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rRNA-protein neighbourhood in *Escherichia coli* 70 S ribosomes and 70 S initiation complex

Probing by bifunctional Pt(II)-containing reagent

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The cleavable homobifunctional reagent dichloro[*N,N,N',N'*-tetrakis(2-aminoethyl)-1,6-hexamethylenediamminediplatinum(II)] dichloride was used for studying rRNA-protein cross-links in free ^{35}S-labelled 70 S ribosomes and within initiation complex ribosome · $AUGU_6$ · fMet-$tRNA_f^{Met}$. It was shown that the sets of proteins cross-linked to 16 S and 23 S rRNA in free 70 S ribosomes and in 70 S initiation complex do not differ significantly. The authors are the first to demonstrate most of the 23 S rRNA-protein cross-links and some 16 S rRNA-protein cross-links, in particular those with L7/L12 protein.

rRNA; Ribosomal protein; Initiation complex; Platinum(II) reagent; Cleavable cross-linking

1. INTRODUCTION

Chemical reagents inducing cross-links between RNAs and proteins are widely used for studying ribosomal topography [1]. However, bifunctional chemical reagents have been applied mainly in studies of rRNA-protein contacts in isolated ribosomal subunits. Data on the interaction of rRNA of one subunit with proteins of another within the 70 S ribosome are scarce. In addition, chemical rRNA-protein cross-links within the complexes of ribosomes with tRNA and mRNA still require investigation. Pt(II) compounds are rather promising cross-linking reagents which have been used to realize cross-links between biopolymers within 30 S ribosomal subunits [2], tRNA · aminoacyl-tRNA synthetase [2], initiation factor IF3 · 30 S subunit [3] and elongation factor EF-Tu · Phe-$tRNA^{Phe}$ complexes [4].

Here, rRNA-protein cross-links in free ^{35}S-labelled 70 S ribosomes and within initiation complex ribosome · $AUGU_6$ · fMet-$tRNA_f^{Met}$ have been studied with the use of the reagent dichloro-[*N,N,N',N'*-tetrakis(2-aminoethyl)-1,6-hexamethylenediamminediplatinum(II)] dichloride (Pt_2AmCl_4). The results indicate the absence of significant changes in rRNA-protein neighbourhoods caused by formation of the complex.

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2. MATERIALS AND METHODS

$tRNA_f^{Met}$ was purchased from Boehringer Mannheim. ^{35}S-labelled 30 S and 50 S ribosomal subunits were isolated as in [5]. $AUGU_6$ was synthesized according to [6]. f[^{35}S]Met-$tRNA_f^{Met}$ was obtained as described [6]. 1 A_{260} unit of $tRNA_f^{Met}$ contained 1200 pmol methionine residues and 900 pmol formyl groups. Pt_2AmCl_4 was synthesized as in [7]. The activity of ribosomes in poly(U)-directed $(Phe)_2$ synthesis was determined according to [5]. Initiation complex was obtained as in [8] with

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the following alterations: IF-2 was omitted and Mg^{2+} was at 10 mM. Then the complex and free 70 S ribosomes were treated with 1 mM Pt_2AmCl_4 for 50 min at 20°C. Separation of platinated ribosomes into subunits was performed after dissociation of the initiation complex induced by addition of EDTA as described in [8]. rRNA-protein cross-links were isolated according to [8]. The cross-links were reversed by treatment with 2 M thiourea for 1 h at 37°C and pH 3.5. Ribosomal proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis using a modified method [9]. Unlabelled proteins were used as a carrier. In the first dimension, electrophoresis was carried out in glass capillaries (0.5 × 70 mm). Electrophoresis was performed at 0.5 mA/capillary for 1.5 h in the case of acidic proteins and 1 mA/capillary for 1 h with basic proteins. In the second dimension, electrophoresis was carried out in 0.5 × 70 × 90 mm slabs (22% acrylamide, pH 4.3) at 22 mA for 1 h. In some experiments acidic proteins were analyzed in gels containing 18% acrylamide, pH 4.5 [9], in the second dimension. In this case, the time of electrophoresis was longer (4.5 and 3 h in the first and second dimension, respectively) for the separation of proteins L7/L12 and S6.

