MODIFICATION OF AMINO ACID ACCEPTANCE AND TRANSFER CAPACITY OF S-RNA IN THE PRESENCE OF ORGANIC SOLVENTS*

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Sonicated suspensions of polycyclic hydrocarbons have been reported to change the amino acid acceptor capacity of s-RNA (Chang and Bond, 1964). The change observed was not very large, presumably because of the heterogeneous conditions employed. Recently So et al. (1964) studied the effect of organic solvents and of environmental changes on the stimulation or inhibition of polynucleotide (synthetic messenger) directed polypeptide synthesis using an S_{30} fraction isolated from E. coli cells (Nirenberg and Matthaei, 1961).

Considering the possibility that a homogeneous system containing some simple organic solvents could provide better information on the aminoacylation of s-RNA, we have now studied the effect of various organic solvents on the stimulation or inhibition of the aminoacylation of s-RNA, and finally its transfer in the poly U directed polyPhe synthesis. In addition, the effects of organic solvents, which are known to disrupt the hydrophobic regions, thus resulting in a modification of the secondary structure of the polynucleotide or protein, have also been studied by carrying out optical rotatory dispersion (ORD) measurements on aminoacyl synthetase, ribosomes, poly U, s-RNA and a mixture of poly U and ribosomes, with and without the organic solvent.

The aminoacylation of E. coli s-RNA was carried out by a modification of the procedure of Nathans and Lipmann (1961). In a total volume of 0.1 ml were contained 40-50 μg of s-RNA, 20 μl of E. coli enzyme, ATP (0.01 M),

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PEP (0.01 M), PEP kinase 10 μg/ml, Tris (0.1 M) pH 7.6, Mg^{++} (0.02 M), mercaptoethanol (0.05 M), and 0.01 μmoles of the ^{14}C amino acid (with specific activities of Asp, 4.35 x 10^6, Leu, 2.72 x 10^6, Lys, 6.65 x 10^6, Phe, 2.63 x 10^6, Tyr, 2.63 x 10^6, and Val, 5.58 x 10^6 counts/min/μmole). The reaction mixture was incubated at 37° for 30 minutes, and the radioactivity measured as described in an earlier publication (Sarin and Zamecnik, 1964). The results obtained with some of the amino acids in the presence of various solvents are summarized in Table I. The values are based on the control as 100, i.e. the aminoacylation of s-RNA in the absence of an organic solvent.

Table I

Effect of Organic Solvents on the Amino Acid Acceptance Capacity of s-RNA

	Amino Acid Acceptance Capacity (% Control)					
Solvent	Val	Phe	Tyr	Leu	Asp	Lys
Control	100	100	100	100	100	100
Ethylene glycol monomethyl ether (10%)	82	154	52	121	125	101
Diethylene glycol (1%)	101	136	129	53	125	74
Diethylene glycol (5%)	78	119	146	168	125	50
Propylene glycol (5%)	99	97	95	181	114	54
Glycerol (5%)	84	56	116	160	143	37
Benzene (0.1%)	93	70	107	44	122	6
Toluene (0.1%)	89	4	88	35	122	7
Decalin (0.14%)	85	4	112	59	122	4

Solutions of benzene, toluene and decalin were made in ethylene glycol monomethyl ether so as to keep the incubation mixture homogeneous. The incubation mixture in these cases was 10% with respect to ethylene glycol monomethyl ether.

The specific activities expressed as mμmoles per mg s-RNA were: Valyl-, 0.53; Phenylalanyl-, 0.57; Tyrosyl-, 0.35; Leucyl-, 1.7; Aspartyl-, 0.40; and Lysyl-, 0.42.

Different lots of s-RNA showed some differences in the extent of stimulation or inhibition of aminoacylation for various amino acids.

Of particular note in Table I, aspartyl-s-RNA shows an increase in the acceptance of aspartic acid and remains so throughout the range of solvents studied. Lysyl-s-RNA shows a decrease in the acceptance of amino acid in the presence of various solvents, maximal inhibition of lysine acceptor capacity being observed in the presence of small amounts of benzene, toluene and deca-

lin. Valine acceptor capacity of s-RNA shows only a very small overall effect in the presence of the various organic solvents studied. It is of interest that the aminoacylation of s-RNA with Phe, Leu and Lys is inhibited in the presence of benzene, toluene and decalin, whereas aminoacylation of s-RNA with respect to Val, Tyr and Asp is only slightly affected. It is possible that the effect of these organic solvents is to modify the secondary structure of a particular aminoacyl-RNA or its aminoacyl synthetase (cf. Table III); or it may be that the disruption of hydrophobic regions brings about varying degrees of modification in the different s-RNA's and the aminoacyl synthetases.

