MODIFICATION OF THE AMINOACID CODE AFTER BROMINATION OF TRANSFER RNA*

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In a previous work (Weil et al., 1964) we have studied the effects of bromination on the accepting and transferring capacities of s-RNA**. We have shown that bromination alters the capacity of s-RNA to transfer lysine but does not affect the capacity to transfer phenylalanine, in the presence of the corresponding polynucleotides.

We have extended these studies of the effects of bromination and we are presenting here results of experiments which show that, after bromination of s-RNA, other aminoacids than phenylalanine are incorporated in the presence of poly U.

The s-RNA was extracted from yeast by the method of Monier et al. (1960), followed by DEAE-cellulose chromatography. The quaternary ammonium salt of transfer RNA was preparated as previously described (Weil and Ebel, 1962) and was brominated in dimethylformamide solution according to Duval and Ebel (1965). After regeneration of the sodium salt of transfer RNA, aminoacids were attached to transfer RNA and the transfer experiments were performed as described in our previous paper (Weil et al., 1964).

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** Abbreviations used: s-RNA=soluble ribonucleic acid; DEAE-cellulose=diethylaminoethyl-cellulose; A=adenine; G=guanine; C=cytosine; U=uracil; poly A=polyadenylic acid; poly U=poly - uridylic acid; BrC=5-bromocytosine; BrU=5-bromouracil; FU=5-fluorouracil; poly BrC=polybromocytidylic acid; poly BrU= polybromouridylic acid.

We have compared, in the presence of poly U, the transfer of several aminoacids from s-RNA which had been brominated to various extents, with that of phenylalanine under similar conditions.

For 4 aminoacids: glutamic acid, isoleucine, lysine and valine, which are not normally transferred in the presence of poly U, some transfer occurs when the s-RNA is brominated (table I). It is interesting to note that in several cases, the percentage of transfer increases for the mild brominations of s-RNA, but decreases again when the amount of bromine added to the reaction mixture is greater than 1 Br/nucleotide. In our previous communication (Weil et al., 1964) we had already shown that for these relatively strong brominations, the transfer of phenylalanine decreased, although the anticodon AAA of phenylalanyl-s-RNA could not have been modified. These decreases in the rate of transfer are not specific and are probably due to a modification in the secondary structure of s-RNA, as shown by the alteration of the melting-curve(Duval and Ebel, 1965).

For the other aminoacids studied, we did not observe any transfer in the presence of poly U after bromination of s-RNA, except for glycine and tyrosine, and only in a few experiments (table I).

As we have usually modified the transfer RNA before attaching the aminoacid, the observed abnormal incorporations could have been due to modifications of the accepting capacity of transfer RNA: some s-RNA molecules specific for phenylalanine could for instance, after bromination, accept isoleucine and then transfer it in the presence of poly U. We have therefore first attached isoleucine to transfer RNA and then performed the brominations on isoleucyl-s-RNA. With these brominated isoleucyl-s-RNA's, we have obtained, in the presence of poly U, the same results as those shown on table I. This suggests that the abnormal incorporation is caused by a perturbation of the transfer mechanism.

Several hypotheses may be proposed to explain the incorporation of certain aminoacids in the presence of poly U after bromination of s-RNA. One could first suppose that bromination produces a modification of the shape of the s-RNA molecule, involving an alteration in the base-pairing between s-RNA and messenger-RNA on the ribosomes; some nucleotides located beyond the anticodon might then become able to exchange bonds with the messenger-RNA.

