PROTEINS S14 AND S19 ARE NEAR-NEIGHBORS IN THE *E. COLI* RIBOSOME

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1. Introduction

We have previously reported data from cross-linking experiments indicating that S7 and S9 as well as S13 and S19 are pairs of near-neighboring proteins in the 30 S ribosomal subunit [1]. This same group of proteins are ones which cooperate with each other during assembly in vitro and which are associated with a common region of the 16 S RNA. Thus, S7 will bind to 16 S RNA in the absence of all other proteins [2–4] and its presence enhances the binding of S 9, S 10, S 13, S 14 and S 19 to the RNA [2,5]. Similarly, degradation of the 30 S ribosomal subunit with nuclease releases ribonucleoprotein fragments which contain various combinations of these very same six proteins [6,7].

If these six proteins and their associated RNA binding sites form a well defined neighborhood in the 30 S ribosomal subunit, pairs of these proteins other than S 7 and S 9 or S 13 and S 19 should be cross-linkable in the intact ribosome. Furthermore, it should be possible to obtain neighborhoods within the same group of proteins which overlap with each other. The data described here confirm both expectations. We have found that dimethyl suberimidate dihydrochloride (DMS) can be used to crosslink S 14 to S 19.

2. Materials and methods

The reagents DMS and dimethyl adipimidate dihydrochloride (DMA) were obtained from Pierce Chemical Company, Rockford, Illinois, USA. The 30 S ribosomal subunits were purified as described earlier from *E. coli* MRE 600 [8,9]. A typical cross-linking incubation mixture contains 50 A_{260}/ml 30 S subunits in TEAMK-buffer (5 mM $MgCl_2$, 50 mM KCl, 10 mM triethanolamine–HCl, pH 8.5) and 10 mM DNA or DMS. The mixture was incubated at room temperature (approx. 22°C) for 30 min and the reaction was stopped by the addition of NH_4Cl to a final concentration of 0.2 M. The pH of the mixture was monitored and adjusted during the reaction by the addition of 1 M triethanolamine–HCl (pH 8.5). The acetic acid extraction of the proteins from reacted subunits has been previously described [8] well as the purification of the cross-linked complex by chromatography on phosphocellulose [8], molecular sieve chromatography through Sepahadex G100 [8], and preparative gel electrophoresis [1,8]. Samples were characterized by gel electrophoresis in polyacrylamide as described in detail earlier [1,8]. In addition, antibodies raised against the individual 30 S proteins [10] were employed to characterize the cross-linked proteins. The details of Ouchterlony procedure as well as the column fractionation with antibodies are described elsewhere [1,8].

3. Results and discussion

Fig. 1 shows the electrophoretic pattern obtained

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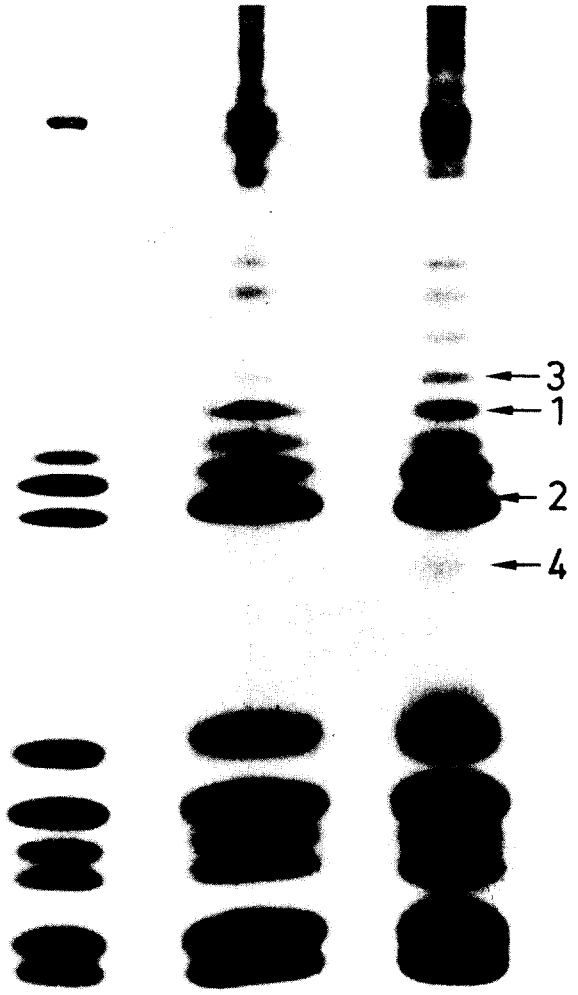

