EFFECTS OF CHEMICAL MODIFICATIONS ON THE BIOLOGICAL PROPERTIES OF s-RNA*

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We have initiated a study of the accepting and transfering capacities of brominated and methylated s-RNA's in an attempt to obtain informations on the mechanism of action of transfer RNA. The s-RNA was isolated from baker's yeast in the logarithmic growth phase by the method of Monier et al. (1960) followed by DEAE-cellulose chromatography. In order to remove the attached aminoacids, it was finally incubated with 0.1 M Na_2CO_3 pH 10 at 37° for 1 hour and then dialyzed overnight at 4° against 10^{-3}M NaCl.

Methylation of s-RNA was performed according to Brookes and Lawley (1961) with dimethylsulfate in 0.4 M potassium acetate for 22 hours at 4°; the s-RNA was then precipitated by addition of 0.1 volume of 20 % potassium acetate pH 5 and 2 volumes of ethanol, redissolved and dialyzed overnight at 4° against 10^{-3}M NaCl.

Bromination of s-RNA was performed according to Duval and Ebel, (1964, in preparation) on the quaternary ammonium ribonucleate in dimethylformamide solution. Precipitation of s-RNA with trimethylhexadecylammonium bromide and dissolution of the so-obtained quaternary ammonium ribonucleate in dimethylformamide were carried out as previously described (Weil and Ebel, 1962). Bromine dissolved in dimethylformamide was added, allowed to react for 30 seconds, and the sodium salt of s-RNA was immediately regenerated by addition of 0.25 volume of a saturated NaBr solution in dimethylformamide; we used this solution of NaBr in dimethylformamide rather than an aqueous solution of NaCl, in order to avoid the reaction with bromine which occurs in the presence of water. After rege-

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neration the s-RNA was washed twice with a cold mixture of 0.1 N NaCl 1 volume - ethanol 2 volumes, redissolved and dialyzed overnight at 4° against 10^{-3}M NaCl.

Methylated or brominated s-RNA's were analyzed after perchloric hydrolysis by two-dimensional paper chromatography in order to determine the percentage of methylated or brominated bases. Methylation gives rise to N^7-methylguanine and, to a lesser extent, to N^1-methyladenine and N^1-methylcytosine, while uracil is not methylated. When bromination is performed on the quaternary ammonium ribonucleate in dimethylformamide, Ebel, Duval and Bollack (1963) showed that there is formation of 8-bromoguanine, 5-bromouracil and 5-bromocytosine, while adenine is never brominated; these compounds are different from those obtained when bromination is performed in the presence of water, as was done by Yu and Zamecnik (1963); in that case there is addition of Br on the C_5 and of OH on the C_6 of the pyrimidine ring with saturation of the C_5, C_6 double bond and loss of the U.V. absorption.

It has been checked (Ebel et al., in preparation) that bromination and methylation, under the mild conditions selected here, do not cause any degradation of the s-RNA molecule : ultracentrifugation gave the same S_{20w} values for modified and control s-RNA's and DEAE-cellulose chromatography did not show the appearance of mono- or oligonucleotides. The melting curves of control and modified s-RNA's are similar, except after strong methylations or brominations.

For each series of bromination and methylation, the modified s-RNA's have been compared, when testing their biological properties, to control s-RNA's which had undergone the same treatments but without addition of bromine or dimethylsulfate. We had previously checked (Weil, Ebel and Monier, 1961) that the quaternary ammonium treatment does not alter the biological activity of s-RNA, nor does it seem to alter the helical configuration (Mommaerts et al., 1964). Each series included, besides the corresponding control s-RNA, 3 brominated s-RNA's (obtained after addition of respectively 0.25 , 0.50 and 1 Br/nucleotide unit) and 3 methylated s-RNA's (obtained after addition of respectively 2.4 and 6 methyl groups/nucleotide unit).

