COVALENT BINDING OF URIDINE-OLIGONUCLEOTIDES TO 70 S *E. COLI* RIBOSOMES

R. LÜHRMANN, U. SCHWARZ and H.G. GASSEN
Institut für Biochemie der Westfälischen, Wilhelms Universität, 44 Münster Orleansring 23a, W. Germany

Received 22 February 1973

1. Introduction

In the course of our studies of the mechanism of codon—anticodon interaction in protein synthesis we became interested in a detailed aspect of this process, namely the specific binding of the mRNA into the active sites of the ribosome [1]. Since, however, short chain oligonucleotides and polynucleotides containing a stable secondary structure are bound to the ribosome only in the presence of their corresponding tRNA's [2], we tried to bind oligonucleotides covalently into one of the active sites of the ribosome. It has been shown by Traut and Haenni [3] and by Moore [4], that sulfhydryl reagents like *N*-ethyl maleimide and iodoacetamide are bound to an active sulfhydryl group of the 30 S subunit. In contrast to *N*-ethyl maleimide, iodoacetamide is bound to the ribosome in stoichiometric amounts without blocking the poly(U) dependent binding of Phe-tRNA. Therefore we used iodoacetylchloride as a bifunctional reagent to bind uridine-oligonucleotides containing a 3′-terminal 5-aminouridine to 70 S ribosomes (fig. 1). We report here the preparation of the "programmed" ribosomes and their activity in Phe-tRNA binding.

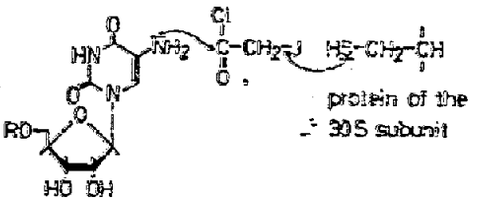

Fig. 1. Iodoacetylchloride as a bifunctional reagent for the crosslinking of oligonucleotides to proteins.

2. Materials and methods

Labeled compounds were purchased from the Radiochemical Centre, Amersham. [^{3}H]UDP was adjusted to a specific activity of 50 mCi/mmole, [^{3}H]Urd to 30 mCi/mmole, [^{3}H]Phe to 1 Ci/mmole and the specific activity of [^{14}C]Val was 260 mCi/mmole and of [^{14}C]Phe 450 mCi/mmole. *E. coli* tRNA was obtained from Boehringer, Mannheim, and 5-bromouridine from Zellstoffabrik Waldhof, Mannheim. Iodoacetylchloride was prepared from iodoacetic acid with thionylchloride. Polynucleotide phosphorylase was isolated from *Micrococcus luteus* as described earlier [5]. Enzymatic phosphorylation of 5-aminouridine was performed with phosphotransferase from carrots [6]. 70 S ribosomes were prepared from *E. coli* MRE 600 cells (Whitman, London) and were washed 5 times with 0.5 M NH_4Cl. tRNA from *E. coli* was charged with labeled amino acids by the usual procedure [7]. Oligonucleotide dependent binding of AA-tRNA's to ribosomes was measured as described earlier [1].

* The compounds are symbolized, in harmony with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (European J. Biochem. 15 (1970) 203) as follows:
$(nh_2)^5U$ 5-aminouridine;
$(nhr)^5U$ = 5-(iodoacetylamino) uridine;
CPT = carrot phosphotransferase;
$PNPase^P$ = primer dependent polynucleotide phosphorylase.

