The proteins of the messenger RNA binding site of *Escherichia coli* ribosomes

O.I.Gimautdinova, G.G.Karpova, D.G.Knorre and N.D.Kobetz

Institute of Organic Chemistry, Siberian Division of the Academy of Sciences of the USSR, Novosibirsk, 630090, USSR

Received 8 June 1981

ABSTRACT

Oligo(U) derivatives with $[^{14}\text{C}]-4-(\text{N}-2\text{-chloroethyl-N-methylamino})\text{benzaldehyde}$ attached to 3'-end cis-diol group via acetal bond, $\text{p(Up)}_{n-1}\text{UCHRC1}$ as well as with $[^{14}\text{C}]-4-(\text{N}-2\text{-chloroethyl-N-methylamino})\text{benzylamine}$ attached to 5'-phosphate via amide bond, $\text{ClRCH}_2\text{NHpU(pU)}_6$ were used to modify 70S *E.coli* ribosomes near mRNA binding centre. Within ternary complex with ribosome and tRNA^{Phe} all reagents covalently bind to ribosome the extent of modification being 0.1-0.4 mole/mole 70S. $\text{p(Up)}_{n-1}\text{UCHRC1}$ alkylates either 30S ($n=5,7$) or both subunits ($n=6,8$). rRNA is preferentially modified within 30S subunit. $\text{ClRCH}_2\text{NHpU(pU)}_6$ alkylates both subunits the proteins being mainly modified. The distribution of the label among proteins differ for various reagents. S4, S5, S7, S9, S11, S13, S15, S18 and S21 are found to be alkylated within 30S subunit, proteins L1, L2, L6, L7/L12, L19, L31 and L32 are modified in the 50S subunit.

Most proteins modified within 30S subunit are located at the "head" of this subunit and proteins modified within 50S subunit are located at the surface of the contact between this subunit and the "head" of 30S subunit at the model of Stoffler.

INTRODUCTION

The general methods of the attachment of the reactive aromatic 2-chloroethylamino group either to 3'-end or to 5'-end of oligoribonucleotides were elaborated by N.I. Grineva and coworkers. The former is based on the attachment of 4-(N-2-chloroethyl-N-methylamino)-benzaldehyde to 3'-end cis-diol group via acetal bond¹⁻². The latter uses the binding of 4-(N-2-chloroethyl-N-methylamino)-benzylamine to 5'-phosphate via amide linkage³. The oligo(A) derivatives of the former type $(\text{Ap})_{n-1}\text{ACHRC1}$ were demonstrated to stimulate Lys-tRNA binding to ribosomes in the case of $n > 3$ ⁴. In the ternary complex 70S ribosome $(\text{Ap})_{n-1}\text{ACHRC1}$ Lys-tRNA the reagent was

found to alkylate 30S subunit 16S rRNA being modified preferentially⁵. Similar results were obtained with oligo(U) derivatives⁶. With the reagent bearing reactive group at 5'-end, ClRCH₂NHpa(pa)₆ affinity labelling of ribosomes has resulted in the significant modification of 50S subunit thus indicating that mRNA-binding centre is located in the vicinity of 50S subunit⁷.

The present paper deals with the determination of the proteins which are alkylated by the reagents of both above mentioned types within ternary complex of 70S ribosome of E.coli, oligouridilate derivative and tRNA^{Phe}.

MATERIALS AND METHODS.

Polyuridilic acid, tRNA and ribosomes from E.coli MRE600 were from Special Technology Design Bureau for Biologically Active Compounds (Novosibirsk). E.coli tRNA^{Phe} (Boehringer, Mannheim, F.R.G.), [¹⁴C]-phenylalanine 180-200 mCi/mmol (Chemapol, CSSR) were used.

Oligouridilates were obtained as described in⁸. [¹⁴C]-4-(N-2-chloroethyl-N-methylamino)-benzaldehyde with labelled formyl group was obtained as described in⁹.

E.coli tRNA was charged with [¹⁴C]-phenylalanine by commonly used procedure¹⁰.

The following buffers were used:

A: 0.1M tris-HNO₃, pH 7.3, 0.05M LiCl, 0.03M Mg(NO₃)₂,

B: 0.05M tris-HCl, pH 7.5, 0.1M NH₄Cl, 0.5mM MgCl₂,

C: 0.01M tris-HCl, pH 7.8, 7M urea,

D: 0.05M NaH₂PO₄, pH 5.8, 6M urea, 0.012M methylamine,

0.012M 2-mercaptoethanol,

E: 0.01M tris-HCl, pH 7.4, 0.01M MgCl₂,

F: 0.05M Na₂B₄O₇, pH 9.02, 7M urea.

