INFLUENCE OF pH AND S-RNA CONCENTRATION ON CODING AMBIGUITIES*

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Since the discovery of the ambiguity exhibited by poly U by promoting -- in an in vitro system -- the incorporation of leucine (Bretscher and Grunberg-Manago, 1962; Nirenberg and Jones, 1963), and poly UC for the incorporation of threonine (Bretscher and Grunberg-Manago, 1962), many other cases of ambiguity have been reported. Coding ambiguities may be induced by streptomycin (Davies et al., 1964), base analogs Grunberg-Manago and Michelson, 1964), Mg (Davies et al., 1964; Szer and Ochoa, 1964), and solvants (So and Davies, 1964). This puts a shade on the confidence that can be had in the coding units as they have been determined in the in vitro system. The purpose of this work is to determine the conditions for suppressing this ambiguous coding. This study may also help to clarify the cause of ambiguities and give some insight into the translation mechanism.

METHODS

The in vitro amino acid incorporation system used, was a slightly modified version of the Nirenberg and Matthaei system (1960), as previously described (Grunberg-Manago and Michelson, 1964), except that NH_4 was substituted for K.

Polynucleotides were synthesized from the appropriate nucleoside pyrophosphate using a preparation of polynucleotide phosphorylase from Azotobacter agilis (S.A. = 40, expressed as exchange units as defined by Grunberg-Manago et al.(1956). C^{14}-isoleucine CA 18, lysine and serine were obtained from

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Amersham; and C^{14}-isoleucine CB 18, and leucine were obtained from the French Atomic Energy Commission. The buffer used was Tris maleate (pH 6.5) from Sigma, or Tris HCl. The pH of the incubation mixture obtained by addition of buffer pH 6.5, 7, 7.8, were respectively, 6.53, 7.06, 7.9. The polymers AU and UC have been prepared from incubation mixtures of UDP, ADP or UDP, CDP (UDP/ADP = 2; UDP/CDP = 1), and have the following base composition after alkaline hydrolysis : U/A = 3.7, C/U = 1.2. ADP, UDP and CDP were commercial preparations from Sigma. S-RNA was prepared from E.coli B, the same strain as the one used for the preparation of the in vitro system, by the method of Nathans and Lipmann (1961).

RESULTS

a) Influence of Mg

As has been previously reported (Davies et al., 1964; Szer and Ochoa, 1964), higher concentrations of Mg favor the incorporation of isoleucine, serine and leucine in the presence of poly U, with S_{30} fraction of E.coli or with ribosomes plus supernatant (Fig. 1). It should be noted that while the optimum concentration of Mg is higher in the presence of poly U for the incorporation of isoleucine and serine than in the presence of copolymers containing their respective codons with high frequency (AU and UC), this is not the case for leucine. We found that the incorporation of leucine with the S_{30} fraction, in the presence of poly U, is optimum with 0.016 M Mg, and that optimum is the same in the presence of poly AU. The incorporation of threonine in the presence of poly CU is also favored by high Mg concentrations (0.020 M optimum) (Fig. 1), in agreement with the previous statement that the observed incorporation is due to an ambiguity and not to a codon containing C and U (Bretscher and Grunberg Manago, 1962).

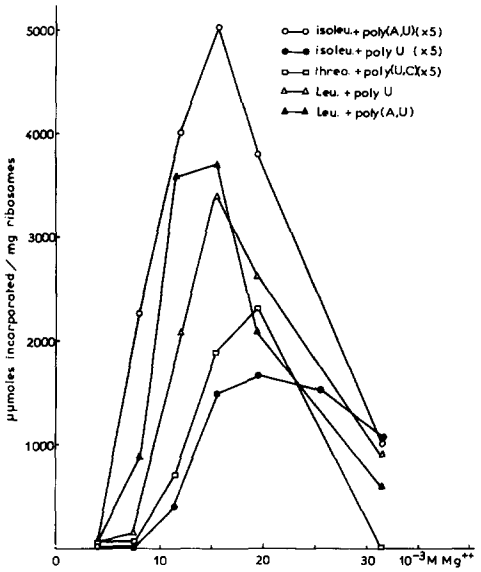

Fig. 1. Influence of Mg on amino acid incorporation; same conditions as for Table I

