SYNTHETIC POLYNUCLEOTIDES AND THE AMINO ACID CODE, V[*].

BY

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In the preceding paper of this series substantial agreement was found between the experimentally determined letters of the genetic code[1-4] and amino acid replacements in nitrous acid mutants of tobacco mosaic virus[5-7]. Due to deamination of certain bases, treatment of nucleic acids with HNO_2 leads to base substitutions in the polynucleotide chains and hence to changes of their nucleotide sequence[8,9]. Guanine is converted to xanthine, adenine to hypoxanthine, and cytosine to uracil. Uracil is not changed. In the case of tobacco mosaic virus RNA, controlled HNO_2 treatment can lead to deamination of a single base and, as a result of the base change, to the substitution of an amino acid for another at a specific location in the polypeptide chain of the protein coat. It has been assumed[6] that the conversion of guanine to xanthine, a base which is not present in nucleic acids, is reverted (guanine taking the place of xanthine) when the RNA is replicated so that no mutation occurs. In a similar way, when adenine is deaminated to hypoxanthine, which like xanthine is not normally present in nucleic acids, guanine is supposed to take the place of hypoxanthine after replication of the RNA. In this case, however, guanine is substituted for the original adenine (A→G replacement) and mutation occurs. Deamination of cytosine to uracil leads directly to a C→U replacement.

Hypoxanthine can be substituted for guanine, i.e. inosinic acid (I) for guanylic acid (G), in synthetic polynucleotides with retention of their coding characteristics for, as previously noted[4], poly UI (5:1) was equivalent to

poly UG (5:1) in regard to those amino acids (cysteine, glycine, tryptophan, and valine) which are coded by U- and G-containing letters. In line with this observation, treatment with HNO_2 conferred upon poly UA (5:1) the coding characteristics of poly UG (5:1), e.g. cysteine and valine incorporation was substituted for isoleucine and tyrosine incorporation. This is the test tube counterpart of amino acid replacements in HNO_2 mutants of tobacco mosaic virus. On the other hand, deamination of guanine to xanthine by treatment of poly UG (5:1) with HNO_2 eliminated the stimulation of valine incorporation caused by the untreated polymer. Thus, contrary to hypoxanthine, xanthine cannot replace guanine in the genetic code. An account of these experiments is given in this paper.

Preparations and methods. - These were the same as in previous work[4] unless otherwise specified. Poly UI (5:1) was prepared with Azotobacter polynucleotide phosphorylase[1] from a mixture of uridine 5'-diphosphate and inosine 5'-diphosphate in molar ratio 5:1. Its sedimentation coefficient was 6.3 S.

Deamination of polynucleotides. - 720 mg of sodium nitrite were added to a solution of 6 mg of polymer in 9 ml of 20% acetic acid. After standing at room temperature with occasional shaking for one hour (experiment 2, Table 2) or two hours (experiment 1, Table 2), the solution was dialyzed for 5-6 hours against distilled water, with several changes, and the polymer recovered by lyophilization. In the case of poly UA (5:1), deamination of the

adenine residues appeared to be near completion in 30 minutes as judged by extensive loss of the capacity to stimulate the incorporation of isoleucine into acid-insoluble products in the Escherichia coli system.

Results.- Experiments with poly UI: As shown in Table 1 poly UI (5:1) stimulated the incorporation of phenylalanine, cysteine, valine, glycine, and tryptophan (experiment 1) and leucine (experiment 2). Although the activity of this polymer was somewhat lower than that of poly UG (5:1) (cf. Table 2), the phe/cys, phe/val, phe/gly, phe/try, and phe/leu incorporation ratios, given in the last column of the table, were in reasonable agreement with the corresponding ratios for poly UG (5:1) (cf. Table 2 of preceding paper[4]). This proves that hypoxanthine can replace guanine in the genetic code.

Experiments with HNO_2-treated polymers: The results of experiments with poly UA, UG, and UC are shown in Table 2. In addition to the changes that can be specifically ascribed to deamination, treatment with HNO_2 resulted in a pronounced overall decrease in the activity of the polymers. This was reflected by an 85% and 70% decrease of the capacity of poly UA (5:1) to stimulate the incorporation of phenyalanine after treatment with HNO_2 for 2 hours (experiment 1) and 1 hour (experiment 2), respectively. The reason for this decrease in activity is unknown. Nevertheless, the change in coding characteristics of poly UA, due to A→I conversion by deamination, was readily apparent. Before deamination, poly UA promoted the

incorporation of isoleucine, leucine, and tyrosine but not that of cysteine and valine. Deamination largely eliminated the capacity to stimulate incorporation of isoleucine and tyrosine and brought forth stimulation of the incorporation of cysteine and valine. Stimulation of leucine incorporation was largely retained in agreement with the finding[4] that leucine is coded by 2U1A and 2U1G (or 2U1I, Table 1) besides 2U1C letters.

Treatment of poly UG (5:1) with HNO_2 (guanine→xanthine conversion) virtually eliminated all activity of this polymer. Phenyalanine incorporation was drastically decreased and valine incorporation was wiped out[10]. Thus, in sharp contrast to hypoxanthine, xanthine is unable to substitute for guanine in the genetic code.