All buffers were stirred for 1 hour with 2% (w/v) bentonite and centrifuged at 15000 rpm as described in¹¹.

[¹⁴C]-4-(N-2-chloroethyl-N-methylamino)benzylidene derivatives of oligouridilates p(Up)_{n-1}UChRCl, n=5,6,7,8 with specific radioactivity 12 mCi/mmol were obtained according to¹. 0.1M oligonucleotide solution in dimethylformamide was treated at -70° with [¹⁴C]-4-(N-2-chloroethyl-N-methylamino)-ben-

zaldehyde and 0.5M dimethoxypropane. The reaction mixture was incubated at 20° for 45 min., neutralised with triethylamine at -70° and then $p(\text{Up})_{n-1}\text{UCHRC1}$ was precipitated with ether as $(\text{C}_2\text{H}_5)_3\text{NH}^+$ salt. The precipitate was dissolved in methanol and precipitated with ether. The product contained 85-95% of $p(\text{Up})_{n-1}\text{UCHRC1}$ and 5-15% of $(p\text{U})_n$.

$[^{14}\text{C}]\text{-5'-P-4-(N-2-chloroethyl-N-methylamino)benzylamide of } (p\text{U})_7$ was prepared as described in³.

Ribosomal subunits were separated by centrifugation in the sucrose gradient 10-30% in the buffer B similar to¹².

The oligouridilate derivative dependent binding of $[^{14}\text{C}]\text{-Phe-tRNA}$ to ribosome was carried out as follows: 100 μl of the reaction mixture containing 220 pmol of each of 30S and 50S subunits, 7-8 A_{260} units of $[^{14}\text{C}]\text{-Phe-tRNA}$ (40 pmoles of $[^{14}\text{C}]\text{-Phe}/A_{260}$ unit), 10-20 μg of either poly(U) or oligouridilate derivative in buffer A were incubated at 25° for 20 min. Complex was separated by gel-filtration on Sephadex G-50 (0.4 x 10 cm) equilibrated with buffer A, retained on nitrocellulose filter according to Nirenberg and Leder¹³ and counted. Data are given in the Table 1.

Affinity labelling of ribosomes was carried out in the ternary complex "70S ribosome. oligouridilate derivative. tRNA^{Phe} ". 1 ml of the reaction mixture containing 0.002 moles of each of ribosomal subunit, 0.04-0.08 moles of the oligouridilate derivative and 0.008-0.01 moles of tRNA^{Phe} (E.coli) in the buffer A was incubated for 20 min. at 25°. In the experiments with the competitive inhibition of the affinity labelling by poly(U) and $(p\text{U})_{14}$ were taken in the four-fold weight excess to the reagent.

The ternary complex was separated by gel-filtration on the column with Sephadex G-50 (1x20 cm) equilibrated with buffer A. Alkylation of ribosomes proceeded within the ternary complex for 24 hours for derivatives bearing the reactive group at 3'-end and for 19 hours for the reagent with the reactive group attached to 5'-end phosphate.

Analysis of the extent of modification of ribosomal subunits. The modified ribosomes were precipitated with 0.7 volume of ethanol. The precipitate was dissolved in buffer B

and ribosomal subunits were separated by centrifugation in the sucrose gradient 10-30% in the same buffer (at 25000 rev/min in Spinco SW 27 rotor for 17 hours). 1 ml fractions were collected and absorbancy at 260 nm and radioactivity of each fraction were determined. Fractions containing subunits were joined, magnesium concentration was raised up to 0.01M and subunits were precipitated with ethanol.