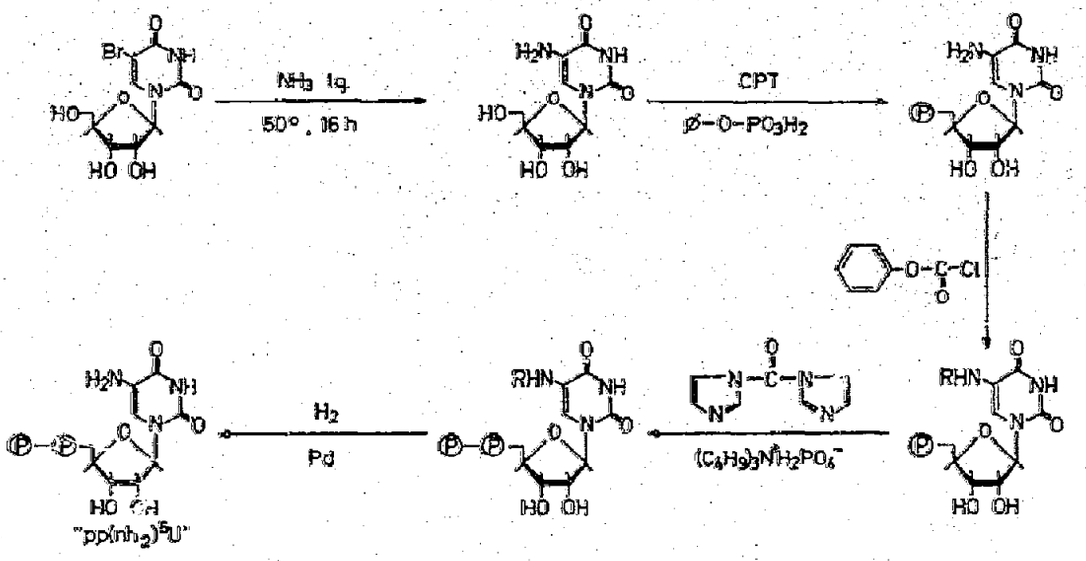

Fig. 2. Synthetic procedure for the synthesis of 5-aminouridine-5'-phosphate.

3. Results

For the synthesis of the U-[^{3}H]U–U–$(nh_2)^5$U, the 5-aminouridine-5'-diphosphate had to be synthesized. The procedure which was used is shown in fig. 2. Since the amino group reacted with carbonyl-diimidazole [8], it was protected with the benzyloxycarbonyl function according to the method of Ivanovics et al. [9]. The protective group was split off quantitatively by hydrogenation at the level of the nucleoside diphosphate. The starting dinucleoside phosphate U-[^{3}H]U was prepared by RNAase A catalyzed condensation [10]; it was elongated by two consecutive PNPase-catalyzed steps [5] (fig. 3). The amino group of the terminal uridine was acetylated in 0.5 M $NaHCO_3$ using a tenfold excess of ICH_2COCl at 37° for 20 min. Since the 5-aminouridine shows on protonation a shift in the UV-spectrum from 294 nm to 265 nm, whereas the monosubstituted aminouridine shows a pH independent maximum at 275 nm (fig. 4), the kinetics of the acetylation were followed spectrophotometrically. The U-[^{3}H]U–U–$(nhr)^5$U reacted quantitatively with the sulfhydryl group of glutathione. The binding of the oligonucleotide to 70 S ribosomes was performed in 14 mM $MgCl_2$, 45 mM KCl and 8 mM Tris-HCl pH 7.2. The kinetics of the binding are shown in fig. 5. About 35 pmoles of oligonucleotide were bound per 100 pmoles of ribosomes at saturating amounts of oligonucleotide (fig. 6). In order to separate the unreacted oligonucleotide from the ribosomes, the programmed ribosomes were chromatographed over a Sephadex G-75 column. The ribosomes eluted in the exclusion volume were used to examine the Phe-tRNA binding capacity of the programmed ribo-

