FIDELITY IN PROTEIN SYNTHESIS: PROLINE MISCODING IN A THERMOPHILE SYSTEM

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Received October 15, 1965

Our previous studies indicated that specificity in the translation of messenger RNA's in a subcellular system derived from Bacillus stearothermophilus is influenced to a considerable extent by the temperature and cationic environment during protein synthesis (Friedman and Weinstein, 1964). This apparent variation in the read-out of the genetic code has been referred to as the problem of "fidelity." A similar influence of temperature (Szer and Ochoa, 1964), cations (Szer and Ochoa, 1964; Davies et al., 1964), aminoglycoside antibiotics (Davies et al., 1964), organic solvents (So and Davie, 1964), and pH (Grunberg-Manago and Dondon, 1965) on the translation of synthetic messenger RNA's has been described in the E. coli system.

The results obtained in the thermophile system with poly U, poly UG, and poly UC indicated that when miscoding occurred it was restricted to groups of amino acids which were characteristic for each polymer (Friedman and Weinstein, 1964). The pattern of response suggested that polycations induce miscoding by stabilizing the alignment of S-RNA's to codons which contain two rather than three complementary nucleotides. The present study describes an even greater degree of miscoding. A preliminary account of part of this work has been reported (Friedman and Weinstein, 1965a).

METHODS

A brief description of the thermophile system has appeared (Friedman and Weinstein, 1964) and a more detailed one is forthcoming (Friedman and Weinstein, 1965b). The components of the reaction mixtures are presented in the legend to Table I. CTP and UTP are not required and their omission does not affect the present results.

RESULTS AND DISCUSSION

According to data obtained in the E. coli system, codon assignments for proline include CCC, CCU, CCA, and CCG (Nirenberg et al., 1965). On the basis of our former studies, one would not expect polymers devoid of cytidylic acid to code for proline, even in a high ambiguity situation. We have observed, however, a small but significant and reproducible stimulation of proline incorporation directed by poly UG (Table I). This stimulation occurred only in the presence of 0.018 M Mg^{++} and 6.9×10^{-4} M dihydrostreptomycin (DHSm). Addition to the incubation mixture of 1 μM of C^{12}-proline completely blocked the incorporation of C^{14}-proline. This finding, coupled with the fact that our incubation mixture always contains a full complement of C^{12}-amino acids excluding the radioactive amino acids being tested, indicates that the incorporation we detect is not due to contamination of C^{14}-proline with other C^{14}-amino acids.

Polyproline is somewhat soluble in 5% TCA but insoluble in 20% TCA (Wahba et al., 1963). When samples were precipitated with 20% rather than the usual 5% TCA, there was a decrease in the recovery of C^{14}-proline from 4.6 to 1.1 μμmole (Table I). Thus, the protein being synthesized behaves more like a polypeptide composed of several amino acids than it does polyproline.

Table I. Effect of dihydrostreptomycin (DHSm) on poly UG and UGA-directed proline incorporation.

Polymer	DHSm (6.9×10^{-4} M)	C^{14}-proline incorp. (μμmoles)
Experiment 1		
---	---	0.5
---	+	0.6
100 μg poly UG	---	0.6
"	+	4.6
"	+	0 (C^{12}-proline)
"	+	1.1 (20% TCA)
Experiment 2		
---	---	0.5
---	+	0.4
10 μg poly UGA	+	5.1
50 μg poly UGA	+	15.6
100 μg poly UGA	+	15.7
"	---	2.0
"	+	0 (C^{12}-proline)
"	+	7.2 (20% TCA)

Reaction mixtures contained in a 0.4 ml volume the following components (in μmoles unless otherwise specified): Tris-HCl buffer, pH 7.8, 4.0; potassium chloride, 24.0; β-mercaptoethanol, 2.4; ATP, 0.25; GTP, 0.01; CTP, 0.01; UTP, 0.01; phosphoenolpyruvate, 1.25; phosphoenolpyruvate kinase, 12 μg; a mixture of C^{12}-L-amino acids, excluding proline, 0.0125 of each; C^{14}-L-proline (sp. act. 186 mc/mM), 1.8 mμmoles; and the iS-30 fraction of B. stearothermophilus (approximately 0.62 mg protein and 0.37 mg RNA). Magnesium acetate was added at a final concentration of 0.018 M. The mixtures were incubated at 37°C for 20 min. in the absence and presence of poly UG (5:1) and UGA (10:3:1). The base ratios of the synthetic polyribonucleotides (Miles Laboratories) reflect the input ratios of the nucleoside-5'-diphosphates used for their synthesis. Reactions were stopped with TCA, heated at 85°C for 30 min., washed by membrane filtration, and assayed for radioactivity as previously described (Friedman and Weinstein, 1965b). The values represent μμmoles C^{14}-proline incorporated per reaction mixture.

A UGA copolymer also stimulated proline incorporation (Table I). The degree of stimulation was dependent upon the polymer concentration, reaching a plateau at 15.6 μμM with 50 μg poly UGA per incubation mixture. In this particular experiment, there was a small stimulation of proline incorporation in the absence of DHSm, but with most extracts tested the stimulation occurred only in the presence of DHSm. Again the reaction was quenched by C^{12}-proline and the product was considerably less soluble in 5% than in 20% TCA.

