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Three-dimensional location of ribosomal protein BL2 from *Bacillus stearothermophilus*, a key component of the peptidyl transferase center

W. Hackl, M. Stöffler-Meilicke and G. Stöffler[+]

Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Ihnestraße 73, D-1000 Berlin 33 (Dahlem), Germany and [+] *Institut für Mikrobiologie, Medizinische Fakultät, Universität Innsbruck, Fritz-Pregl-Strasse 3, A-6020 Innsbruck, Austria*

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Protein BL2 from *Bacillus stearothermophilus* has been localized by immunoelectron microscopy on the interface side of the 50 S subunit, beneath the angle formed between the central protuberance and the L1 protuberance. The immunoelectron microscopic data suggest that the interface region of the 50 S particle is not as flat as most of the proposed three-dimensional models suggest, but instead there is a significant concavity. Since several studies demonstrated that BL2 is implicated in peptidyl transferase activity or at least located close to the peptidyl transferase center, the location of protein BL2 also provides information as to the location of this important functional domain.

Ribosomal topography; Ribosomal protein BL2; Peptidyl transferase center; Immunoelectron microscopy

1. INTRODUCTION

Protein L2 is the largest ribosomal protein of the 50 S subunit from *Escherichia coli*. L2 appears to be highly conserved as judged by amino acid sequence analysis [1] and immunological crossreactivity [2], pointing to an essential role for ribosome function. Protein EL2 is known to be a 23 S RNA-binding protein and belongs to the group of proteins essential for the assembly of the 50 S subunit [3]. Several lines of evidence suggest that EL2 is a key component of the peptidyl transferase [4,5]. Thus, information on the location of this protein within the intact 50 S ribosomal subunit could give indirect evidence as to the location of the peptidyl transferase center. In this study we have used antibodies specific for protein BL2 from *Bacillus stearothermophilus* to localize this important protein on the ribosomal surface.

Correspondence (present) address: M. Stöffler-Meilicke, Institut für Mikrobiologie, Medizinische Fakultät, Universität Innsbruck, Fritz-Pregl-Strasse 3, A-6020 Innsbruck, Austria

Abbreviations: IEM, immunoelectron microscopy; TP50, total ribosomal protein from 50 S subunits

2. MATERIALS AND METHODS

Ribosomes and ribosomal subunits from *B. stearothermophilus* strain 799 were prepared as described [6]. Single protein BL2 from *B. stearothermophilus* was prepared by the acetic acid extraction procedure, followed by ion-exchange column chromatography on CM-cellulose [1] and was kindly provided by Dr M. Kimura. Antibodies against purified protein BL2 from *B. stearothermophilus* were raised in a rabbit. Characterization of the antiserum, sucrose gradient centrifugation and electron microscopy were performed as described elsewhere [7].

3. RESULTS

3.1. *Characterization of the antibody*

The antiserum raised against protein BL2 from *B. stearothermophilus* reacted exclusively with protein BL2 and no other ribosomal protein, as judged by double immunodiffusion, modified immunoelectrophoresis (not shown) and im-

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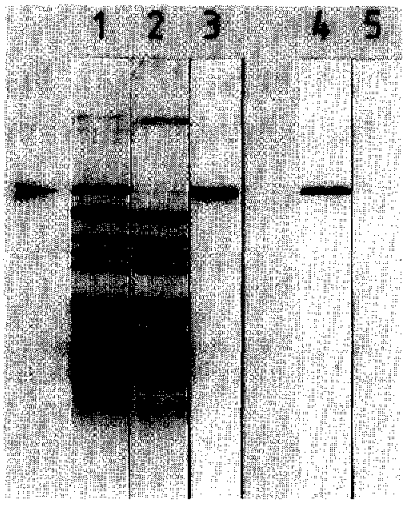

Fig.1. One-dimensional SDS-polyacrylamide gels and immunoblots. Tracks 1 and 4: 10 μg and 5 μg of TP50 extracted with acetic acid. Tracks 2 and 5: 10 μg and 5 μg of TP50 extracted with 2 M LiCl/4 M urea. Track 3: 0.5 μg of protein BL2. Tracks: 1–3, SDS-polyacrylamide gel electropherogram stained with Coomassie brilliant blue; 4,5, nitrocellulose sheets to which the proteins have been electrophoretically transferred and which have been incubated with anti-BL2 and subsequently immunostained with alkaline phosphatase. The arrowhead marks the position of BL2. Tracks 3 and 5 demonstrate that BL2 is quantitatively absent from TP50 that has been extracted with 2 M LiCl/4 M urea.

munoblotting (fig.1, lane 4). When the antibodies were tested for their reactivity with intact 50 S subunits by sucrose gradient centrifugation, up to 28% of the subunits were dimerized (fig.2a). The antibodies did, however, not react with 70 S monosomes (fig.2a).