The aminoacid accepting capacities of brominated and methylated s-RNA's have been up to now studied for 6 aminoacids (leucine, lysine, phenylalanine, proline, serine and valine) under the following conditions : 200 µg of control or modified s-RNA were incubated at 37° in a mixture which contained, in a final volume of 1 ml buffered at pH 7.5 : ATP 10 µM, $MgCl_2$ 1.85 µM, glutathione 2.5 µM, serum albumin 0.1 mg,

dialyzed 105,000 x g supernatant from yeast 0.1 ml, Tris 17.5 μM, ^{14}C-valine 0.2 μM (corresponding to 1 μC). After 30 minutes of incubation, the reaction was stopped by addition of 1 ml 10 % trichloracetic acid. The precipitate was washed twice with 5 % trichloracetic acid, twice with alcohol, and dissolved in 1 ml 4 M NH_4OH. An aliquot was plated, dried and its radioactivity counted. For each series a blank was run with no s-RNA, and its radioactivity was substracted from the values obtained with the various tested s-RNA's. All the tubes, including the blank, were run in dupplicate. Although we have made a great number of experiments, we have obtained irregular results. In some experiments increasing brominations and methylations have caused a proportional decrease of the aminoacid accepting capacities and this decrease was parallel for all the aminoacids tested. However very often we have found, as Yu and Zamecnik (1963) have described it after bromination in aqueous medium, a non-parallel decrease, suggesting that specific sites had been touched. These discordant results are for the moment difficult to interpretate and we are extending our studies in this field, trying especially to eliminate the possibility of a non-specific attack on the terminal CCA triplet.

However in some cases, these experiments have provided interesting results. We have found a decrease in the capacity of accepting one aminoacid after a modification which was not expected to modify the corresponding anticodon, showing that the decrease in accepting capacity was not due to an attack on the anticodon. For instance bromination, which should not alter the anticodon (AAA) of phenylalanyl-s-RNA, produces a decrease in the capacity of accepting phenylalanine, while on the other hand methylation, which does not modify the anticodon (UUU) of lysyl-s-RNA, produces a decrease in the capacity of accepting lysine.

These results, which suggest a dissociation of the sites responsible for the accepting capacity and the transfering capacity in the s-RNA molecule, have been confirmed by our studies on the influence of chemical modifications on the transfering capacity. We have especially studied the transfer of lysine and phenylalanine. First, uniformly labelled ^{14}C-lysine (54 mc/mM) or ^{14}C-phenylalanine (80 mc/mM) were attached on the control and modified s-RNA's, according to von Ehrenstein and Lipmann (1961) in the presence of a dialyzed 105,000 x g supernatant from yeast. The charged s-RNA's were isolated by phenolic extraction, followed by alcoholic precipitation of the aqueous phase; the precipitates were washed twice with a mixture of 0.1 M NaCl 1 volume - alcohol 2 volumes, redissolved and dialyzed overnight at 4°

against 10^{-3}M NaCl. We have obtained highly labelled aminoacyl-s-RNA's (about 10,000-12,000 CPM/mg for lysyl-s-RNA's and 12,000-15,000 CPM/mg for phenylalanyl-s-RNA's).

The aminoacyl-s-RNA was incubated at 37° in a mixture which contained, in a final volume of 1.7 ml buffered at pH 7.3 : ATP 1 μM, potassium phosphoenolpyruvate 10 μM, pyruvate kinase 0.5 mμM, KCl 146 μM, $MgCl_2$ 17 μM, Tris 10 μM, the 19 other ^{12}C-aminoacids 0.03 μM of each, 2-mercaptoethylamine 1.8 μM, GTP 0.3 μM, preincubated extract from E. coli (prepared according to Nirenberg and Matthaei, 1961) 0.3 ml and either 100 μg poly U in the case of phenylalanyl-s-RNA or 50 μg poly A in the case of lysyl-s-RNA. After 30 minutes 20 μg of pancreatic ribonuclease are added and allowed to act for 15 minutes at 37°. To isolate polyphenylalanine, 5 ml of 10 % trichloracetic acid are added, the precipitate is washed twice with 5 ml 10 % trichloracetic acid for 20 minutes at 90° and twice with 5 ml alcohol for 20 minutes at 60°. To isolate polylysine, precipitation and washings are carried out with 5 % trichloracetic acid pH 1.7 containing 0.025 % sodium tungstate. Finally, in both cases, the precipitate is dissolved in 1 ml 4 M NH_4OH; an aliquot is plated, dried and counted for radioactivity. For each control or modified aminoacyl-s-RNA, we ran a blank without polynucleotide and this value was substracted from the one obtained in the presence of the polynucleotide.

