30S RIBOSOMAL PROTEINS ASSOCIATED WITH THE 3′-TERMINUS OF 16S RNA

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1. Introduction

Although it has proven very difficult to develop a generally applicable technique for cross-linking ribosomal proteins to their corresponding RNA binding sites in situ, a very restricted procedure has been available for quite some time. Thus, oxidation of the 3′-terminal ribose of RNA followed by reduction of the product formed with the ε-amino group of lysine can create a stable bond between the RNA and an appropriately positioned protein [1]. Indeed it has been suggested that the ribosomal protein S1 is situated in the intact 30S subunit at a site very near to the 3′-terminus of the 16S RNA [2]. The evidence for this surmise consists of the observation that the proteins extracted from periodate oxidized 30S subunits are deficient in the electrophoretic component corresponding to S1. Nevertheless no evidence was presented to demonstrate that the missing protein was in fact bound to the 3′-end of the oxidized/reduced intact 16S RNA [2].

Our reexamination of the topographic relationship between 30S protein S1 and the 3′-terminus of the 16S RNA was prompted by recent experiments which suggest a well defined functional relationship between these ribosomal components. Thus, nucleotide sequences at the 3′-end of the 16S RNA are apparently involved in the initiation of protein synthesis [3,4]. Furthermore, the original suggestion that S1 is involved in the binding of mRNA to the ribosome [5] has now been rather extensively verified [6,7]. It seems, therefore, likely that S1 and 3′-end of the 16S RNA make up a binding site for mRNA.

Accordingly, we have studied the proteins attached to 16S RNA after oxidation and reduction of 30S ribosomal subunits. Several such proteins have been identified. Of these, S1 and S21 are the only ones for which evidence could be obtained which indicates that they are bound at or near the 3′-end of the 16S RNA.

Abbreviations: SDS, sodiumdodecylsulfate; Tris, Tris (hydroxymethyl)-aminomethane; EDTA, (ethylenedinitrilo) tetra-acetic acid disodium salt; AS, antisera.

2. Materials and methods

30S ribosomal subunits (5 mg), prepared as described earlier [8], were oxidized in 1 ml of the following solution: 17 mM sodium metaperiodate (Merck); 40 mM sodium acetate (pH 6.2); 40 mM sodium-chloride and 20 mM magnesium acetate (buffer 1) for 50 min at 20°C in the dark (this step was omitted in the control). Excess periodate was removed by either of two methods. One consisted of precipitating the subunits in ethanol and then washing the precipitate two times in 66% ethanol-buffer 1 solution containing 1% glycerol and one time in the same mixture without glycerol. Alternatively the oxidized subunits were dialyzed against buffer 1 containing 1% glycerol and then against the same buffer without glycerol.

After removal of the periodate, the pH of the subunit solution was raised to pH 8.2 by addition of 1 M Bicine buffer (20 mM magnesium acetate 40 mM sodium chloride pH 9.8). The solution was adjusted to 10 mM $NaBH_4$ to reduce the protein–RNA linkage and after 30 min at 4°C, ethanol was added to precipitate the subunits. The precipitate was dissolved in a solu-

tion containing 2% SDS, 30 mM EDTA 20 mM Tris pH 7 and warmed at 37°C for 5 min. This mixture was then layered over a sucrose gradient (5 to 30%) containing 0.2% SDS and 10 mM sodium acetate, pH 6.5 and centrifuged for 19 h at 27 000 rev/min at 17°C (SW27 rotor). Most of the protein not covalently bound to the 16S RNA was removed in this way, and the SDS was removed from the RNA by ethanol precipitation. Proteins were released from the RNA by digestion with ribonuclease A and ribonuclease T1 according to [9] or by alkali treatment: 0.3 M sodium hydroxide for 25 min at 37°C. Under these conditions no hydrolysis of proteins can be observed [7]. Ouchterlony double diffusion tests were performed as described earlier [10]. The rest of the ethanol precipitate was dissolved in a solution containing 3%

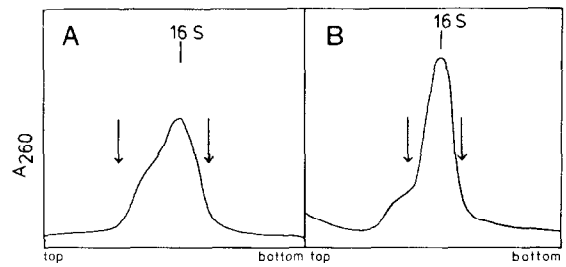

Fig.1. Sedimentation profile of 16S RNA. The RNA was pooled as indicated by the arrows. (A) SDS-containing sucrose gradient (5 to 30%) of 16S RNA after oxidation and reduction of 30S subunits as described in Materials and Methods. (B) Sarkosyl containing sucrose gradient (5 to 30%) of the pooled 16S RNA of gradient 1A. The RNA was heated to 56°C before layered over the gradient, see Materials and methods.