In order to demonstrate that the reactive epitopes within the 50 S subunits were indeed provided by protein BL2, absorption experiments were performed (fig.2b). Preincubation of the antibody with 120 pmol of single protein BL2 completely inhibited dimer formation. Preincubation with TP50 had the same effect. In contrast, preincubation with TP50 lacking protein BL2 had no effect on dimer formation. For this latter experiment, TP50 has been extracted from 50 S subunits with 2 M LiCl/4 M urea, since it has previously been shown that under these conditions protein BL2 remains bound to the 23 S rRNA [8]. Consequently, the protein mixture of TP50 lacks protein BL2 quantitatively as judged by SDS-polyacrylamide gel electrophoresis and immunoblotting (fig.1, lanes 2 and 5).

3.2. *Electron microscopy*

Electron micrographs of 50 S subunits reacted with anti-BL2 are shown in fig.3. The majority of the subunits were present as dimeric immunocomplexes; altogether, more than 800 of such complexes have been evaluated. Almost 50% of the immunocomplexes did not allow identification of the characteristic features of the 50 S subunits nor to discern the connecting antibodies, since they were superimposed by features of the

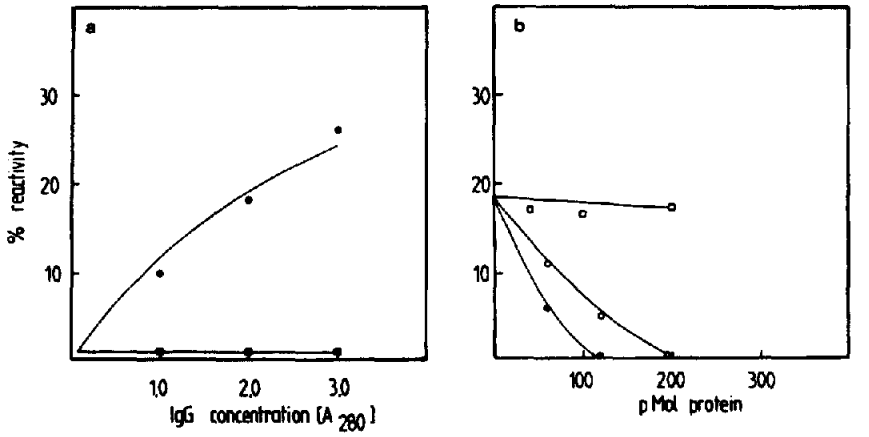

Fig.2. Antibody-ribosome-complex formation in sucrose gradients. (a) Reactivity of 50 S (●—●) and 70 S (■—■) ribosomes from *B. stearothermophilus* with BL2-specific antibodies, plotted against IgG concentration. (b) Inhibition of dimer formation by preincubation of anti-BL2 with increasing amounts of single protein BL2 (●—●), of TP50 (○—○) and TP50 lacking protein BL2 (□—□). The reactivity was determined by planimetry of the area under the dimer peak [19].