b) Influence of pH

At 20° the incorporation of leucine, isoleucine or serine, which occurs in the presence of poly U at pH 8.1 (0.018 M, $MgCl_2$), disappears, or is markedly reduced at pH 6.5 (Table I); it should be noted that in some experiments only 70% and not 100% of inhibition was found. Phenylalanine incorporation, however, is higher at pH 6.5 than at pH 8. At 37° there is a slight inhibition of phenylalanine incorporation at acid pH (6.5), but not over 20%. The drop of activity for leucine, isoleucine and serine, could not therefore be explained by a reduced affinity of ribosomes for poly U at acid pH. It is important to note, moreover, that leucine, serine and isoleucine incorporations are not very sensitive to pH in the presence of copolymers containing their respective codons for the amino acid, in high frequency (UA, UC). Threonine incorporation also disappears at pH 6.5 in the presence of copolymer UC, while that of serine does occur at this pH in the presence of the same copolymer, though it is reduced.

TABLE I

INFLUENCE OF pH ON AMINO ACID INCORPORATION

Amino acids	Poly.	Incorp. μμmoles/mg ribosome protein		
		pH		
		6.5	7	8
phenylalanine	A	6100 (165)	4050 (110)	3700 (100)
	AU	3840 (97)	3560 (90)	3950 (100)
leucine	U	0 (0)	460 (65)	712 (100)
	AU	470 (62)	340 (45)	759 (100)
isoleucine	U	0 (0)	13 (7)	195 (100)
	AU	690 (92)	630 (83)	759 (100)
serine	U	0 (0)	43 (67)	64 (100)
	UC	1560 (48)	3220 (99)	3233 (100)
threonine	UC	0 (0)	204 (135)	150 (100)

Values in parentheses indicate % incorporation at pH 8
The control values were as follow, respectively at pH 6.5, 7, 8: phenylalanine, 53, 54, 64; leucine, 135, 63, 168; isoleucine, 135, 122, 157; serine, 42, 39, 41; threonine, 40, 42, 39.
The incubation mixture (0.5 ml) contained in μmole/ml : Mg acetate, 18 (the Mg used for S_{30} preparations was taken into account); Tris, pH 7.8, 50; KCl, 70; thioglycerol, 55; ATP, 1.08; GTP, 0.25; PEP K, 4.46; 0.04 of each of the amino acids labelled with C^{14} (specific activities in μc/μmole : phenylalanine, 11.4; leucine, 19.3; isoleucine, 20; serine, 22.4; threonine, 25.8); 0.04 of each of the other 19 amino acids; PEP kinase, 8 μg; polymers, 150 μg; E.coli S-ARN, 2 mg; S_{30}, 2.78 mg. The samples are incubated 40 minutes at 20° C. The incorporation is measured by the amount of radioactive material precipitated by hot TCA.

c) Influence of S-RNA

Ambiguous amino acid incorporation appears to be sensitive to an excess of S-RNA to an extent varying with the factor promoting the ambiguity. Isoleucine incorporation in the presence of polymer analogues is suppressed by the addition of 1 mg E.coli B S-RNA per mg ribosomes/0.5 ml (Fig.2) as has already been reported (Grunberg-Manago and Michelson, 1964). The observed isoleucine incorporation in the presence of poly U is also markedly inhibited (70-80%) by 2 mg S-RNA. On the contrary,

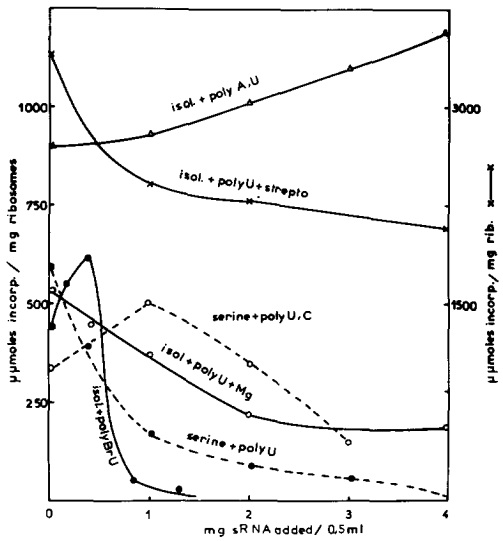

Fig.2. Influence of S-RNA concentration on coding ambiguities. Same conditions as for Table I with the following exceptions :$MgCl_2$ concentration was 0.016 M;in the experiment with poly BrU, 2 mg ribosomes and 2 mg supernatant were used; the amount of streptomycin used was 20 µg/ml.