Analysis of the extent of modification of ribosomal proteins and ribosomal RNA within subunit. The subunit pellets were dissolved in buffer B. Equal volume of 4M LiCl in 8M urea, containing 0.5 mM 2-mercaptoethanol was added to the modified subunit solution and the mixture was incubated at 0° during 24 hours. The precipitate of ribosomal RNA was separated from ribosomal protein supernatant by centrifugation at 5000 rev/min for 10-15 min, washed twice with 2M LiCl in 4M urea and dissolved in 0.01M tris-HCl, pH 7.2. The solution was shaken with equal volume of 80% phenol for 15 min at 4°C and centrifuged for 20 min at 5000 rev/min. The aqueous layer was taken and RNA was precipitated by the addition of 2 volumes of absolute ethanol. The RNA-pellet was resuspended in a small volume of 0.01M tris-HCl, pH 7.2 and then RNA was precipitated once more by 2 volume of ethanol. The precipitate was washed twice by ethanol and ether to remove phenol, dissolved in 0.01M tris-HCl, and radioactivity of the solution was measured. To determine the radioactivity bound to ribosomal protein aliquote of the protein supernatant in 2M LiCl - 4M urea was incubated with 5% TCA at 90° for 15 min to hydrolyze RNA, afterwards the precipitate of proteins was collected by filtration on a nitrocellulose membrane filter (AUFS, "Chemapol", CSSR) and washed three times with 5 ml of 5% TCA, the filter was dried and the radioactivity sorbed on the filter was determined in the scintillation counter (Mark-2, "Nuclear Chicago", USA).

Preparation of ribosomal proteins.

Method 1 was that of Hardy S.J.S. and Kurland C.G.¹⁴ One-tenth volume of 1M MgCl₂ was added to one volume of ribosomes followed by rapid addition of two volumes of glacial acetic acid at 0-2° under vigorous stirring. The suspension was

stirred in the cold for 45 minutes. The RNA precipitate was removed by centrifugation at 6000 rev/min for 15 minutes. The RNA pellet was washed with 67% acetic acid containing 32 mM MgCl_2 , and two supernatants were pooled. Supernatant, containing proteins modified with $[\text{}^{14}\text{C}]\text{-ClRCH}_2\text{NHpU(pU)}_6$ was incubated for 3 hours at 40° to cleave phosphamide bond. Supernatant, containing proteins modified with $[\text{}^{14}\text{C}]\text{-p(Up)}_{n-1}\text{UCHRC1}$ was incubated for 1 hour at 25° to cleave acetal bond. The above treatments resulted in the splitting of the oligonucleotide moiety off the proteins. (Ribosomal proteins were shown to be stable under conditions as revealed by subsequent 2-dimensional gel electrophoresis). Then 4 volumes of cold acetone were added to protein supernatant, mixture was incubated at -20°C for 20-24 hours and protein pellet was separated by centrifugation, washed by acetone and dried in vacuum.

Method 2 was used for identification of proteins of 30S subunits modified with $[\text{}^{14}\text{C}]\text{-p(Up)}_{n-1}\text{UCHRC1}$ to enhance radioactivity of these proteins by reductional alkylation. The LiCl-urea protein supernatant (see "Analysis of modification of ribosomal proteins and rRNA") containing modified proteins with oligonucleotide derivative covalently bound with protein was exhaustively dialyzed against buffer C and applied to column ($0.6 \times 5 \text{ cm}$) with DEAE-cellulose equilibrated with buffer C. Unmodified proteins (except S1 and S6) were eluted by buffer C and then modified proteins containing oligonucleotide fragment were eluted by 0.5M NaCl in the same buffer. Fractions, containing $[\text{}^{14}\text{C}]\text{-radioactivity}$ were pooled and after that the cleavage of the oligonucleotide moiety off the proteins was performed. Supernatant containing proteins modified with $[\text{}^{14}\text{C}]\text{-p(Up)}_{n-1}\text{UCHRC1}$ was incubated at 40° , pH 4 for 1 hour; supernatant containing proteins modified with $[\text{}^{14}\text{C}]\text{-ClRCH}_2\text{NHpU(pU)}_6$ was incubated at 40° , pH 1 for 3 hours. Then solutions, containing proteins were neutralized with alkali to pH 7, dialyzed against buffer F, concentrated with Ficoll ("Pharmacia", Sweden) and subjected to reductional alkylation and subsequent 2-dimensional gel electrophoresis (see below). The mixture of unmodified 30S proteins was subjected to the same set of procedures and only S1 and S6 were found

to be retained by the resin in control experiment.

Reductional alkylation of 30S modified proteins.