3. RESULTS

Binding of fMet-$tRNA_f^{Met}$ to ribosomes in the initiation complex was investigated using unlabelled ribosomes and f[^{35}S]Met-$tRNA_f^{Met}$ via the nitrocellulose filtration technique. Under the desired conditions, 0.5 mol labelled tRNA binds per mol ribosomes; without template, binding is not detected at all. In order to promote rRNA-protein cross-links, free 70 S ^{35}S-labelled ribosomes and the initiation complex 70 S ^{35}S-ribosome · $AUGU_6$ · fMet-$tRNA_f^{Met}$ were treated with 1 mM Pt_2AmCl_4 (20°C, 50 min). In a parallel experiment with unlabelled ribosomes and f[^{35}S]Met-$tRNA_f^{Met}$, it was shown that the level of tRNA binding does not change during the course of incubation with the reagent. Moreover, even preliminary incubation of ribosomes with Pt_2AmCl_4 does not lead to significant inactivation [at least in the system of poly(U)-directed synthesis of $(Phe)_2$]. Therefore, the level of [^{14}C]Phe-$tRNA^{Phe}$ binding to untreated risobomes and those incubated with Pt_2AmCl_4 is 1.77 and 1.36 mol tRNA per mol ribosomes, respectively; the level of $(Phe)_2$ formation on treated ribosomes was not less than 60% of that of the untreated samples. After incubation with Pt_2AmCl_4 ribosomes were dissociated into subunits by centrifugation in a sucrose density gradient (15–30%) under dissociating conditions (1 mM Mg^{2+}). About 10% of 70 S ribosomes were not dissociated into subunits, evidently due to intersubunit cross-links. Isolated subunits as well as undissociated 70 S ribosomes were separated into rRNA and proteins by centrifugation in sucrose density gradient (5–20%) in the presence of EDTA and SDS [8]. rRNA-protein cross-links were detected as ^{35}S-containing material with a sedimentation coefficient somewhat higher than those of the corresponding rRNAs (in control experiments, when Pt_2AmCl_4 was omitted, intersubunit cross-links and ^{35}S radioactivity in rRNA fractions were not detected). When free 70 S ribosomes were treated with Pt_2AmCl_4, 30–40% of ^{35}S radioactivity was found in the fractions of rRNA-protein cross-links in 50 S and 30 S subunits as well as in 30 S-50 S intersubunit cross-links. The corresponding percentage of cross-linking within the initiation complex was found to be somewhat lower (26–33%). ^{35}S-proteins cross-linked to rRNA were analyzed by two-dimensional electropheresis after complete removal of coordinated rRNA by thiourea treatment. The results are shown in figs 1 and 2. A typical electropherogram of ^{35}S-ribosomal proteins after staining of the gel with Coomassie is given in fig.3. In some experiments, additional analysis of acidic proteins (see section 2) was performed to separate proteins L7/L12 and S6.

4. DISCUSSION

The sets of proteins cross-linked to 16 S and 23 S rRNA by Pt_2AmCl_4 in free 70 S ribosomes and 70 S initiation complex are rather similar. Detectable differences concern mainly proteins corresponding to the relatively slight spots in autoradiograms (see figs 1,2). This indicates that the identified rRNA-protein contacts in ribosomes are not significantly altered by interaction with the template and initiator tRNA. Recently, proteins S3, S4, S11, S13/S14 and S18 have been found to be coordinated to 16 S rRNA after treatment of 30 S subunits with *trans*-$Pt(NH_3)_2Cl_2$ [2]. Most (S3, S4, S14, S18) are present in the set of proteins cross-linked to 16 S rRNA by Pt_2AmCl_4. It should be mentioned that the use of Pt_2AmCl_4, containing a flexible spacer group (the distance between the cross-linked functional groups of biopolymers being $\lesssim$15 Å), provides the opportunity to produce other variants of specific cross-links compared to *trans*-$PT(NH_3)_2Cl_2$ [2] which has a rigid, linear