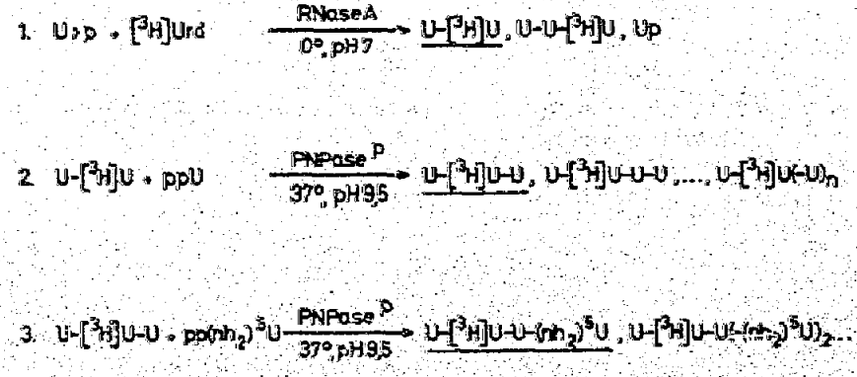

Fig. 3. Synthetic scheme for the synthesis of labeled oligonucleotides containing 5-aminouridine in the 3'-position.

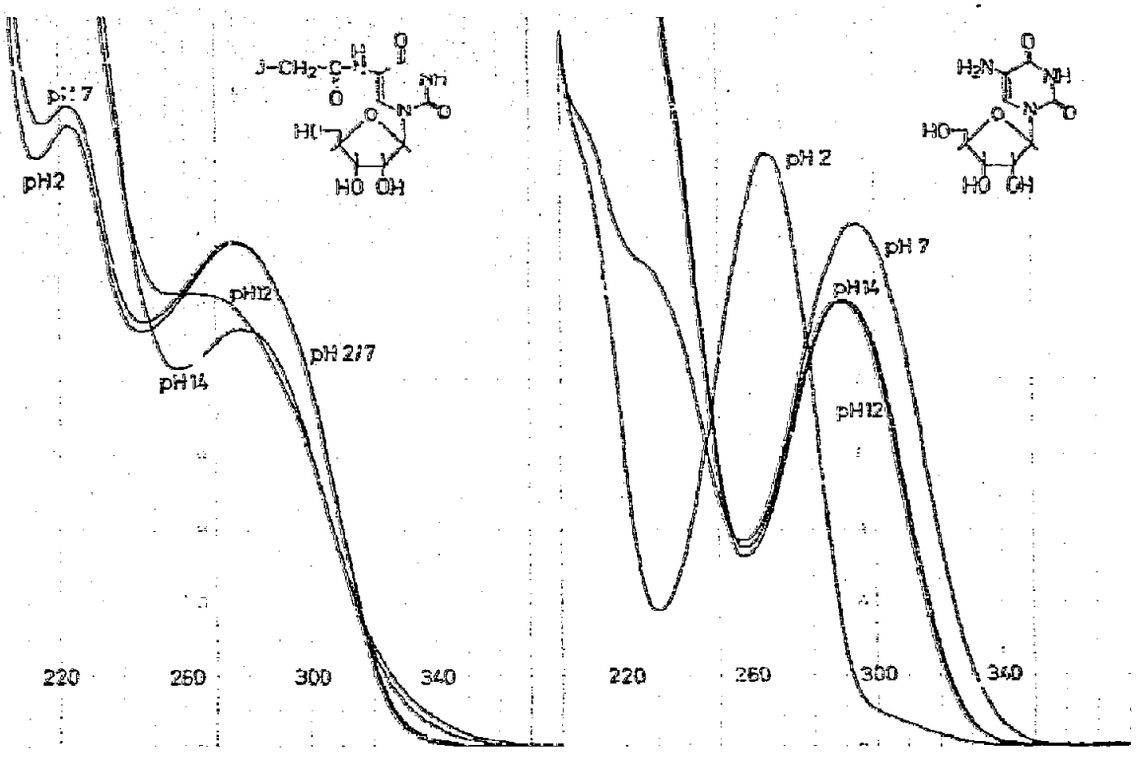

Fig. 4. UV-spectra of 5-aminouridine and 5-iodoacetylaminouridine at different pH-values. Only 5-aminouridine shows a shift between pH 7 and pH 2.

somes (fig. 7). Since only a small amount of Phe-tRNA was bound, we tested whether the modified ribosomes were capable of binding poly([^{3}H]U) and poly(U) dependent additional Phe-tRNA. It can be seen from table 1, that although the poly(U) binding was not affected, no additional Phe-tRNA was bound.