As the control and modified aminoacyl-s-RNA's of a given series have specific activities which decrease as the percentage of bromination or methylation increases, we introduced in our reaction mixtures, not the same number of mg of s-RNA, but the same number of counts, in other words the same number of charged s-RNA molecules. Under these conditions, if the transfering activities are not modified, we should obtain the same percentage of transfer within one series, providing that an excess of uncharged modified s-RNA does not affect the transfer; we checked this point and found that the addition of uncharged brominated or methylated s-RNA does not modify in our experimental conditions the transfer of the aminoacid from the aminoacyl-s-RNA to the polypeptides.

The results of the experiments where we studied the influence of methylation on the transfering activity of s-RNA are summarized in table I and expressed as a percentage of the transfer observed with the control s-RNA. Methylation does not affect the transfer of lysine; the polynucleotide which stimulates best the incorporation of lysine is poly A, therefore the anticodon of lysyl-s-RNA should be UUU, which cannot be methylated. On the other hand methylation affects the transfer of phenylalanine; the polynucleotide which is messenger for phenyl-

alanine is poly U, so that the anticodon of phenylalanyl-s-RNA should be AAA, which can be methylated. These results suggest that methylation affects the transfering capacities only when it is able to affect the anticodon of the corresponding aminoacyl-s-RNA.

Table I. Effects of methylation on the transfer of lysine and phenylalanine.

	lysyl-s-RNA				phenylalanyl-s-RNA			
	Control	Number of methyl groups added per nucleotide unit			Control	Number of methyl groups added per nucleotide unit		
		2	4	6		2	4	6
% transfer	100	94	100	92	100	76	65	53

The results obtained with bromination, which are summarized in table II, are also in favor of this conclusion: Bromination, which touches U, produces a very sharp decrease of the transfering capacity of lysyl-s-RNA where the anticodon is UUU. But bromination, which does not touch A, is not able to decrease the transfering capacity of phenylalanyl-s-RNA where the anticodon is AAA; this is true at least for the bromination up to 0.5 Br/nucleotide, where the percentage of phenylalanine transfered is still 84 %, whereas it is only 47 % for lysine; when we reach 1 Br/nucleotide, there is a decrease of the transfer even for phenylalanine, but it should be pointed out that under these conditions a very distinct modification of the melting curve is observed (Duval and Ebel, 1964, in preparation) and it is probable that the drop in transfering capacity in this case is not due to an attack of the anticodon but to a degradation in the secondary structure of the molecule.

Our experiments lead us to conclude that in the s-RNA molecule the sites responsible for the accepting capacity are different from the sites responsible for the transfering capacity. We have seen that some s-RNA molecules have a decreased accepting capacity while their anticodon could not have been touched by the modification; on the other hand, some modified s-RNA molecules are still able to accept

Table II. Effects of bromination on the transfer of lysine and phenylalanine.

	lysyl-s-RNA				phenylalanyl-s-RNA			
	Control	Number of Br per nucleotide unit			Control	Number of Br per nucleotide unit		
		0.25	0.50	1		0.25	0.50	1
% transfer	100	68	47	9	100	93	84	44

the aminoacid but are not able to transfer it any more. Furthermore our experiments are in favor of the codes which have been proposed for lysine and phenylalanine.

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REFERENCES

Brookes, P. and Lawley, P., Biochem.J., 80, 496 (1961).
Ebel, J.P., Duval, J. and Bollack, C., Bull.Soc.Chim.Biol., 45, 449 (1963).
Mommaerts, W., Brahms, J., Weil, J.H. and Ebel, J.P., C.R.Acad.Sci. (in press).
Monier, R., Stephenson, M. and Zamecnik, P., Biochim.Biophys.Acta, 43, 1, (1960).
Nirenberg, M. and Matthaei, J., Proc.Nat.Acad.Sci. US, 47,1580 (1961).
Von Ehrenstein, G. and Lipmann, F., Proc.Nat.Acad.Sci. US, 47, 941 (19
Weil, J.H., Ebel, J.P. and Monier, R., Nature, 192, 169 (1961).
Weil, J.H. and Ebel, J.P., Biochim.Biophys.Acta, 55, 836 (1962).
Yu, C. and Zamecnik, P., Biochem.Biophys.Res.Comm., 12, 457 (1963).