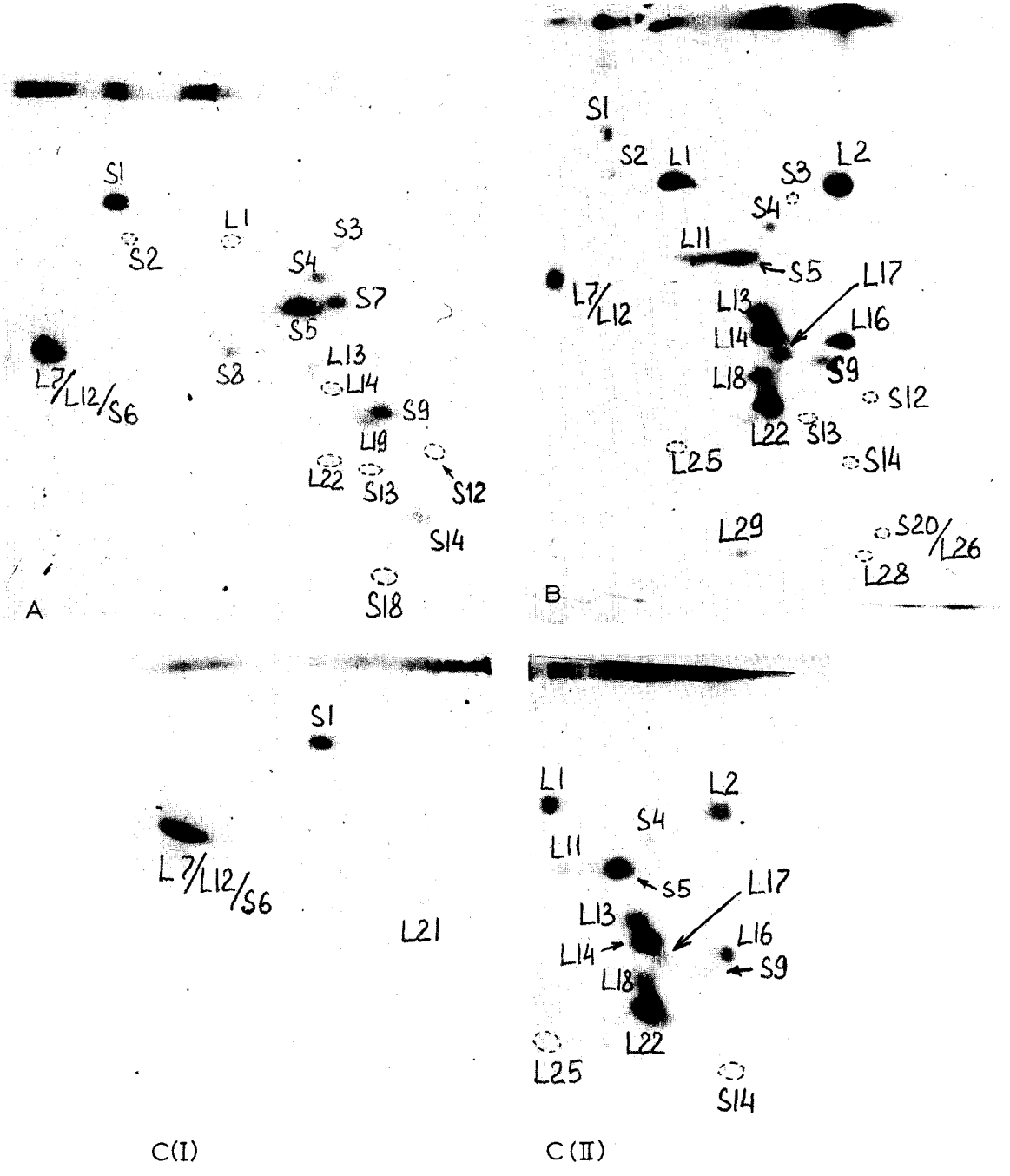

Fig.1. Identification by two-dimensional polyacrylamide gel electrophoresis of ^{35}S-proteins cross-linked to rRNA after treatment of 70 S ^{35}S-ribosomes with Pt_2AmCl_4: in 30 S subunits (A); 50 S subunits (B); 30 S-50 S intersubunit cross-links (C) [I, acidic proteins; II, basic proteins]; autoradiography of the gel.