Table I

TRANSFER OF AMINOACIDS IN THE PRESENCE OF POLY U AFTER BROMINATION OF S-RNA

Aminoacid	Number of experiments	Amount of Br added to s-RNA in the reaction mixture (in moles of Br per nucleotide)					
		0	0.25	0.5	0.75	1	1.25
Phenylalanine	5	30	28	28.2	-	13.2	-
Glutamic acid	6	0	0.2	0.7	0.8	1.2	0.9
Isoleucine	12	0	0	0.6	1.0	0.8	0.4
Lysine	5	0	0	0.4	0.3	0.3	0.2
Valine	14	0	-	0.3	0.3	0.9	0.2
Alanine	3	0	-	0	0	0	-
Histidine	3	0	-	0.3	0	0	0
Serine	9	0	0	0	0.1	0.2	0.1
Threonine	5	0	-	0	0.1	0.1	0
Aspartic acid	9	0	-	0	0	0	0
Glycine	8	0	-	0	0	0	0
	2	0	-	0.2	0.8	0.2	0.9
Tyrosine	5	0	-	-	0	0.1	0
	3	0	-	-	1.3	1.4	2.4

For each aminoacid, several experiments with different batches of s-RNA were performed. The percentage of radioactivity transferred from the normal or brominated ^{14}C-aminoacyl-s-RNA to the polypeptides was measured. On this table the figures represent the averages of the transfer percentages observed in the various experiments.

Without assuming any change in the shape of the s-RNA molecule, the abnormal transfers could be explained if certain bromi-

nated nucleotides acquired other base-pairing properties. The 4 aminoacids which are transfered in the presence of poly U after bromination of s-RNA have, according to several authors (Bretscher and Grunberg-Manago, 1962; Jones and Nirenberg, 1962; Wahba et al., 1963), the three following bases in their anticodons: A, U and C. A is not brominated and thus remains able to pair with the U of poly U. But the question arises, whether U and C may eventually, after bromination, pair with U and thus allow a base-pairing between the modified anticodon and poly U. Grunberg-Manago and Michelson (1964) have shown that poly BrU can stimulate the incorporation of isoleucine, as if BrU could play the role of A, and that poly BrC can stimulate the incorporation of threonine, as if BrC could also play the role of A. Our experiments could be interpreted by a similar mechanism. After bromination, the anticodons of some glutamyl-s-RNA molecules could be ABrUBrC or BrUBrUBrC, those of some isoleucyl-s-RNA molecules AABrU, those of some lysyl-s-RNA molecules BrUBrUBrU, and those of some valyl-s-RNA molecules BrCAA.

For the aminoacids alanine, histidine, serine and threonine, the usually assumed anticodons contain G; according to some authors, the anticodons for aspartic acid, glycine and tyrosine, also contain G. All these aminoacids (except glycine and tyrosine in some experiments) are not transferred in the presence of poly U after bromination of s-RNA. In contrast to the first group of 4 aminoacids (glutamic acid, isoleucine, lysine, valine), where the transfer could be explained by a possible modification of the base-pairing properties of BrU and BrC, it seems that the presence of BrG in the anticodon prevents any binding to poly U. It is interesting to note that glycine and tyrosine gave ambiguous results. With some brominated s-RNA preparations we observed an incorporation of these two aminoacids in the presence of poly U; with other preparations we obtained no incorporation. At present we are unable to explain these discordant results.

Our experiments can be compared to those performed on FU incorporation into RNA. The information contained in the messenger RNA can be modified by the incorporation of this analog, and this causes some changes in the aminoacid composition of proteins and the appearance of altered enzymes (Bussard et al., 1960; Naono and Gros, 1960; Gros and Naono, 1963). Champe and Benzer (1962) have observed that FU causes phenotypic reversions in certain mutants of bacteriophage T_4 and they suggested that this analog behaves

like U in the transcription of DNA into messenger RNA, but like C in coding for aminoacid incorporation.

The induction of errors at the code level by a chemical modification of s-RNA may represent a picture of what happens in the suppressor mutants where a modification of the s-RNA is a possible explanation for the mutation observed (Yanofsky et al., 1961).

Furthermore our results could allow us to induce errors in the in vitro synthesis of a well-defined protein by introduction of modified aminoacyl-s-RNA's in the system.

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