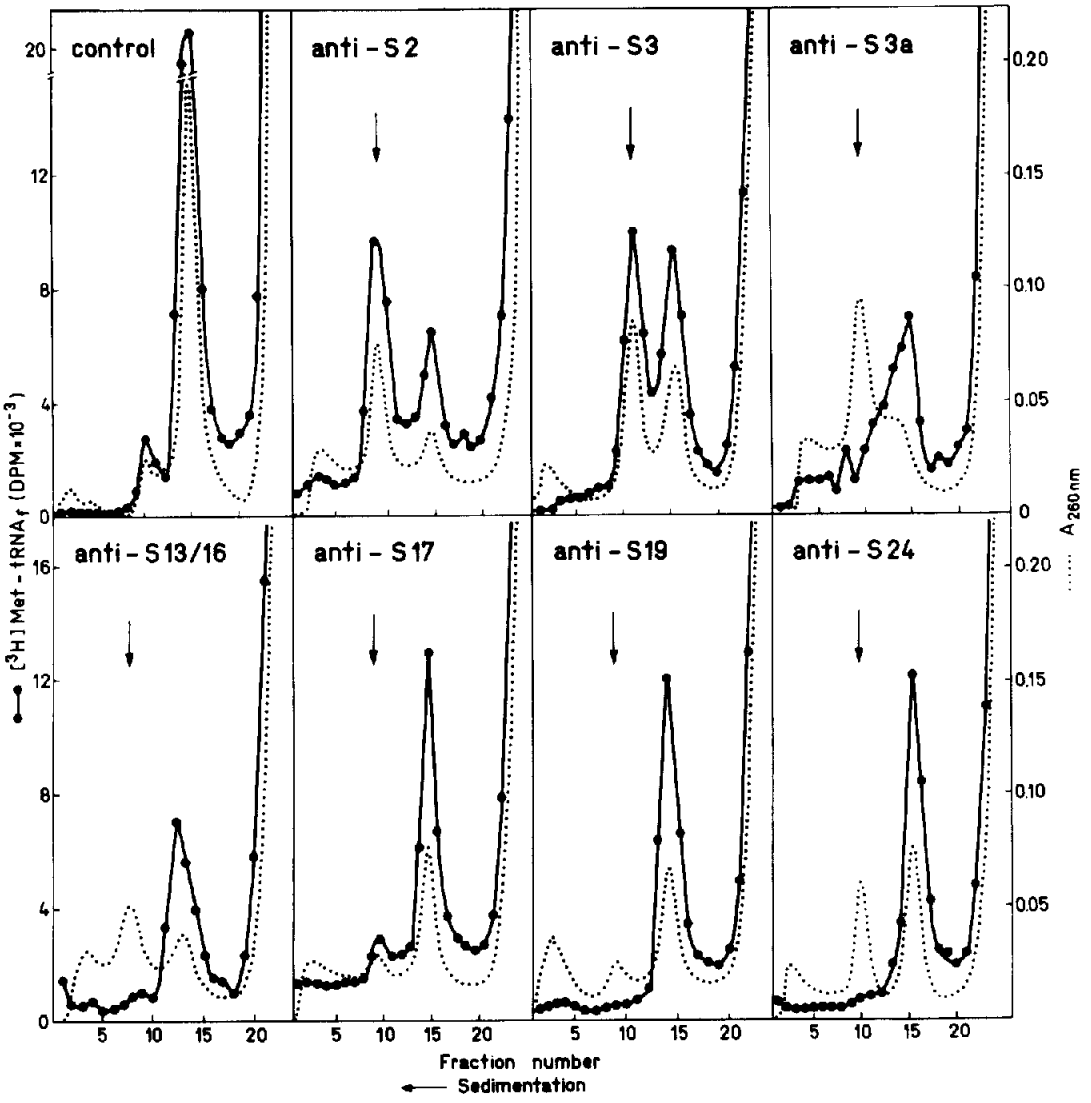

Fig.1. Sucrose gradient analysis of 40 S immune complexes prepared using antibodies against ribosomal proteins as indicated, and incubated with ternary initiation complexes [eIF-2 × GMPPCP × [^{3}H]Met-tRNA$_f$] as described in detail in section 2. Antibodies were applied in the following amounts: 123 μg anti-S2, 70 μg anti-S3, 25 μg anti-S3a, 222 μg anti-S13/16, 232 μg anti-S17, 104 μg anti-S19, 222 μg anti-S24. The distributions of [^{3}H]Met-tRNA$_f$ (●——●) and of the absorbance at 260 nm (···) in the gradients are shown. Arrows indicate positions of dimeric 40 S immune complexes. Control: without antibodies.

able to repeat the experiment under the conditions described in this paper, because some of the antibody preparations used in the earlier study, anti-S6 included, were after repurification no longer able to form dimeric immune complexes with 40 S ribosomal subunits. But our earlier conclusion that protein S6 participates in eIF-2 binding [4] is supported by crosslinking experiments [1,2].