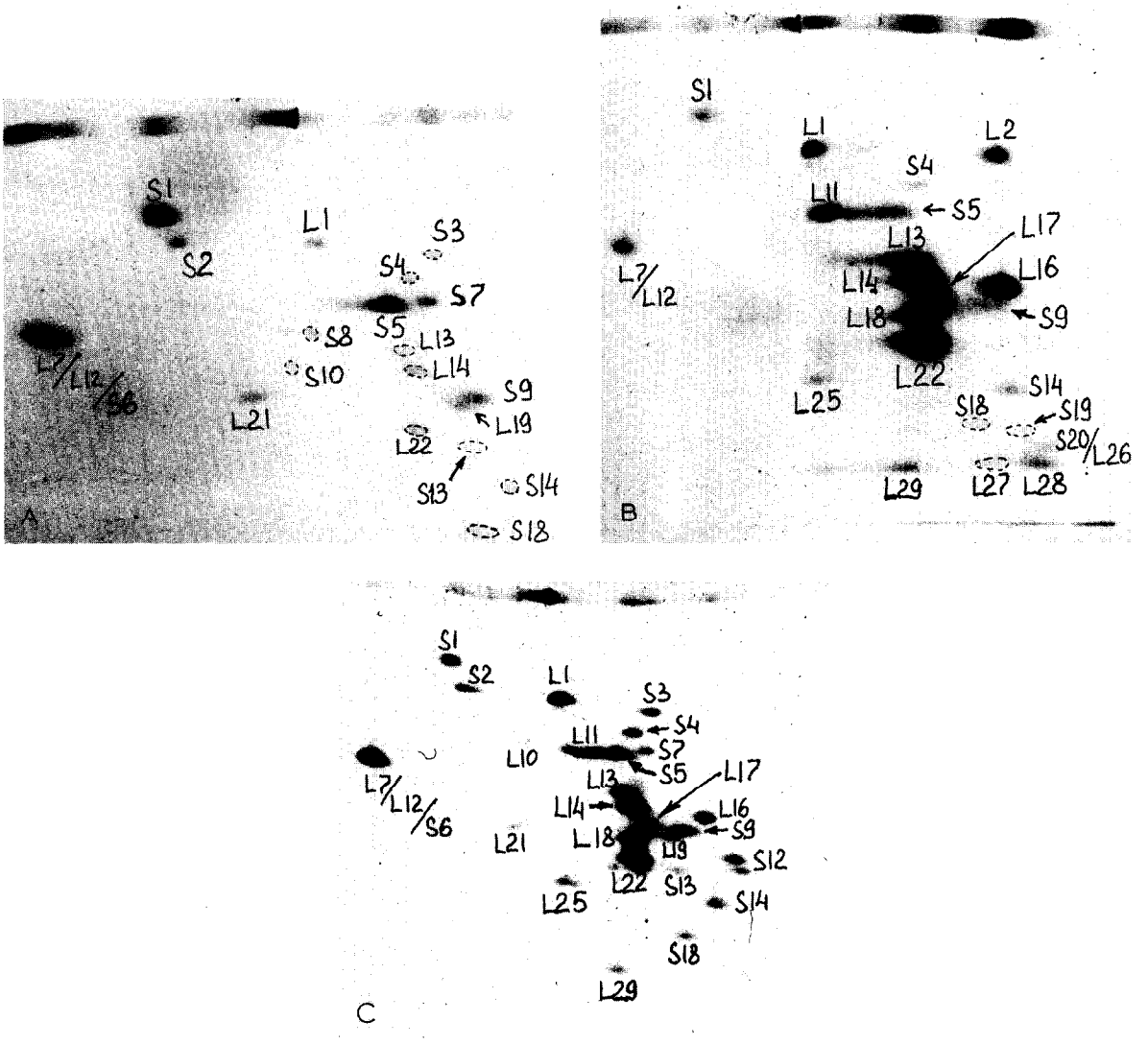

Fig.2. Identification by two-dimensional polyacrylamide gel electrophoresis of ^{35}S-proteins cross-linked to rRNA after treatment of the 70 S initiation complex with Pt_2AmCl_4: in 30 S subunits (A); 50 S subunits (B); 30 S-50 S intersubunit cross-links (C); autoradiography of the gel.

structure (the distance between the cross-linked groups being ~5 Å).

Cross-linking of the majority of proteins to 23 S rRNA (see above) has thus been detected for the first time. Proteins L1, L27 and L29 were found earlier to cross-link to 23 S rRNA [1,10]. In contrast, the neighbourhood of proteins S3, S4, S7, S9 and S18 with 16 S rRNA, and L1 and L2 with 23 S rRNA was previously demonstrated through direct UV-induced RNA-protein cross-links [11–13]. The vicinity of proteins S1, S4, S7, S8 and S12 to 16 S rRNA is supported by chemical cross-linking