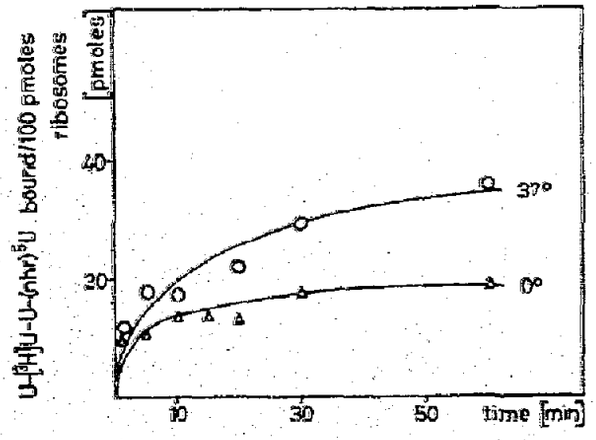

Fig. 5. Kinetics of the covalent binding of U-[^{3}H]U–U–(nhr)^{5}U to 70 S ribosomes at 0° and 37°. The incubation mixture contained in a total volume of 100 µl: 214 pmoles ribosomes, 30 nmoles oligonucleotide (specific activity 30 mCi/mmole), 14 mM $MgCl_2$, 45 mM KCl and 8 mM Tris-HCl pH 7.2. Aliquots of 5 µl were withdrawn at appropriate times and filtered over Selectron BA 85 0.45 µm filters.

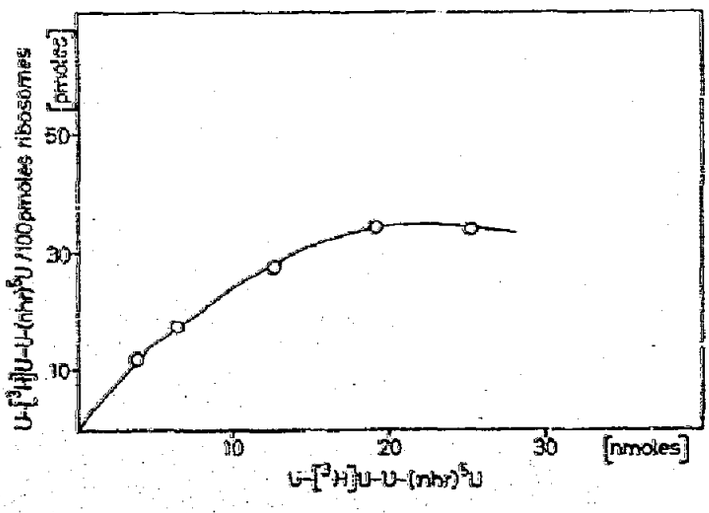

Fig. 6. Binding of U-[^{3}H]U–U–(nhr)^{5}U to 70 S ribosomes. The reaction mixtures were incubated for 20 min at 37°. 100 pmoles of ribosomes were used per assay.