2% solution of HCHO was added to solution of modified proteins in buffer F to concentration $0.001M^{15}$. This mixture was incubated at $0-4^{\circ}$ for 2 minutes, $[^3H]$ - $NaBH_4$ (specific activity 1 Ci/mmol, "Isotop", USSR) was added to concentration 0.002M. The procedure was repeated twice. $[^3H]$ -proteins were exhaustively dialyzed against buffer D and then passed through the column with phosphocellulose (Mannex P, High capacity) in the same buffer to separate $[^3H]$ -proteins from other radioactivity. The latter was eluted by buffer D and $[^3H]$ -proteins were eluted by 1M NaCl in buffer D and dialysed against "Starting buffer"¹⁶.

2-dimensional gel electrophoresis of ribosomal proteins was performed according to Kaltschmidt and Wittmann¹⁶ with small changes: dimensions of 1D and 2D-gels were approximately as recommended by Howard and Traut¹⁷ with respective changes in current and voltage as well of the running time and amount of proteins in the sample. This system gives complete resolution of the whole set of 70S ribosomal proteins except L8 and L9.

Proteins were eluted from the gel by 0.5% solution of SDS. Radioactivity was determined in scintillation counter.

RESULTS.

It was demonstrated earlier that $(Ap)_{n-1}ACHRC1$ stimulates Lys-tRNA binding with E.coli ribosomes⁴. At $n=3,4$ the stimulation is lower than that of respective $(Ap)_{n-1}A$. This may be due to some distortion of conformation of two nucleotide residues adjacent to benzylidene moiety of the reagent resulting in the decrease of their ability to base pairing with anticodon of tRNA. At $n=5$ there is no difference between Lys-tRNA binding with ribosomes in the presence of either $(Ap)_4A$ or $(Ap)_4ACHRC1$. Therefore in the present investigation we have chosen $p(Up)_{n-1}UCHRC1$ derivatives with $n=5-8$.

Affinity labelling of ribosomes was performed using 70S ribosomes obtained by association of 30S and 50S subunits as described in "Materials and Methods". The extent of reassociation is not lower than 95%. Typical results demonstrating

the ability of ribosomes to bind $[^{14}\text{C}]$ -Phe-tRNA in the presence of oligo(U) derivative as well as to bind $[^{14}\text{C}]$ -p(Up)_{n-1} UCHRC1 in the presence of non-acylated tRNA are presented in the Table 1.

It is seen that the ability of reassociated ribosomes to bind $[^{14}\text{C}]$ -Phe-tRNA in the presence of poly(U) is nearly the same as that of starting 70S ribosomes. Binding of $[^{14}\text{C}]$ -Phe-

Table 1.

Mutual stimulation of $[^{14}\text{C}]$ -Phe-tRNA and $[^{14}\text{C}]$ -p(Up)₄UCHRC1 non-covalent binding to ribosomes.

30S 50S	70S	p(Up) ₄ - UCHRC1	polyU	Phe-tRNA	tRNA	moles bond per 1 mole of ribosomes	
						Phe-tRNA	p(Up) ₄ UCHRC1
+	-	-	+	+	-	0.300*	
-	+	-	+	+	-	0.310*	
+	-	+	-	+	-	0.236**	
+	-	+	-	-	+		0.61***

* Backgrounds corresponding to the binding of $[^{14}\text{C}]$ -Phe-tRNA in the absence of poly(U) (0.05mol./mole ribosomes) are subtracted.

** Backgrounds corresponding to the binding of $[^{14}\text{C}]$ -Phe-tRNA in the absence $[^{14}\text{C}]$ -p(Up)₄UCHRC1 (0.05 mol./mole of ribosomes or 3600 cpm per 8 A₂₆₀ units of ribosomes) and backgrounds corresponding to the binding of $[^{14}\text{C}]$ -p(Up)₄UCHRC1 in the presence of non-labelled tRNA (2900 cpm per 8 A₂₆₀ units of ribosomes) are subtracted.

*** Backgrounds corresponding to the binding of $[^{14}\text{C}]$ -p(Up)₄-UCHRC1 in the absence of tRNA (0.06 mol. per 1 mole of ribosomes) are subtracted.

-tRNA in the presence of $p(\text{Up})_4\text{UCHRC1}$ is 25% lower than that in the presence of poly(U). The results obtained with other reagents used in this paper are nearly the same.

In the presence of non-acylated tRNA ribosomes bind 0.6 moles of $[^{14}\text{C}]-p(\text{Up})_4\text{UCHRC1}$. The level of binding of other reagents does not differ significantly. This level may be enhanced up to 0.7-0.8 moles per mole ribosomes using tRNA^{Phe} instead of unfractionated tRNA.