data [1,10,14]. Close contact between L1 and 16 S rRNA was supported by UV-induced rRNA-protein cross-linking in free 70 S ribosomes as well as within pre- and posttranslocational complexes [12]. Cross-linking of proteins L7/L12 to 16 S rRNA has not previously been observed. It seems

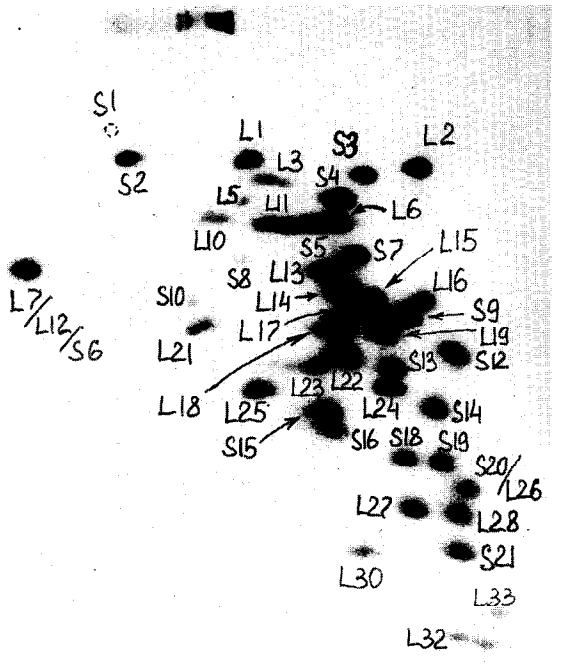

Fig.3. Typical two-dimensional polyacrylamide gel electropherogram of ^{35}S-proteins after staining of the gel with Coomassie.

unlikely that this cross-link arises from the coordination of some 30 S protein to 16 S rRNA, since thus far, no L7/L12-30 S protein cross-links are known to occur. Hence, we assume that the L7/L12 stalk (one of the L7/L12 dimers) is in the vicinity of one of the 16 S rRNA sites (distance $\lesssim 15$ Å) in free 70 S ribosomes as well as in the 70 S initiation complex.

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