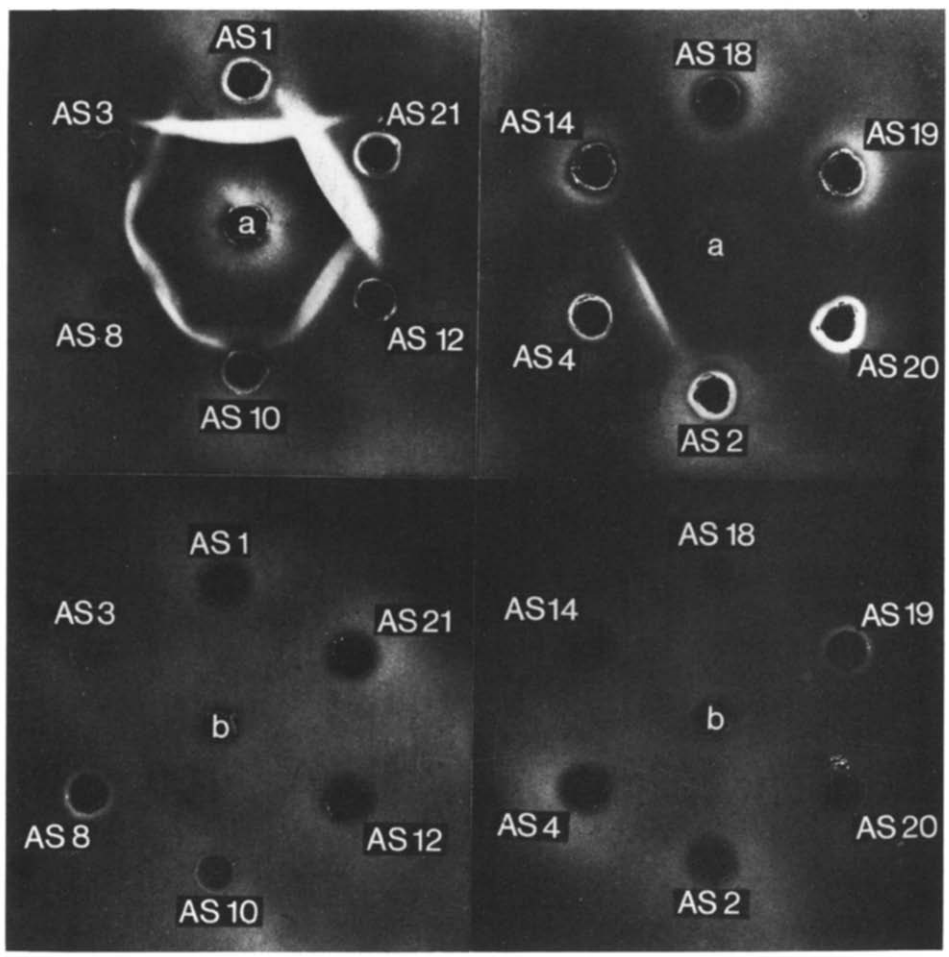

Fig.2A

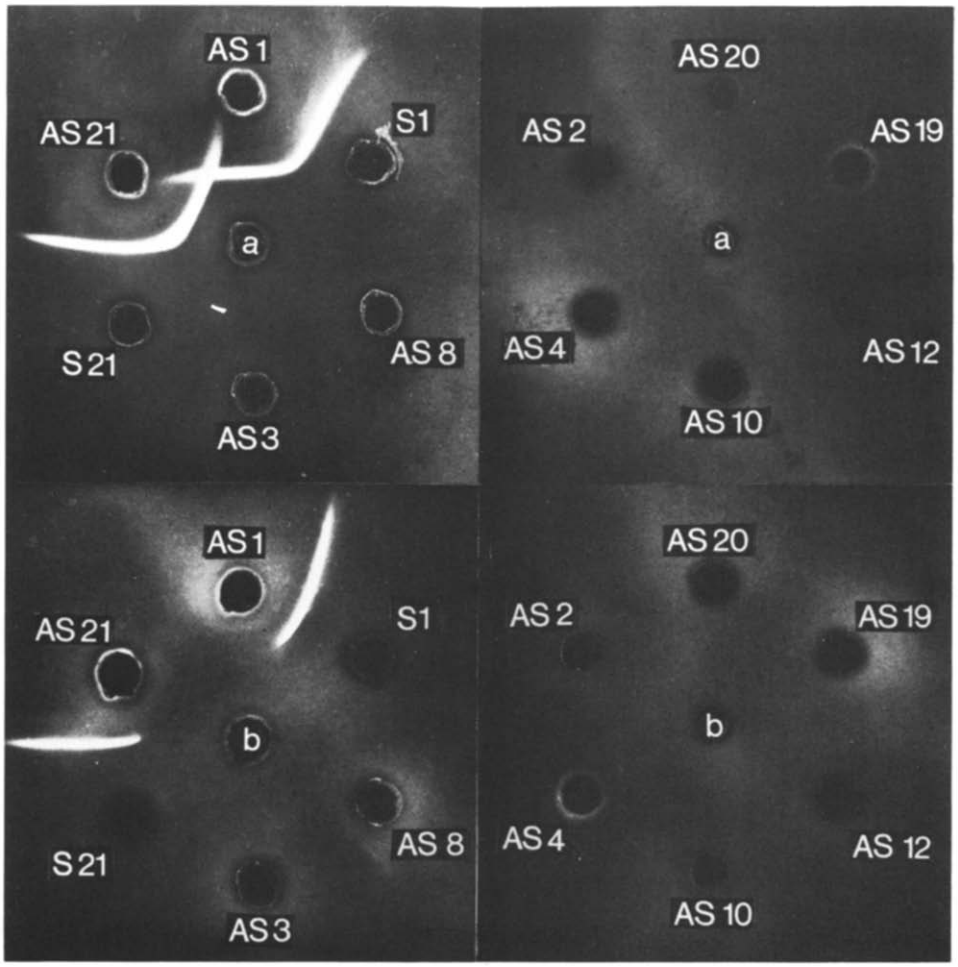

Fig.2B

Fig.2. Ouchterlony double diffusion tests are shown with antisera raised against the proteins indicated. For details see [10]. (2A) Proteins isolated from the material of the gradient shown in fig.1A. (a) Protein solution obtained after oxidation/reduction of the 30S subunits and (b) protein solution obtained without oxidation of the 30S subunits (control). (2B) Proteins isolated from the material of the gradient shown in fig.1B. In the two holes indicated with S1 and S21, respectively, 1.5 μg of single protein were applied. (a) and (b) as in fig.2A.

Sarkosyl (Ciba-Geigy) 30 mM EDTA and 10 mM Tris pH 7 and warmed at 56°C for 5 min. This mixture was then layered over a sucrose gradient (5 to 30%) containing 0.2% Sarkosyl and 10 mM sodium acetate pH 6.5 and centrifuged for 20 h at 27 000 rev/min at 3°C (SW27 rotor). In this way all the protein not covalently bound was removed and the RNA as well as RNA-protein complex was precipitated by ethanol precipitation. The same procedure as described above was used to isolate the proteins.

3. Results and discussion

Aliquots of RNA from oxidized/reduced 30S subunits were purified on a sucrose gradient containing SDS. As can be seen in fig.1A, this RNA consists of a major 16S peak and a slower more heterogenous population of molecules that appear as a pronounced shoulder to the 16S peak. When the proteins associated with pooled RNA (see fig.1A) are analyzed by gel electrophoresis after alkali treatment (data not shown)

or by Ouchterlony immunodiffusion after RNase treatment, several components can be identified: S1, S21 and S4 are always there, but there are also variable amounts of S3, S8, S10 and S12 (see fig.2A (a)). In contrast, a control sample of RNA which was extracted from subunits that had not been oxidized, but otherwise treated in the same way as the experimental sample, did not yield any detectable proteins (see fig.2A (b)). Similar results were obtained by Overbeek and Van Duin (personal communication). The recovery of at least seven different proteins associated with the oxidized/reduced RNA suggested to us that single strand nicks in the RNA would permit proteins to become attached to the RNA at more than one point in the 16S sequence. Were this so, the proteins associated with artificial 3′-termini should be found on fragments which sediment slower than 16S, while those associated with the native 3′-end should be recovered on the intact 16S RNA.