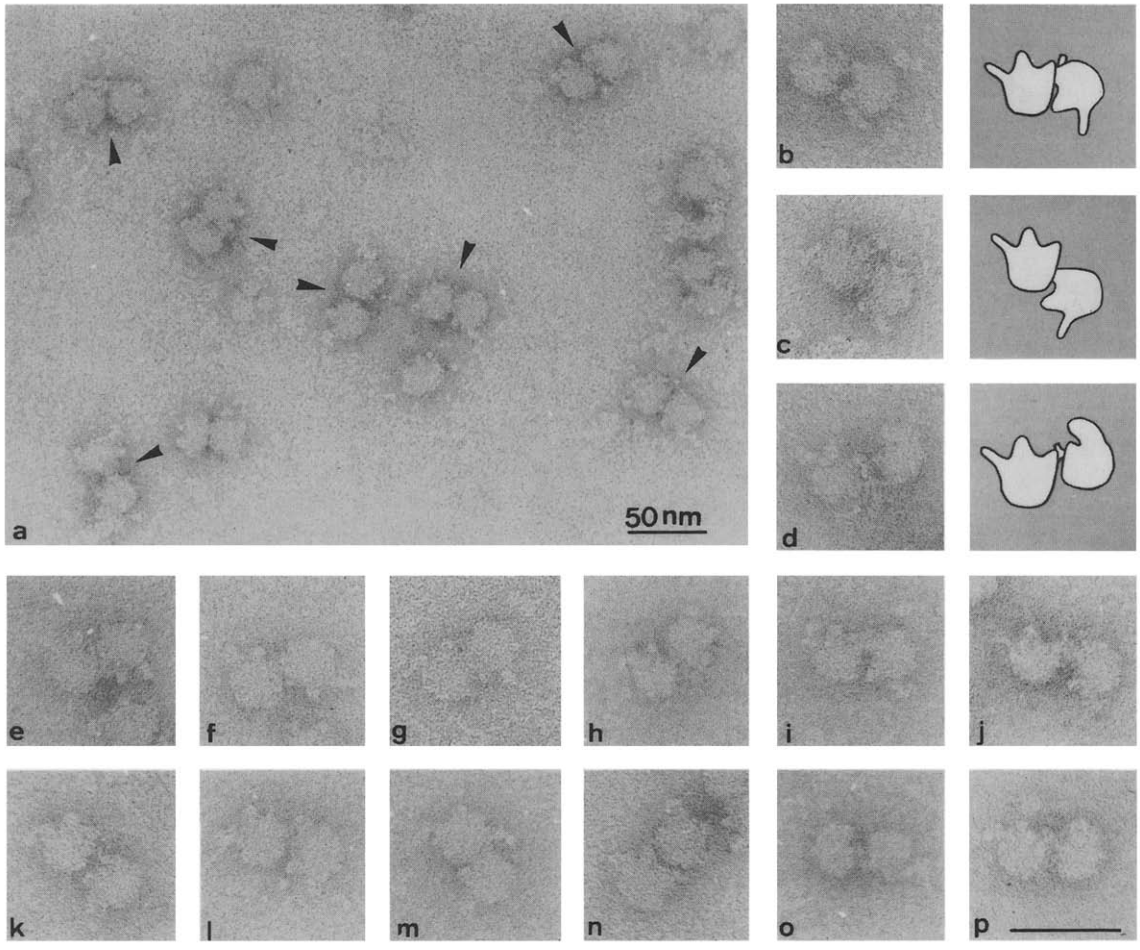

Fig.3. Electron micrographs of 50 S subunits from *B. stearothermophilus* reacted with antibodies specific for protein BL2. (a) General field: arrowheads mark typical immunocomplexes. (b–p) Selected electron micrographs; the interpretative schemes represent the micrographs to their left.

50 S particles. Only 12% of the dimeric immunocomplexes allowed an interpretation of both subunits.

In most dimers displayed in the crown projection, the two L1 protuberances are in close contact (fig.3b,d,e–g,i–l). Only in some of the immunocomplexes, the Fc parts of the connecting IgG molecules are visible (fig.3b,d,h–l), indicating that the actual antibody-binding site must be located some 70 Å away from the contour line of the L1 protuberance (taking into account that the length of one Fab arm is approx. 70 Å). Frequently, the central protuberance of one subunit is in proximity to the base of the other particle (fig.3c,i–n). In the kidney view, the Fab arms of the IgG molecules bind on the blunted end of the interface side of the 50 S particle, close to the notch (fig.3d,o,p).

The electron micrographs described above led us to locate protein BL2 on the three-dimensional model as shown in fig.4. Due to the difficulties of interpretation, the anti-BL2-binding site covers a relatively large area. Protein BL2 is located at the interface region of the 50 S subunit, a result which is consistent with the finding that the antibodies specific for BL2 do not react with 70 S monosomes (see above). In addition, the fact that antibody binding to the kidney views reveals little of the Fab arm to be seen suggests that the interface region of the 50 S particle is not as flat as most of the three-