The location of the respective ribosomal proteins on the surface of the 40 S subunit as demonstrated by immunoelectron microscopy [6–8] together with the recently observed eIF-2-binding area [9] are shown in a schematic drawing in fig.3. These results clearly support the conclusion drawn from the inhibition experiments with antibodies (figs 1 and 2). Ribosomal proteins S3a and S24 are (at least partially) located at the eIF-2-binding region, whereas proteins S2 and S3,

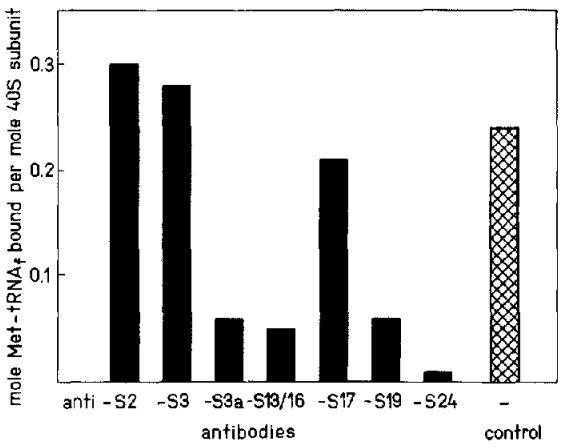

Fig.2. Specific Met-tRNA$_f$-binding activity of the dimeric immune complexes of 40 S subunits formed by antibodies against different ribosomal proteins. The columns represent quotients between the amount of Met-tRNA$_f$ and the amount of 40 S subunit present in the dimeric complexes as calculated from the experiments documented in fig.1. As a control, Met-tRNA$_f$ binding to monomeric 40 S particles in the absence of antibodies (control gradient in fig.1) is shown.

the antibodies which do not inhibit eIF-2 binding, are located on the opposite side of the 40 S particle (fig.3). Proteins S13/16, S17 and S19 are closely neighboured in a distinct region of the 'head' of the 40 S subunit near one end of the eIF-2-binding site (fig.3). Our observation that only antibodies against one of these ribosomal proteins (anti-S17) are nearly unable to inhibit eIF-2 binding (figs 1 and 2) suggests a very distal location of S17 in this particular region at the end of the eIF-2-binding area.

Another approach to investigate the interaction between 40 S ribosomal proteins and initiation factor eIF-2 was chemical crosslinking [1,2]. The results of the experiments indicated that eIF-2 interacts with ribosomal proteins S3, S3a, S6, S13/16, S19 and S24. (The nomenclature of ribosomal proteins used in this paper follows that of McConkey et al. [14], whereas in [1,3] an earlier nomenclature was applied [15].) This is in agreement with the results of our antibody inhibition experiments, with the exception of protein S3. The observed crosslinking of protein S3 to eIF-2 [1] could be explained by indirect crosslinking via protein S3a, because in all crosslinking studies with 40 S ribosomal subunits proteins S3 and S3a were identified as neighboured proteins (for review see [5]).

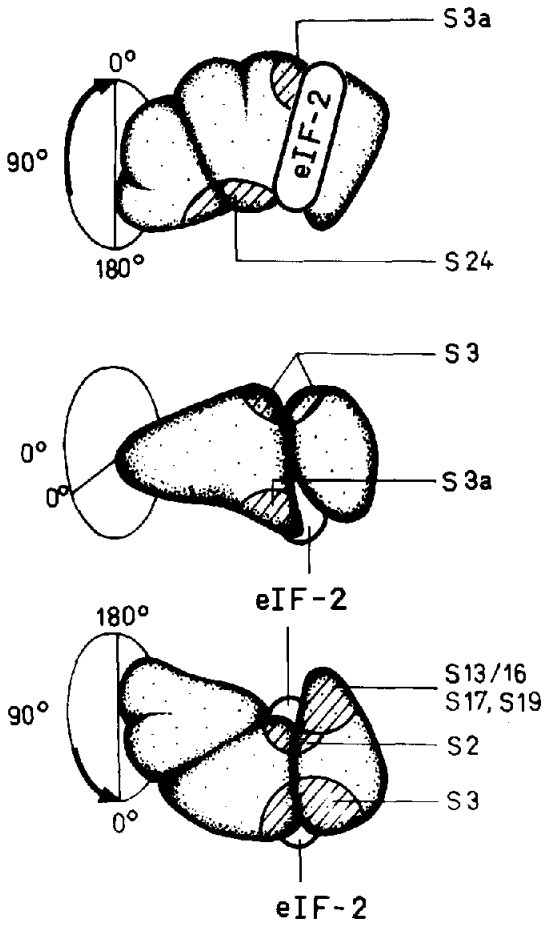

Fig.3. Three-dimensional model of the 40 S ribosomal subunit demonstrating the location of the antibody-binding sites of ribosomal proteins S2, S3, S3a, S13/16, S17, S19 and S24 (results taken from [6–8]) and the location of the eIF-2-binding area (results taken from [9]).

In general, the data obtained by chemical crosslinking, by the antibody inhibition assay, and by immunoelectron microscopy are in good agreement, and the picture emerges that at least proteins S3a, S13/16, S19 and S24 are involved in eIF-2 binding to the 40 S ribosomal subunit.

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