the incorporation of isoleucine in the presence of an AU copolymer is not inhibited, but slightly enhanced by S-RNA in a comparable amount. The incorporation of serine in the presence of poly U and high Mg is also more sensitive to an excess of S-RNA than in the presence of copolymer UC (Fig.2). When isoleucine incorporation is induced by streptomycin, it is less sensitive to S-RNA, nevertheless a small, but definite, inhibition does occur, but this may well be a non-specific effect.

DISCUSSION

One of the explanations offered on ambiguities observed in the presence of halogenated polymers involves the increased stability of the complexes formed with the anti-codon of the S-RNA by messengers containing analogues (Grunberg-Manago and Michelson, 1964; Michelson and Grunberg-Manago, 1964). Two out of three bases might be enough for the formation of the complex, or alternatively pairing mistakes with adjacent bases might occur. Increasing complexing ability of poly U at low temperature and high Mg was also suggested to explain pairing mistakes between S-RNA

and polynucleotide templates (Szer and Ochoa, 1964). The Mg effect, however, is difficult to explain only on the basis of an increasing complexing ability of poly U, as no increase of the stability of poly A + poly U complexes is observed for concentrations of Mg higher than 0.010 M (Massoulié, unpublished) (Maximum T_m = 80°, 0.01 M $MgCl_2$, 0.1 M or 0.5 M NaCl). Moreover, the effect of pH also renders this explanation improbable. The stability of poly U + poly A complexes (poly (A:U:U) or poly (A:U)) is the same at pH 6.5 and pH 8 (Massoulié, unpublished), but the incorporation of leucine, serine and isoleucine is nevertheless suppressed at acid pH. A more plausible explanation for pH and Mg effect would be a change in ribosome conformation, as is probably the case in the presence of streptomycin (Davies et al., 1964). This change might result in decreasing the stability of the ribosome - S-RNA-messenger complex.

The inhibitor effect of S-RNA is not very clear; it might be due to a competition of different S-RNAs for the messenger-ribosome complex. The higher affinity would be for the S-RNA containing the right anti-codon. In the presence of a large excess of this S-RNA, no other S-RNA could complex with the messenger on the ribosomes. This explanation is supported by the recent discovery of Nirenberg and Leder (1964)that phenylalanine S-RNA is bound with high efficiency to ribosome-poly U complex.

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REFERENCES

M.S.Bretscher, M.Grunberg-Manago, Nature, 195, 283 (1962)
J.Davies, W.Gilbert, L.Gorini, Proc.Natl.Acad.Sci., 51, 883 (1964)
M.Grunberg-Manago, P.J.Ortiz, S.Ochoa, Biochim.Biophys.Acta, 20, 269('56)
M.Grunberg-Manago, A.M.Michelson, Biochim.Biophys.Acta, 80, 431(1964)
J.Massoulié (unpublished results)
A.M.Michelson, M.Grunberg-Manago, Biochim.Biophys.Acta, 87, 593(1964)
D.Nathans, F.Lipmann, Proc.Natl.Acad.Sci., 47, 497 (1961)
M.W.Nirenberg, J.H.Matthaei, Proc.Natl.Acad.Sci., 47, 1580(1960)
M.W.Nirenberg, O.W.Jones, in "Informational Macromolécules" (Vogel, Bryson, Lampen, eds.)Academic Press Inc., p.451(1963)
M.W.Nirenberg, P.Leder, Science, 145, 1399 (1964)
A.G.So, E.W.Davie, Biochemistry, 3, 1165 (1964)
W.Szer, S.Ochoa, J.Mol.Biol., in press