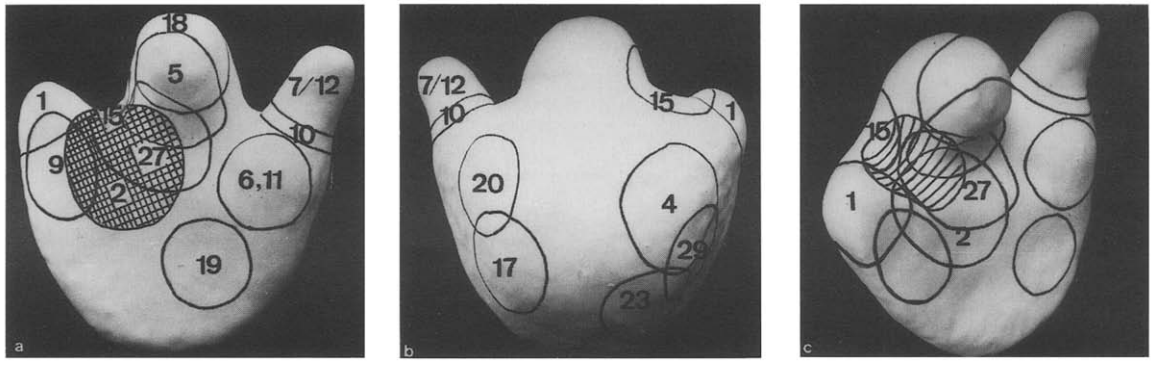

Fig.4. (a,b) Three-dimensional model of the 50 S subunit from *E. coli* to which the location of protein BL2 from *B. stearothermophilus* has been transferred (cross-hatched area). The numbers give the locations of the centers of antibody-binding sites of the other ribosomal proteins from *E. coli* which have so far been determined by immunoelectron microscopy in our laboratory. (c) The hatched area corresponds to the binding site of puromycin and chloramphenicol respectively [14,15]. The numbers give the locations of the proteins which have been labeled by puromycin and analogues of puromycin [14,18].

dimensional models would suggest, but instead there is a significant concavity.

4. DISCUSSION

In previous papers [7,9] we have shown that the three-dimensional arrangement of the ribosomal proteins from *E. coli* and *B. stearothermophilus* is identical. This finding was of general importance, since it now allows us to incorporate the data obtained on the location of ribosomal protein L2 from *B. stearothermophilus* into the three-dimensional ribosome model of *E. coli*. Out of six antisera specific for *E. coli* EL2, none formed stable immunocomplexes with intact 50 S subunits which would have allowed the immunoelectron microscopic localization of protein EL2 on the ribosomal surface. Antibodies specific for protein BL2, however, did react with intact 50 S particles, thus enabling us to localize BL2 on the interface side of the 50 S subunit, at the base of the central protuberance close to the angle formed between this feature and the L1 protuberance (fig.4a).

In good agreement with the location of protein L2, as determined in this study, are results from crosslinking experiments obtained by ourselves [10] and by others [11,12], who found that in *E. coli* protein EL2 can be crosslinked to protein EL9 by a variety of crosslinking reagents. Surface determinants of protein EL9 have been mapped by IEM in an area very close to the surface determinants of protein L2 (fig.4a). In this context it is noteworthy that antibodies specific for protein EL9 reacted with 70 S monosomes, whereas the antibodies specific for protein BL2 did not. Traut and co-workers [13] recently described two monoclonal antibodies specific for protein L2 of which one reacted with 70 S monosomes whereas the other did not.

Several lines of evidence suggest that protein EL2 is involved in peptidyl transferase activity [4,5]. The peptidyl transferase inhibitors chloramphenicol and puromycin have been localized by IEM in the angle formed between the central protuberance and the broader lateral protuberance, close to the position where we locate protein L2 (see fig.4c and [14,15]). In addition to protein L2, various other ribosomal components, including proteins L1, L14, L15, L16, L23 and L27, have also been suggested to be involved in peptidyl transferase activity or to be located close to this functional domain [16]. Of these proteins, L1, L15 and L27 have also been located in this same area (fig.4c). Protein L16, which has not yet been mapped by IEM, was found to be crosslinked to L27 [10], and we have preliminary IEM data that protein L14 is located close to proteins L2 and L27. Only protein L23 has antigenic determinants exposed far away from the primary puromycin-binding site [9,18]. Our IEM data, however, as well as results from crystallography [17] suggest that the interface region of the 50 S particle is not as flat as most of the three-dimensional models would suggest, but instead there is a significant

concavity. Thus, although the antigenic site found for L23 lies at the back of the 50 S particle (fig.4b), it is quite possible that this protein could extend through the subunit in the direction of the cavity. We propose therefore that all the proteins that have been shown to be implicated in peptidyl transferase activity are located around the rim or within this concavity.

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