Cross-linking Study on Localization of the Binding Site for Elongation Factor 1\(\alpha\) on Rat Liver Ribosomes*

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During protein biosynthesis in eukaryotic cells, the binding of aminoacyl-tRNA to the aminoacyl (A) site of the ribosome-mRNA complex is mediated by EF-1\(\alpha\) (1, 2) and the translocation of newly synthesized peptidyl-tRNA from the A site to the peptidyl (P) site is catalyzed by another factor EF-2 (3-5). However, little is known of the functional roles of individual eukaryotic ribosomal proteins in these elongation steps. For understanding of molecular mechanisms of protein biosynthesis on mammalian ribosomes, it is essential to identify the binding sites for both the elongation factors on ribosomes.

In a recent paper (6), we described the EF-2 binding sites on rat liver 80 S ribosome, employing the cross-linking reagent 2-iminothiolane. Seven 60 S subunit proteins and two 40 S subunit proteins were identified as components cross-linked to EF-2. In this report, we describe binding sites for EF-1\(\alpha\) on 80 S ribosomes by using 2-iminothiolane and discuss the relationship between the binding sites for EF-1\(\alpha\) and EF-2.

**MATERIALS AND METHODS AND RESULTS**

**DISCUSSION**

We have investigated protein neighborhoods in rat liver ribosomal 40 S (14, 16) and 60 S (12, 13, 17) subunits and at the interface of both the subunits by using chemical cross-linking techniques as described in the preceding paper (27). Recently, we identified nine ribosomal proteins cross-linked to EF-2 employing the cross-linking reagent 2-iminothiolane (6). This paper describes the results of an investigation on the binding site for EF-1\(\alpha\) on rat liver 80 S ribosomes. We identified three 60 S subunit proteins and two 40 S subunit proteins as the components cross-linked to EF-1\(\alpha\), indicating that the EF-1\(\alpha\) binding sites are situated at the interface between 40 S and 60 S subunits.

Locations of the proteins cross-linked to EF-1\(\alpha\) on 80 S ribosomes are shown in a possible model in Fig. 8A. Cross-links within 40 S subunits (14, 16, 18, 19) and 60 S subunits (6, 12, 13) and those at the subunit interface (preceding paper) have been identified so far. L12 was cross-linked to LA33 (6). Although cross-links involving L23 and L39 have not yet been found, the present cross-linking results suggest that L23 and L39 are located near L12 in 60 S subunits. S26-S6 cross-linking was identified by Tolan and Traut (18). S23-S24 cross-linking was reported by Terao et al. (14), S23/S24-S6 by Gross et al. (19), and S24-S4 by Tolan and Traut (18). These findings indicate that both S23 and S24 are located near S6 and S4, although we could not distinguish S23 and S24 in this experiment.

Results obtained here were compared with our previous results of cross-linking between EF-2 and ribosomal proteins (6). Fig. 8B shows the locations of proteins cross-linked to EF-2. Proteins L12, L23, and S23/S24 were cross-linked to both EF-1\(\alpha\) and EF-2, indicating that the binding sites for EF-1\(\alpha\) and EF-2 are partially overlapping at or near the A site of ribosomes.

The topographical relation between the binding sites for EF-1\(\alpha\) and EF-2 on 80 S ribosomes has been investigated in experiments on the competition between elongation factors for their binding region on ribosomes. EF-1-dependent \(^{14}C\) phenylalanyl-tRNA binding was prevented when ribosomes were precharged with EF-2 and GuoPP(CH\(\_\))P (20-22). Inversely, EF-2-dependent \(^{3}H\)GDP binding was inhibited when ribosomes were preincubated with EF-1, GTP, and

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1 The abbreviations used are: EF, eukaryotic elongation factor; GuoPP(CH\(\_\))P, guanylyl(\(\delta,\gamma\)-methylene)diphosphonate; SDS, sodium dodecyl sulfate; Phe, phenylalanine.

2 Portions of this paper (including "Materials and Methods," "Results," Figs 1–8, and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-302, cite the authors, and include a check or money order for $6.00 per set of photocopies. Full-size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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phenylalanyl-tRNA (21). Results from similar experiments using tritiated EF-1 and EF-2 by Nolan et al. (15) also suggested that the two factors have a common site or closely overlapping sites on ribosomes. The results of our cross-linking experiment prove these results more directly.

Proteins cross-linked to EF-2 distribute widely on 80 S ribosomes compared to those cross-linked to EF-1α. The higher affinity of EF-2 to ribosomes than EF-1α (15) may be ascribed to the interaction with the wide area on ribosomes, and such a wide interaction may be necessary for the translocation of peptidyl-tRNA from the A site to the P site by EF-2.

In the case of prokaryotic ribosomes, proteins L1, L3 and L7/L12 have been chemically cross-linked to both the elongation factors EF-Tu and EF-G (23, 24).

In conclusion, we identified the binding site for EF-1α on rat liver 80 S ribosomes which is situated at the subunit interface and partially overlapping with the EF-2 binding site.

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REFERENCES


SUPPLEMENTAL MATERIAL

TO CROSS-LINKING STUDY ON LOCALIZATION OF THE BINDING SITE FOR EF-Lα ON RAT LIVER RIBOSOMES

by Tomoh Uchiumi and Kikio Ogata

MATERIALS AND METHODS

Chemicals—2-iminothiolane was purchased from Pierce Chemicals. Hydrogen peroxide (30%) was from Nitrogen Gas Chemicals (Tokyo). L-[methyl-3H]valine (14 Ci/mmol, [3H]-valine) was obtained from New England Nuclear. Poly(U), catalase and dithiothreitol were from Sigma. Guanidinoethylmethylethanolamine (GEMEHCl) was from Bioskine, Philadelphia. All other chemicals were of analytical grade.