To perform specific alkylation of ribosomes the ternary complex " $70\text{S} \cdot [^{14}\text{C}]-p(\text{Up})_{n-1}\text{UCHRC1} \cdot \text{tRNA}^{\text{Phe}}$ " was separated from the excess reagent by gel-filtration. Complex was incubated at 25° for 24 hours. This time corresponds approximately to 1.5 half times of the conversion of 2-chloroethylamino group to ethylenimmonium cation¹⁸ which is the rate limiting step of the overall alkylation process

The more prolonged incubation results in the essential loss of the reagent off the complex.

It is worth mention that concentration of the complex in the incubation mixture $4\mu\text{M}$. Consequently concentration of the reagent outside the complex is still lower and any modification outside the complex is negligible.

The incubated mixture was centrifuged in the sucrose gradient at low magnesium concentration to separate the ribosomal subunits. In these conditions the unreacted oligonucleotide derivative is completely separated from the subunits⁵. Therefore the radioactivity found in the subunit fractions represents covalently bound reagent. The extents of modification of subunits by the reagents are presented in the Table 2.

In order to demonstrate the specificity of alkylation similar experiments were performed in the presence of 4-fold excess (in moles of the nucleotide residues) of poly (U). The data are given in the same table. It is seen that poly (U) significantly protects ribosomal subunits against modification.

To estimate the distribution of the label between rRNA

Table 2.

The extent of modification of ribosomal subunits

R e a g e n t	Extent of modification of subunits in moles of reagent per mole of ribosomal subunit			
	30S		50S	
	without poly(U)	with poly(U)*	without poly(U)	with poly(U)
$[^{14}\text{C}] - \text{p}(\text{Up})_4 \text{UCHRC1}$	0.100	0.038	0.018	0.014
$[^{14}\text{C}] - \text{p}(\text{Up})_5 \text{UCHRC1}$	0.130	0.028	0.230	0.036
$[^{14}\text{C}] - \text{p}(\text{Up})_6 \text{UCHRC1}$	0.053	0.026	0.011	0.009
$[^{14}\text{C}] - \text{p}(\text{Up})_7 \text{UCHRC1}$	0.158	0.018	0.240	0.040
$[^{14}\text{C}] - \text{ClRCH}_2\text{NHpU}(\text{pU})_6$	0.130	0.006	0.120	0.006

* $(\text{pU})_{14}$ was used as inhibitor in the case of

and proteins within subunits RNA was precipitated by adding to the modified subunit solution the equal volume of 4M LiCl in 8M urea and the radioactivity of both precipitate and supernatant was counted. The results are shown in the Table 3.

It is seen that within 30S subunits 16S rRNA is predominantly modified with $\text{p}(\text{Up})_{n-1} \text{UCHRC1}$ whereas proteins are mainly labelled with $\text{ClRCH}_2\text{NHpU}(\text{pU})_6$. Proteins are modified predominantly within 50S subunit by the reagents of both types.

To determine the distribution of the label among proteins of the 30S subunit modified with $\text{p}(\text{Up})_{n-1} \text{UCHRC1}$ we have separated most of the labelled proteins by DEAE-chromatography at neutral pH in 7M urea. It is known that among native proteins of the small subunit only S1 and S6 are retained at the column in these conditions ¹⁹. The binding of 6-9 additional negative charges present in the reagents results in the change of the net charge of all 19 remaining proteins from positive to negative as revealed by the amino acid composition of these

Table 3.

Distribution of radioactive label among structural components of ribosomal subunits

Reagent	the extent of modification (in %) of the rRNA and ribosomal proteins within subunit			
	30S		50S	
	rRNA	protein	rRNA	protein
$[^{14}\text{C}]\text{-p(Up)}_4\text{UCHRC1}$	88	12	-	-
$[^{14}\text{C}]\text{-p(Up)}_5\text{UCHRC1}$	80	20	15	85
$[^{14}\text{C}]\text{-p(Up)}_6\text{UCHRC1}$	70	30	-	-
$[^{14}\text{C}]\text{-p(Up)}_7\text{UCHRC1}$	90	10	20	80
$[^{14}\text{C}]\text{-ClR-CH}_2\text{NHpU(pU)}_6$	10	90	18	82

proteins ²⁰. Therefore the above procedure permit to separate all modified proteins from nonmodified ones except S1 and S6. Really all ^{14}C radioactivity in the labelled 30S subunit proteins is retained on the DEAE-column. This radioactivity may be eluted by 0.5M NaCl. The separated modified proteins were additionally labelled by reductive alkylation with CH_2O in the presence of $[\text{}^3\text{H}]\text{-NaBH}_4$ ¹⁵.