This conjecture was verified by an additional experiment. The 16S RNA peak was taken from the SDS gradient, and then heated at 56°C in Sarkosyl. This was done in order to disrupt complexes of nicked RNA as well as noncovalently bound proteins. Then the heated RNA was fractionated a second time on a sucrose gradient which in this case contained Sarkosyl. Most of the RNA in this gradient sediments at a reasonably sharp 16S peak (see fig.1B).

When the 16S RNA obtained from the second gradient fractionation is degraded and the released proteins are analyzed, only S1 and S21 can be detected; S3, S4, S8, S10 and S12 are not observed (fig.2B (a)). Finally, control samples of RNA extracted from subunits that had not been oxidized did not show any detectable amounts of either S1 or S21 (fig.2B (b)).

Accordingly, we conclude that both S1 and S21 are located near to the 3′-terminus of the 16S RNA in the purified 30S subunit. However, we cannot be more precise than to say that these three elements are in the same neighborhoods of the ribosome. The principal ambiguities inherent in the interpretation of the present experiments are that the shapes of S1 and S21 are not known, and that the flexibility of the 30S structure is not known. Nevertheless, the finding that the 3′-terminus of the 16S RNA as well as S1 and S21 share to some extent a common neighborhood in the 30S subunit is in good agreement with recent functional studies which show that these same three components are likely to be involved in the binding of a mRNA to the ribosome [3,4,7,11].

The same proteins, S1 and S21, can be recovered crosslinked to 16S RNA if 70S ribosomes ('tight couples') are oxidized/reduced under conditions similar to those described above (data not shown). Preliminary investigations did not show any detectable amounts of these proteins bound either to 5S RNA or 23S RNA which is an additional indication of the very specific, close arrangement of the three components S1, S21 and the 3′-end of 16S RNA.

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