Fig. 1. The electrophoretic pattern obtained in discontinuous 15% slab polyacrylamide gels containing SDS [13] are shown with the following samples: left, protein from untreated 30 S subunits; middle, protein from DMA-treated 30 S subunits; and right, protein from DMS-treated 30 S subunits. The four DMS cross-linked complexes whose components have been identified are numbered.

with proteins treated with the diimidoesters DMA and DMS. The complexes DMS-1, DMS-2, and DMS-3 have been previously identified as S 5-S 8, S 13-S 19 and S 7-S 9, respectively [1,8]. The object of the present report is DMS-4, which is not obtained with DMA. DMS-4 elutes from phosphocellulose columns at 0.5 M NaCl but is heavily contaminated with S 15, S 19, DMS-2 as well as other components. It can be obtained pure, as shown in fig. 2, by preparative gel electrophoresis.

Samples of DMS-4 were examined using the Ouchterlony double diffusion procedure with antisera raised against twenty of the twenty-one purified 30 S proteins (anti-S 17 was not available). DMS-4 produced fusing precipitation lines with anti-S 14 and anti-S 19 (fig. 3). A mixture of S 14 and S 19 produces, in contrast, precipitation lines that cross (fig. 3).

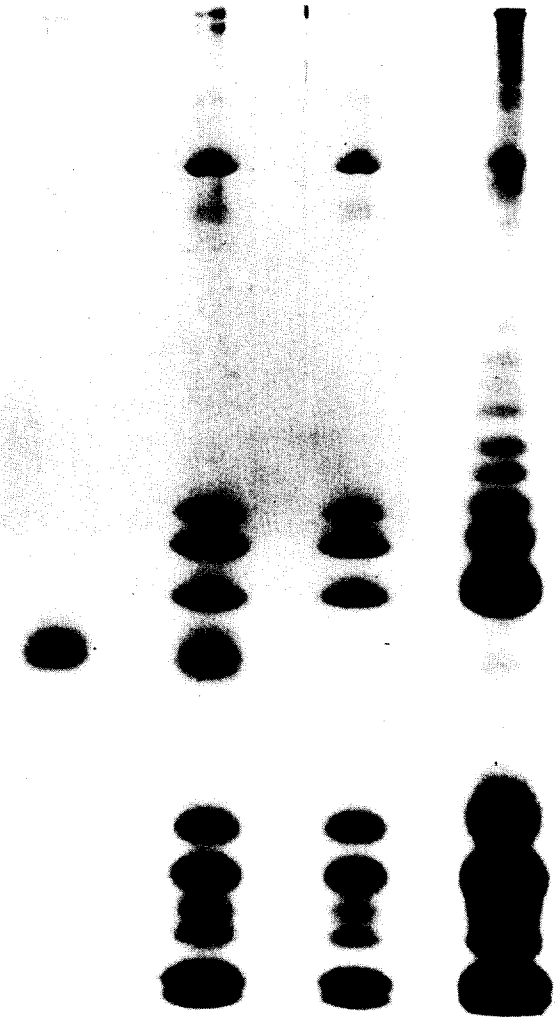

Fig. 2. The electrophoretic patterns from slab gels are shown with the following samples: purified DMS-4 (first well); a mixture of untreated 30 S protein and DMS-4 (second well); the protein from untreated 30 S subunits (third well); and the protein from DMS-treated 30 S subunits (fourth well).

Occasional contamination was detected with anti-S 13 and anti-S 15, but these reactions are weak. That S 14 is indeed cross-linked to S 19 was shown in experiments with radioactive S 19.

^{3}H-labelled S 19 was used to reconstitute 30 S subunits which contained no other radioactive components. These subunits were treated with DMS, and the protein extracted from the subunits. As can be seen in fig. 4, the radioactive S 19 elutes in the included volume of a Biogel A 0.5 M column whether or not