The proteins from the 30S subunit modified with $\text{ClRCH}_2\text{NHpU(pU)}_6$ as well as proteins of the 50S subunit were extracted from the subunits by conventional treatment with 67% acetic acid.

The proteins were separated using the traditional two-dimension gel electrophoresis procedure ¹⁶. Prior to electrophoresis the oligonucleotide moiety of the reagents was splitted off by treatment at acidic pH.

The distribution of the radioactivity among proteins of the small subunit is given in the Fig.1. It is seen that the sets of proteins are different for the reagents of different length. The single protein modified by all $\text{p(Up)}_{n-1}\text{UCHRC1}$ is

Fig.1. Distribution of radioactive label among 30S-ribosomal proteins modified with: a) $[^{14}\text{C}]\text{-p(Up)}_4\text{UCHRC1}$, b) $[^{14}\text{C}]\text{-p(Up)}_5\text{UCHRC1}$, c) $[^{14}\text{C}]\text{-p(Up)}_6\text{UCHRC1}$, d) $[^{14}\text{C}]\text{-p(Up)}_7\text{UCHRC1}$, e) $[^{14}\text{C}]\text{-ClRCH}_2\text{NHpU(pU)}_6$

S9. The most narrow distribution of the label was obtained with $\text{p(Up)}_6\text{UCHRC1}$. It is worth mention that the reagent of the same length bearing the reactive group at the opposite end of the oligonucleotide chain modifies completely different set of proteins.

The distribution of the label between the proteins of the large subunit is presented in Fig.2. Again it is seen that the set of the labelled proteins varies with the change of the length of the reagent as well as of the point of the attachment of the reactive group.

DISCUSSION

The alkylating derivatives of oligouridilates used in the

Fig.2. Distribution of radioactive label among 50S-ribosomal subunits modified with : a) $[^{14}\text{C}]\text{-p(Up)}_5\text{UCHRC1}$, b) $[^{14}\text{C}]\text{-p(Up)}_7\text{UCHRC1}$, c) $[^{14}\text{C}]\text{-ClRCH}_2\text{NH}_2\text{(pU)}_6$.

present paper are rather efficient in the alkylation of ribosomes 0.06–0.4 moles of the reagent being covalently bound to ribosome. The alkylation is specific for the mRNA binding region as revealed by the protective action of poly(U) and oligouridilate.

Three reagents alkylate both ribosomal subunits in nearly equal extent or even with some preference to 50S subunit. This means that mRNA analogs occupy the region adjacent to the area of the contact between subunits.

Nine proteins (S1, S3, S4, S5, S11, S12, S13, S18, S21) were found to be labelled by reactive mRNA analogs in previous investigations. The survey of the data is given in ²¹. We did not look by our technique the labelling of S1. In no case we have seen the labelling of S3 found in ²¹ and of S12 found in ^{22,23}. In addition to previously described proteins we have obtained significant modification of S7, S9, S14, S15.

The set of proteins modified by the reagents depends both on the point of attachment of the reactive group and on the length of oligouridilate moiety. The latter result differs

from those presented in ²¹ for the set of oligoadenylate bearing photoreactive p-azido benzoylhydrazide residue attached to the oxydised by NaIO₄ 3'-end of oligoadenylates. These derivatives were found to modify only proteins S3 and S5 irrespective to the length of the oligoadenylate moiety. However it should be mention that in ²¹ the level of the oligonucleotide binding to ribosomes (0.1-0.2 mole per 1 mole ribosomes) and especially the extent of modification (0.007 mole of the reagent covalently attached to 1 mole of ribosomes) were very low as compared with our data. Therefore, some proteins could be missed in the course of investigation.

The difference in the set of proteins modified with p(U)_{n-1}UCHRC1 of various length means that position of the reactive group in the ternary complex with ribosome and tRNA depends on the length of oligonucleotide moiety of the reagent.