In order to determine whether the increased amount of Phe accepted by s-RNA would also be transferred into polypeptide in the poly U directed polyPhe synthesis, the cell-free E. coli system was studied in the presence of ethylene glycol monomethyl ether (5% and 10%). The E. coli cells (4% mid-log phase, General Biochemicals, Chagrin Falls, Ohio) were disrupted and fractionated according to an earlier procedure (Lamborg and Zamecnik, 1960), and the ribosomes separated from the supernatant protein by centrifugation at 105,000 x g. The complete incorporation system was similar to that of Nirenberg and Matthaei (1961), and contained 0.1 μmole ^{14}C Phe (specific activity, 5 x 10^6 counts/min/μmole). The results of poly U directed polyPhe synthesis are presented in Table II. All assays were run in duplicate.

It becomes apparent from Table II that the presence of ethylene glycol monomethyl ether (5%) stimulates the poly U (40 μg/ml) directed polyPhe synthesis to approximately 150%, whereas ethylene glycol monomethyl ether (10%) in the incorporation system inhibits the polyPhe synthesis, which is 60% of the level of the control system. The inhibitory effect of excess ethylene glycol monomethyl ether can be reversed by the addition of more of the synthetic messenger (poly U). The reversal of the inhibitory effect by the addition of poly U suggests that the presence of the organic solvent may affect the binding of the messenger to the ribosomes. The organic solvent

Table II

Poly U Directed Poly-Phe Synthesis, Using Cell-Free E. coli System

		Poly U/ml	Ethylene Glycol Monomethyl Ether (% Incubation Mixture)	cpm/mg Ribosomal Protein
1.	Blank	-	-	88
	Complete	20 µg	-	870
	Complete	40 µg	-	2680
	Complete	80 µg	-	2650
2.	Blank	-	5	79
	Complete	20 µg	5	2620
	Complete	40 µg	5	3890
	Complete	80 µg	5	2920
3.	Blank	-	10	102
	Complete	20 µg	10	1610
	Complete	40 µg	10	1670
	Complete	80 µg	10	2180

may however also affect the enzyme or the s-RNA, apart from the ribosomes and the messenger.

To gain further insight into the influence of the organic solvents on the various components of the amino acid acceptor and transfer systems, the use of optical rotatory dispersion (ORD) measurements have now been made, thus extending previous observations on the ORD patterns of poly C (Fasman et al., 1964) and of other synthetic polynucleotides and s-RNA (Lamborg et al., 1965; Fasman et al., 1965). The specific rotations at the peak and trough in the ORD of poly U, ribosomes, poly U + ribosomes, s-RNA and valyl synthetase, with and without the organic solvent, are given in Table III.

Table III

ORD of the Various Components of the Amino Acid Acceptor and Transfer Systems

Sample	Peak				Trough				λ_o(mμ)	
	λmμ	$[\alpha]_I$	λmμ	$[\alpha]_{II}$	λmμ	$[\alpha]_I$	λmμ	$[\alpha]_{II}$	I	II
Poly U	285	+2660	285	+3620	258	-5800	258	-4590	273	272
Ribosomes	280	+4330	280	+6290	250	-5340	250	-4300	266	265
Poly U + ribosomes	285	+3040	285	+3540	255	-5330	255	-4710	270	268
s-RNA	280	+3740	280	+5200	250	-3950	252	-2810	264	262
(E. coli)	225	-420	227	+1040	217	-1450	219	0	-	-
Valyl synthetase	-	-	-	-	232	-5900	232	-6670	215	214

All measurements were made on a Cary 60 spectropolarimeter, with conditions similar to those previously reported (Lamborg et al., 1965).

$[\alpha]_I$ represents specific rotations in the respective buffers without ethylene glycol monomethyl ether.