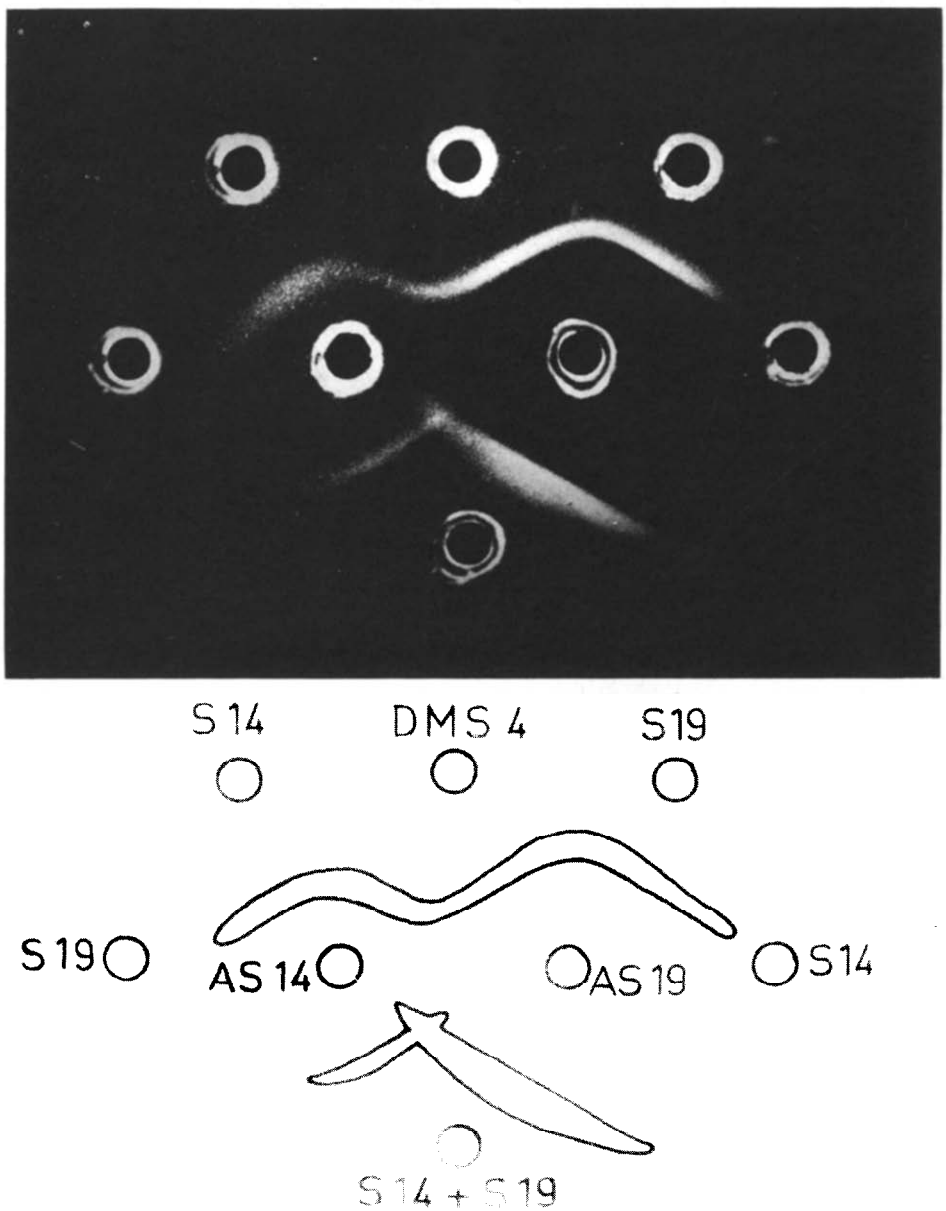

Fig. 3. Ouchterlony double diffusion tests are shown with DMS-4, S 14, S 19 and antisera raised against S 14 as well as S 19. For details see ref. [1,8].