Discussing the possible reasons of these differences it should be taken into account that nucleoside residue bearing the reactive group is unable to participate in the codon-anticodon interactions probably due to severe distortion of the nucleoside conformation²⁴. Therefore, penta- and hexauridilate derivatives may interact only with one anticodon on the ribosome. As deacylated tRNA was used in our experiments this meant that P site was occupied by the reagents²⁵. Two alternative positions of p(U)₄UCHRC1 and three positions of p(U)₅UCHRC1 are schematically presented in Fig.3 (A and B). As we used in our experiments significant excess of tRNA both P and A site should be occupied by p(U)₆UCHRC1 and p(U)₇UCHRC1. In the former case only one possibility exists presented in Fig.3(C). This may explain more sharp distribution of the label in this case (only two proteins are modified in 30S subunit, 50S subunit is not labelled). The strong similarity of the sets of proteins modified by p(U)₅UCHRC1 and p(U)₇UCHRC1 may be due to similar dimensions of the compact double stranded conformation in the latter case and longer flexible single-stranded oligo(U) fragment in the former.

The sets of proteins modified with heptauridilate derivatives with the reactive group at either 5'- or 3'-end are completely different. In the former case S5, S11 and S13 are

Fig.3. Putative location of analogs of mRNA on ribosome at the binding in ternary complex "ribosome . oligo(U) derivative . tRNA".

alkylated. This is in agreement with ²² where it was found that $BrCH_2CONHC_6H_4OpApUpG$ being at P site modifies S11 and S13. When 2-chloroethylamino group was attached to 3'-end of heptauridilate S9 and S18 were modified. S18 was demonstrated to be alkylated inside the ribosome by oligonucleotides containing 3'-terminal 5-haloacetamido uridine ²³ as well as by $BrCH_2CONHC_6H_4OpUpGpA$ ²⁶ supposed to occupy the A site. Thus this protein occupy A-site and 3'-side of the mRNA binding region. S9 was not found in these works probably due to lack of cysteine residues in this protein ²⁷.

Several models of the ribosome structure were proposed describing the position of some of the ribosomal proteins at

the ribosomal subunits ^{28,29}. These models are based mainly on the data of immunoelectron microscopy. In Fig.4 the model of Tischendorf and Stöffler is presented ²⁸ which is the most full as to the number of the localised antigenic determinants. It is seen that all proteins found in our work to be modified with mRNA analogs occupy the "head" of 30S subunit.

The antigenic determinants of S9 and S18 which are found to be at the 3'-side of mRNA binding center are rather far one from the other. However S18 is an extended protein and it may protrude out of the ribosome in some additional points

Fig.4. Location of the proteins modified by oligo(U) derivatives as well as of some proteins discussed in the text on the Tischendorf-Stöffler models of both ribosomal subunit.

not revealed by immunoelectron microscopy located closer to S9. It was also demonstrated that S18 may be cross-linked with rather short bifunctional reagent with S21^{30,31} which is close to S9 at the model under consideration.

The 5'-side of the mRNA binding region contains according to our data the proteins S5, S11 and S13. The antigenic determinants of S5 and S11 are rather close one to another as well as determinant of S12 which was found to react with $\text{BrCH}_2\text{CONHC}_6\text{H}_4\text{OpApUpG}$ in the P site. S13 is cross-linked with S11 and S5³². Therefore again we may suggest that some part of this protein is located close to these proteins in spite of the absence of its antigenic determinants in this region.

As to proteins modified in the 50S subunit L1, L2, L6, L7/L12 and L19 occupy according to Tischendorf and Stöffler the region of the contact between 50S subunit and the head of 30S subunit. The localisation of L31 and L32 found to be alkylated in this work is unknown. However L32 cross-links with L18 and L19 which are in the same region of 50S subunit^{33,34}.

Thus we may conclude that at the considered model mRNA binding center occupies the left side of the "head" of 30S subunit with mRNA extended from the left side (5'-end) to the front of the head (3'-end). Some proteins of 50S subunit either participate in the formation of this center or are quite close to it.