The possibility that our polymers were contaminated with cytidylic acid residues was investigated. Poly UG and UGA were hydrolyzed in 0.3 M KOH, and the resulting mononucleotides were separated by electrophoresis in 0.05 M citrate buffer, pH 3.4, eluted, and their u.v. absorption determined. The predicted mononucleotides were recovered and no cytidine monophosphate (CMP) was detected. Using known amounts of CMP, it was calculated that the maximum amount of this nucleotide which could contaminate poly UG and UGA was less than 1 and 3%, respectively. Further evidence against contamination with CMP is the fact that poly UG did not stimulate the incorporation of threonine and histidine, whereas in a high ambiguity situation a polymer known to contain both U and C residues does code for these two amino acids (Friedman and Weinstein, 1964).

In contrast to the results observed with poly UGA, the homopolymers poly U, poly I, and poly A did not stimulate proline incorporation. Poly I was used in lieu of poly G since it is more readily available, and replacement of guanylic acid with inosinic acid does not change the coding properties of copolymers (Basilio et al., 1962).

The effect of temperature on proline miscoding is presented in Table II. At 37°C, and only in the presence of DHSm, poly UG and poly UGA stimulated proline incorporation. At 65°C, however, both polymers were ineffective in eliciting the proline response. Previous

studies (Friedman and Weinstein, 1964) have demonstrated that with thermophile extracts extensive poly UG-directed phenylalanine incorporation occurs at 65°C. Furthermore, it has been shown (Friedman and Weinstein, 1965b) that the endogenous incorporation of proline is greater at 65°C than at 37°C. Therefore, the inability of poly UG and UGA to code for proline at 65°C cannot be attributed to a general heat-denaturation of the protein synthesizing system nor specifically to the thermolability of proline activation.

Table II. The effect of temperature on proline "miscoding."

Polymer	DHSm (6.9×10^{-4} M)	C^{14}-proline incorp. (μμmoles)	
		37°C	65°C
---	---	1.7	1.0
---	+	2.2	2.3
100 μg poly UG	---	1.9	1.6
"	+	7.0	1.7
50 μg poly UGA	---	1.9	1.0
"	+	10.5	0.3

Incubation temperatures are as indicated. The remaining conditions are as described in Table I.

In view of the ability of DHSm to enhance proline miscoding, it was of interest to examine the effects of this drug on proline incorporation directed by m-RNA's which normally code for this amino acid. In the thermophile system, as with E. coli, poly C directs the synthesis of polyproline (Table III). This reaction was stimulated almost seven-fold by the addition of DHSm. The S-30 fraction was used to test the effects of DHSm on proline incorporation directed by native m-RNA's. In this case, the drug produced a 1.5-fold stimulation of proline incorporation. Further studies indicate that in the latter system DHSm also enhances the incorporation of phenylalanine, arginine, lysine, and isoleucine.

Table III. Effect of dihydrostreptomycin (DHSm) on native m-RNA and poly C-directed proline incorporation.

Extract	Polymer	DHSm (6.9×10^{-4} M)	C^{14}-proline incorp. ($\mu\mu$moles)
S-30	---	---	1.9
S-30	---	+	3.3
iS-30	100 μg poly C	---	11.3
iS-30	"	+	75.7

Where indicated the S-30 fraction (1.4 mg protein and 0.8 mg RNA) was substituted for the iS-30. The products of the poly C reaction were precipitated with 20% TCA (Wahba et al., 1963).

During the preparation of this manuscript it was reported by van Knippenberg et al. (1965) that under certain conditions streptomycin (Sm) also stimulated viral RNA-directed protein synthesis in the E. coli system. The exact relation between miscoding and the stimulatory effects of Sm is not clear. The authors suggest the rate of translation of specific codons may be limited by the amount of the related s-RNA's and that Sm enhances the rate of protein synthesis by permitting other s-RNA's to read these codons.

Based on current concepts of protein synthesis, the following mechanisms may be invoked to explain proline miscoding. DHSm might cause the attachment of proline to a non-proline s-RNA, one which fits codons present in UG-containing copolymers. This would be similar to the anomalous charging of s-RNA which can occur with heterologous mixtures of activating enzymes and s-RNA's (Jacobson et al., 1964). Alternatively, DHSm might alter the assumed interaction between s-RNA and m-RNA by 1) stabilizing non-Watson-Crick type base pairing, such as G-G hydrogen bonding between the anticodon of proline s-RNA and the G residues of our copolymers, or 2) altering the secondary structure of proline s-RNA in a manner which exposes a new anticodon site. Finally, since previous evidence (Davies et al., 1964) showed that the site of

action of streptomycin is on the 30S ribosome subunit, it is possible that DHSm alters an as yet unknown function of the ribosome in coding specificity. Further studies are required to determine which of these mechanisms is involved in proline miscoding. Regardless of the mechanism, this type of miscoding is not unique to the thermophile system since Davies, Gorini and Davis (1965) have recently observed in the E. coli system several cases involving misreading of at least two bases of a codon. In conclusion, it must be emphasized that the absolute stimulation observed in the present report is rather small and it is not known whether this mistake level is of physiologic significance in vivo.

ACKNOWLEDGMENTS

We wish to thank Miss Judith Levitt for her expert technical assistance. This research was supported by U.S.P.H.S. Research Grant No. R10 CA-02332 from the National Cancer Institute. One of us (SMF) is a trainee on Grant 5T4 CA-5011 from the U.S.P.H.S., the other (IBW) is a Career Scientist of the Health Research Council of the City of New York (I-190).

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