Preparation of 80S ribosomes—Poly(U)- and high KCl-treated 80S ribosomes were prepared from rat liver as described previously (16). Obtained 80S ribosomes had no detectable 23S activity when examined by polyacrylamide-SDS gel electrophoresis (8) of the EF-1α preparation showed that it was nearly homogenous and its molecular weight was 45,000.

Preparation of decacylated tRNA and [3H]phenylala—As rat liver tRNA was obtained from purified tRNA (9) by extraction with phenol, saturated with 50% Percoll, pH 7.5, and desalted by fractionation at 37°C for 10 min at pH 8.5 (90). [3H]Phenylalanine (100 Ci/mmol) was obtained as described in Methods (15). Formation of the ribosomes-EF-1α complexes—In a typical experiment 1.20 ml of 80S ribosomes were layered at 37°C for 15 min with 2 mg poly(U) and 12 mg decacylated tRNA in a buffer (46 ml of 12 mM MgCl₂, 0.1 M NaCl, 50 mM NAD, 0.2 mM dithiothreitol and trichloroacetic acid, pH 7.4 (17). The mixture was incubated at 37°C for 5 min as described by Iwasaki and Kaziro (11).
The proteins extracted from the cross-linked complex were separated into 26 fractions by cellulose column chromatography as shown in Fig. 2. Each protein fraction was analyzed by a modified method of diagonal SDS gel electrophoresis which was carried out on the gel containing 9% acrylamide in the first dimension and containing 14% acrylamide in the second dimension. The diagonal patterns of individual fractions showed a number of cross-linked pairs which fell on the vertical lines below the diagonal. Four kinds of new pairs, which contained EF-1α and were not detected when the SDS ribosomes were cross-linked in the absence of EF-1α, appeared in the fractions A to D of the cellulose column chromatography (Fig. 2). The diagonal gel electrophoretic patterns of the fractions A to D are shown in Fig. 4A to Fig. 7A, respectively. Protein spots of each pair were cut out from the gel, and subjected to two-dimensional gel electrophoresis in the basic-acidic system (Fig. 4B) and/or the basic-SDS system (8) with the addition of unlabeled total ribosomes. Fig. 2 shows the two-dimensional gel patterns. All the basic proteins were detected for L17/L17s, S23/S24 and S8/L13 could be identified unambiguously with both the gel systems.

Fig. 4 shows the analyses of the cross-linked pair involving EF-1α in fraction A. The diagonal gel pattern (Fig. 4A) shows the distinct cross-linked pair with the vertical arrow. The protein components of the pair were identified as EF-1α and L12 by autoradiography of the two-dimensional gel with the basic-SDS system (Fig. 4B). It was found that the cross-linked pairs involving EF-1α were formed and completely overlapped as a pair of the diagonal gel (Fig. 5A), since two proteins L13 and L26 had the same molecular weight. The analyses in fraction C, the extremely basic protein L19 was found to be cross-linked to EF-1α (Fig. 6). In the analyses of fraction D, the component cross-linked to EF-1α was identified as S23/S24 with both the basic-acidic and basic-SDS systems.

Results on identification of proteins cross-linked to EF-1α and their molecular weight data are summarized in Table I. The molecular weights of cross-linked protein dimers and their components were estimated from their migration distances in the first and second dimensions of diagonal gel electro- phoresis, respectively (13).
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Fig. 5. Analyses of the cross-linked pair involving EF-1α in fraction B. (A) Diagonal gel electrophoretic pattern. (B) Identification of the cross-linked proteins by two-dimensional gel electrophoresis in the basic-acidic system (upper panel) and the basic-SDS system (lower panel).

Fig. 6. Analyses of the cross-linked pair involving EF-1α in fraction C. (A) Diagonal gel electrophoretic pattern. (B) Identification of the cross-linked proteins in the basic-SDS gel system.

Fig. 7. Analyses of the cross-linked pair involving EF-1α in fraction D. (A) Diagonal gel pattern. (B) Identification of the cross-linked proteins with both the basic-acidic and basic-SDS gel systems.

Fig. 8. Possible arrangement of ribosomal proteins at the binding sites for EF-1α (A) and EF-2 (B). Cross-links within 60S subunit (12, 13) and 40S subunit (14, 15, 16, 17, 18) have been identified until now. Cross-links between 40S and 60S subunit proteins are described in the preceding paper.

Table I

<table>
<thead>
<tr>
<th>Protein Cross-linked to EF-1α</th>
<th>Mr of cross-linked dimer</th>
<th>Sum of Mr of cross-linked components</th>
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<tbody>
<tr>
<td>L12</td>
<td>72,000</td>
<td>64,000</td>
</tr>
<tr>
<td>L23</td>
<td>61,000</td>
<td>63,000</td>
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<tr>
<td>L39</td>
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<td>59,000</td>
</tr>
<tr>
<td>S23/S24</td>
<td>71,500</td>
<td>63,000</td>
</tr>
<tr>
<td>S26</td>
<td>61,000</td>
<td>62,000</td>
</tr>
</tbody>
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