REFERENCES

1. Grineva, N.I., Zarytova, V.F. (1968) Izv.Sib.Otd.Akad.Nauk SSSR, ser.khim.nauk, iss.5, 125-129.
2. Rytte, V.K., Karpova, G.G., Grineva, N.I. (1977) Bioorgan.Khimiya, 3, 31-38.
3. Gimautdinova, O.I., Grineva, N.I., Karpova, G.G., Lomakina, T.S. Shelpacova, E.L. (1978) Bioorgan.Khimiya, 4, 917-927.
4. Budker, V.G., Knorre, D.G., Yasnova, S.N. (1972) Molek.Biol., 6, 581-585.
5. Budker, V.G., Girshovich, A.S., Grineva, N.I., Karpova, G.G., Knorre, D.G., Kobetz, N.D. (1973) Dokl.Akad.Nauk SSSR, 211, 725-728.
6. Budker, V.G., Gimautdinova, O.I., Karpova, G.G., Kobetz, N.D., Teplova, N.M. (1976) Molek.Biol. 10, 340-346.
7. Budker, V.G., Grineva, N.I., Kobetz, N.D., Lomakina, T.S., Maev, S.P. (1978) Biokhimiya, 43, 761-763.

8. Vasilenko, S.K., Serbo, N.A., Venyaminova, A.G., Boldyreva, L.G., Budker, V.G., Kobetz, N.D. (1976) Biokhimiya, 41, 260-263.
9. Belikova, A.M., Vakhrusheva, T.E., Vlassov, V.V., Grineva, N.I., Knorre, D.G., Kurbatov, V.A. (1969) Molek. Biol. 3, 210-220.
10. Knorre, D.G., Sirotiyuk, V.I., Stephanovich, L.E. (1967), Molek. Biol. 1, 837-843.
11. Venkstern, T.V., Li, L., Krutilina, A.I., Axelyrod, V.D., Mirzabekov, A.D., Baev, A.A. (1968) Molek. Biol., 2, 597-611.
12. Gavrilova, L.P., Ivanov, D.K., Spirin, A.S. (1966) J. Mol. Biol. 16, 473-489.
13. Nirenberg, M.W., Leder, P. (1964) Science, 145, 1399-1400.
14. Hardy, S.J.S., Kurland, C.G., Voynow, P., Mora, G. (1969), Biochemistry, 8, 2897-2905.
15. Moans, L.E., Feeney, R.E., (1968) Biochemistry, 7, 2192-2196.
16. Kaltchmidt, E., Wittmann, H.G. (1970) Analyt. Biochem. 36, 401-412.
17. Howard, C.A., Traut, R.R. (1973) FEBS Letters, 29, 177-180.
18. Vlassov, V.V., Grineva, N.I., Knorre, D.G. (1969) Izv. Sib. Otd. Akad. Nauk SSSR, ser. khim. nauk, iss. 1, 104-109.
19. Held, W.A., Mizushima, S., Nomura, M. (1973), J. Biol. Chem. 248, 5720-5730.
20. Craven, G.R., Voynow, P., Hardy, S.J.S., Kurland, C.G., (1969), Biochemistry, 8, 2906-2915.
21. Towbin, H., Elson, D., (1978), Nucl. Acids Res., 5, 3389-3407.
22. Pongs, O., Stöffler, G., Lanka, E. (1975) J. Mol. Biol., 99, 301-315.
23. Luhrmann, R., Gassen, H.G., Stöffler, G. (1976) Eur. J. Biochem. 66, 1-9.
24. Belikova, A.M., Grineva, N.I., Kabasheva, G.N. (1973) Tetrahedron, 29, 2277-2283.
25. Wantabe, S., Janaka, K. (1971) Biochem. Biophys. Res. Commun. 45, 728-734.
26. Pongs, O., Rossner, E. (1976) Nucl. Acids Res. 3, 1625-1633.
27. Chen, R., (1977), Hoppe-Seyler's Z. Physiol. Chem. Bd. 358, 1415-1430.
28. Tischendorf, G.W., Zeichhardt, H., Stöffler, G. (1975) Proc. Nat. Acad. Sci. USA, 72, 4820-4824.
29. Lake, J.A. (1976) J. Mol. Biol. 105, 131-159.
30. Chang, F.N., Flaks, J.G. (1972) J. Mol. Biol. 68, 177-180.
31. Shin, C., Craven, G.R., (1973) J. Mol. Biol. 78, 651-663.
32. Sommer, A., Traut, R.R. (1976) J. Mol. Biol. 105, 995-1015.
33. Kenny, J.W., Traut, R.R. (1979) J. Mol. Biol. 127, 243-263.
34. Expernt-Bezancon, A., Barritault, D., Clegg, J.C.S., Milet, M., Khouvin, Y., Hayes, D.H. (1975) FEBS Letters, 59, 64-69.