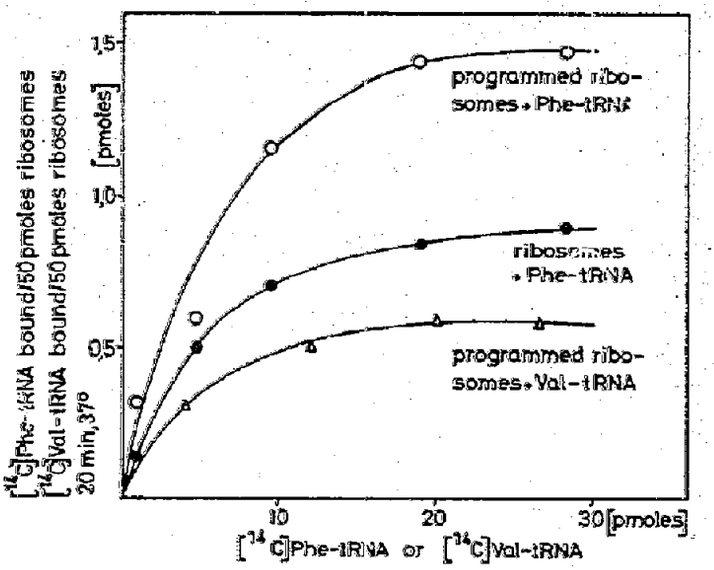

Fig. 7. Binding of Phe-tRNA to programmed ribosomes. The reaction mixture contained in a volume of 100 µl: 50 pmoles ribosomes (20 pmoles labeled with U-[^{3}H]U–U–(nhr)^{5}U), [^{14}C]Phe-tRNA or [^{14}C]Val-tRNA, 14 mM $MgCl_2$, 45 mM KCl, and 8 mM Tris-HCl pH 7.2. As a control, ribosomes were used which were treated under identical conditions, but which were not reacted with the oligonucleotide.

4. Discussion

Oligonucleotides containing 5-aminouridine in the 3'-terminal position can be bound covalently to ribosomes. The stoichiometry obtained – 30% to 50% of the ribosomes are active in oligonucleotide binding – corresponds to other data showing that a similar percentage of ribosomes is active in the poly(U)-dependent binding of Phe-tRNA [11]. The binding of Phe-tRNA was not increased by the addition of poly(U), showing that actually all ribosomes capable of Phe-tRNA binding had reacted with the oligonucleotide.

The programmed ribosomes were active in Phe-tRNA binding but did not stimulate the binding of Val-tRNA. This suggests that the oligonucleotide is bound to one of the active sites of the ribosomes. Whether this is the aminoacyl- or the peptidyl site can not be decided yet. Since the programmed ribosomes are still capable of binding poly(U), but do not bind additional Phe-tRNA, the sulfhydryl group blocked should play an important role in AA-tRNA binding [3]. The amount of Phe-tRNA bound to the labeled ribosomes corresponds rougly to the amount of Phe-tRNA bound by excess U–U–U to ribosomes [1]. This could mean that the complex formation between triplet and ribosome is not the limiting factor in triplet coded binding, but that the nonenzymatic complex formation between codon and anticodon is very unstable.

Table 1

Binding of poly ([^{3}H]U) and poly (U) and U–U–U dependent binding of Phe-tRNA to programmed ribosomes.

	Programmed ribosomes			Ribosomes (control)	
Poly ([^{3}H]U) [pmoles]	13.8			12.5	
	poly(U),	U–U–U,	–	poly(U),	U–U–U
Phe-tRNA [pmoles]	0.8	0.7	0.6	9.9	0.8

50 pmoles of ribosomes were used containing 20 pmoles of oligonucleotide. The average chain length of poly ([^{3}H]U) was calculated to be 30 nucleotides.

References

[1] H.G. Gassen, H. Schetters and H. Matthaei, Biochem. Biophys. Acta 272 (1972) 560.
[2] D. Hatfield, Cold Spring Habor Symp. Quant. Biol. 31 (1966) 619.
[3] R.R. Traut and A.L. Haenni, European J. Biochem. 2 (1967) 64.
[4] P.B. Moore, J. Mol. Biol. 60 (1971) 169.
[5] H. Schetters, H.G. Gassen and H. Matthaei, Biochem. Biophys. Acta 272 (1972) 549.
[6] E.F. Brunngraber and E. Chargaff, J. Biol. Chem. 242 (1967) 4834.
[7] F. Gros and H. Matthaei, in: Practical molecular genetics (Springer Verlag, Berlin, Heidelberg, New York) in press.
[8] D.E. Hoard and D.G. Ott, J. Am. Chem. Soc. 87 (1965) 1785.
[9] G.A. Ivanovics, R.J. Rousseau and R.K. Robins, J. Med. Chem. 14 (1971) 1155.
[10] H.G. Gassen, FEBS Letters 14 (1971) 225.
[11] C.G. Kurland, Ann. Rev. Biochem. 41 (1972) 377.