$[\alpha]_{II}$ represents specific rotations in the respective buffers containing 5% ethylene glycol monomethyl ether in the case of poly U, ribosomes, and poly U + ribosomes; and containing 10% ethylene glycol monomethyl ether in the case of s-RNA and valyl synthetase. Ribosomes were washed with sodium deoxycholate (0.6%) followed by suspension of the pellet in the buffer and centrifugation at 105,000 x g (twice) to remove sodium deoxycholate.

ORD measurements of poly U, ribosomes, and poly U + ribosomes were made in the buffer containing Tris (0.01 M) pH 7.8, Mg^{++} (0.01 M), KCl (0.06 M) and mercaptoethanol (0.006 M). Ribosomes and ribosomes + poly U solutions were centrifuged to remove heavier aggregates (to avoid birefringence) before determination of the ORD.

ORD of s-RNA was measured in 0.14M NaCl and 0.014 M sodium citrate buffer (pH 7.1), and valyl synthetase was studied in Tris (0.05 M), EDTA (0.001 M) and mercaptoethanol (0.001 M) buffer (pH 7.4).

The concentrations of the various solutions for the ORD were: poly U, 0.21 mg/ml; ribosomes in buffer, 0.32 mg protein/ml; ribosomes in buffer + 5% ethylene glycol monomethyl ether, 0.30 mg protein/ml; ribosomes (0.32 mg protein/ml) + poly U (0.21 mg/ml) in buffer; ribosomes (0.30 mg protein/ml) + poly U (0.21 mg/ml) in buffer + 5% ethylene glycol monomethyl ether; s-RNA, 0.48 mg/ml; and valyl synthetase, 0.39 mg/ml.

Partially purified sample of E. coli valyl synthetase was a gift from Dr. M. Lamborg.

From Table III, it appears that the organic solvent brings about a significant effect on the ribosomes and on the enzyme. The increment in amplitude of the Cotton effects in the presence of ethylene glycol monomethyl ether, for ribosomes is 920, for valyl synthetase is 770, and for

s-RNA is 320. A decrease in amplitude of 250 is, however, observed with poly U. An increase in amplitude suggests an ordering of the structure, whereas a decrease implies an opening up of the secondary structure. It is of interest to note that the mixture of poly U + ribosomes probably represents a new conformation of the messenger-bound ribosomes, as the specific rotation of poly U + ribosomes is not the added sum of the specific rotations of poly U and ribosomes taken separately. It appears that the effect of the organic solvent on the amino acid acceptor and transfer systems is a composite one, comprised of modification of the enzyme, ribosomes, and the s-RNA. In addition, the messenger appears to bind to the ribosomes to give a new conformation, which is then effective as a template in protein biosynthesis.

The present observations provide first evidence that organic solvents can modify the amino acid esterification process, producing under a given circumstance stimulation of esterification for one amino acid and inhibition for another. They also offer evidence on the influence of organic solvents on the interaction of ribosomes, messenger, and s-RNA. The latter effect of organic solvents is complementary to that described by So et al. (1964). This is also a first report of the application of optical rotatory dispersion to the study of structural patterns of the ribosomes and their interaction with s-RNA and m-RNA.

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REFERENCES

Chang, M., and Bond, T. J., Nature, 201, 623 (1964).
Fasman, G. D., Lindblow, C., and Grossman, L., Biochemistry, 3, 1015 (1964).
Fasman, G. D., Lindblow, C., and Seaman, E., Private communication.
Lamborg, M., and Zamecnik, P. C., Biochim. Biophys. Acta, 42, 206 (1960).
Lamborg, M., Zamecnik, P. C., Li, T. K., Kagi, J., and Vallee, B. L., Biochemistry, 4, 63 (1965).
Nathans, D., and Lipmann, F., Proc. Nat. Acad. Sci. U. S., 47, 497 (1961).
Nirenberg, M. W., and Matthaei, J. H., Proc. Nat. Acad. Sci. U. S., 47, 1588 (1961).
Sarin, P. S., and Zamecnik, P. C., Biochim. Biophys. Acta, 91, 653 (1964).
So, A. G., and Davie, E. W., Biochemistry, 3, 1165 (1964).
So, A. G., Bodley, J. W., and Davie, E. W., Biochemistry, 3, 1977 (1964).