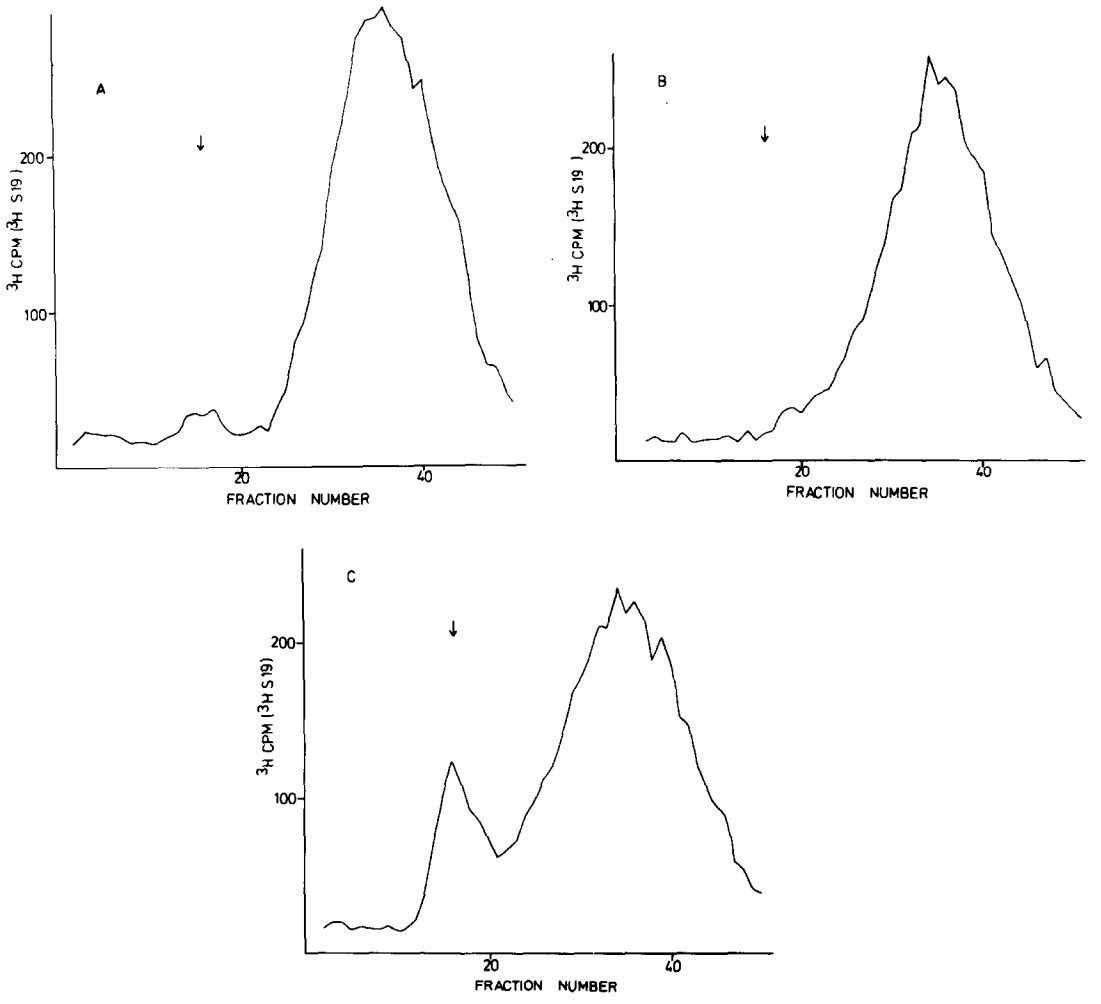

Fig. 4. The elution patterns are shown for protein obtained from 30 S subunits which had been reconstituted with radioactive S 19 and then passed through a Biogel A 0.5 M column. The samples were analyzed as follows: A, protein from untreated 30 S subunits incubated with anti-S 14 antibody; B, protein from DMS-treated 30 S subunits; and C, protein from DMS-treated 30 S subunits incubated with anti-S 14 antibody. The arrows indicate the excluded volume fraction of the columns.

the proteins are cross-linked. However, when the same cross-linked mixture is reacted with anti-S 14, ^{3}H-S 19 appears in the excluded volume fraction (fig. 4). This is expected because the large size of the antigen-antibody complexes restricts them to the excluded volume of the column. Since anti-S 14 does not transfer ^{3}H-S 19 from the included volume to the excluded volume fraction unless the proteins have been crosslinked, we conclude that S 14 is cross-linked to S 19.

DMS-4 coelectrophoreses with protein S 4 on continuous 10% polyacrylamide gels containing SDS, which indicates that DMS-4 like S 4 has a molecular weight close to 26 000. The apparent molecular weights of S 14 and S 19 in the same gel system are 14 000 and 13 000, respectively [11]. Therefore, we conclude that DMS-4 is a bimolecular complex containing S 14 and S 19.

The low yield of DMS-4 seen in fig. 1 makes its recovery in amounts sufficiently large for analysis

difficult. Furthermore, were is not correlated with the assembly data and the fragment analysis described in the introduction, this neighborhood would be suspect precisely because of the limited amounts in which it is produced. Nevertheless, DMS-4 illustrates an aspect of cross-linking experiments which has been discussed earlier. The recovery of large amounts of one member protein in a cross-linked complex precludes the recovery of this same protein in large amounts in other complexes [1]. Thus, up to 30% of S 19 can be recovered in DMS-2 [1], which may account for the low yields of DMS-4. Indeed, it is possible that both S 14 and S 19 will subsequently be found in other low yield complexes produced by DMS.

In conclusion, the identification of the neighborhood S 14-S 19 adds one more member to the group of cooperating proteins around S 7. However, we do not wish to suggest that the cooperative interactions between these proteins are necessarily a consequence of direct protein-protein contacts. The yields of cross-linked complexes created by diimidoesters are surprisingly small. This finding as well as some relevant control experiments have suggested that the 30 S proteins are not packed in RNA-free domains [1]. Accordingly, contacts between pairs of near-neighboring proteins may not be extensive. The data concerning DMS-4 illustrate this quite well. DNA which is between 8 and 9 Å long does not create cross-links between S 14 and S 19, and DMS, which is between 11 and 12 Å long does so to a limited extent. Such data suggest that while S 14 and S 19 may be near one another in the ribosome, contacts between them are quite possibly limited.

Acknowledgements

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