The experiment with poly UC (5:1) showed retention of phenyalanine (code letter UUU) and marked loss of serine (code letter 2U1C) incorporation activity following treatment with HNO_2 for 1 hour (C→U conversion). Since the activity toward phenylalanine was retained, contrary to the marked drop observed with poly UA, it is likely that a non-specific decrease in activity, caused by HNO_2 treatment, was compensated by an increase due to conversion of poly UC, which codes for phenylalanine and other amino acids, to poly U which codes for phenylalanine only.

Discussion. - As shown in this paper HNO_2 treatment of synthetic polynucleotides, used as artificial messengers for protein synthesis in the *E. coli* system, leads to amino acid replacements like those observed in

HNO_2 mutants of tobacco mosaic virus. However, multiplication of the virus involves two processes viz. replication of the RNA and transcription of its code into a polypeptide sequence. Our model experiments with polynucleotides relate only to the effect of HNO_2 on the transcription of the message.

The finding that hypoxanthine can replace guanine in amino acid coding is not surprising in view of the similarity of these two bases with regard to hydrogen bonding. Poly I has been shown to form DNA-like, double-stranded helical complexes with poly C[11,12]. The stability of the hypoxanthine-cytosine pair is of the order of magnitude of that of the adenine-uracil pair as the melting out temperature of poly A + U (61° in 0.15 M NaCl-0.15 M sodium citrate) is only about 10 degrees higher than that of poly I + C[13]. Therefore, hypoxanthine in poly UI triplets (e.g. UUI) would pair with cytosine in complementary "adaptor"[14] triplets (AAC) of cysteine or valine transfer RNA. However, the guanine-cytosine pair, with three hydrogen bonds, is held together more tightly than the hypoxanthine-cytosine pair with only two hydrogen bonds. The lower efficiency of poly UI as compared with poly UG, noted in a previous section, might be a reflection of this difference. Our observations are in line with the finding[15] that deoxy ITP could replace (with 25% efficiency) deoxy GTP in DNA synthesis by DNA polymerase. The further finding reported in this paper that xanthine cannot replace guanine in coding, although apparently

not explainable in terms of hydrogen bonding properties (as xanthine is similar to guanine in this respect), is also in line with the failure of deoxyxanthosine triphosphate to replace deoxy GTP in the DNA polymerase system[15]. In view of these results, reversal of the guanine→xanthine conversion (guanine taking the place of xanthine) on replication of HNO_2-treated tobacco mosaic virus RNA, is unlikely. Deamination of guanine is more likely to yield an RNA that is unable to replicate (lethal mutation).

Modification of the coding characteristics of synthetic polynucleotides with agents other than HNO_2 might throw light on the mode of action of certain mutagens. Several amino acid replacements in tobacco mosaic virus protein have been brought about by treatment of the virus with brominating and alkylating agents[5,7]. However, the relationship between the observed replacements and the chemical effects of these mutagens on the nucleic acid bases is obscure.

Summary.- Hypoxanthine can replace guanine in amino acid coding for, like poly UG (5:1), poly UI (5:1) stimulated the incorporation of cysteine, glycine, leucine, tryptophan, and valine into acid-insoluble products in the *E. coli* system to the same relative extent. Treatment of synthetic polynucleotides with nitrous acid modified their coding characteristics as expected from the deamination of adenine to hypoxanthine and cytosine to uracil. Poly UA lost its coding specificity and acquired that of poly UI, and poly UC lost its activity to stimulate the incorporation of serine but not that of

phenylalanine. Deamination of guanine to xanthine, by treatment of poly UG with nitrous acid, wiped out the activity of this polymer to stimulate valine incorporation. Thus, contrary to hypoxanthine, xanthine cannot replace guanine in amino acid coding.

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TABLE 1

EFFECT OF POLY UI (5:1) ON AMINO ACID INCORPORATION IN *E. COLI* SYSTEM*

Experiment No.	Amino acid	Without poly UI	With poly UI	Net	Ratio+
1	Phenylalanine	0.18	9.14	8.96	---
	Cysteine	0.15	1.28	1.13	7.9
	Valine	0.21	2.24	2.03	4.4
	Glycine	0.17	0.56	0.39	23.0
	Tryptophan	0.12	0.51	0.39	23.0
2	Phenylalanine	0.10	4.91	4.81	---
	Leucine	0.26	1.51	1.25	3.9

*mμmoles/mg ribosomal protein. +Ratio of phenylalanine incorporation to that of the amino acid in question.

TABLE 2

EFFECT OF VARIOUS POLYNUCLEOTIDES, BEFORE AND AFTER TREATMENT WITH HNO_2, ON AMINO ACID INCORPORATION IN *E. COLI* SYSTEM*

Experiment No.	Amino acid	Polynucleotide UA (5:1) Before HNO_2	UA (5:1) After HNO_2	UG (5:1) Before HNO_2	UG (5:1) After HNO_2	UC (5:1) Before HNO_2	UC (5:1) After HNO_2
1	Phenylalanine	15.9	2.4	22.4	0.07	---	---
	Isoleucine	4.0	0.05	---	---	---	---
	Valine	0	0.4	6.1	0	---	---
2	Phenylalanine	11.8	3.6	---	---	15.8	14.1
	Isoleucine	1.5	0.07	---	---	---	---
	Leucine	1.7	0.30	---	---	---	---
	Tyrosine	3.1	0.12	---	---	---	---
	Cysteine	0	0.16	---	---	---	---
	Valine	0	0.33	---	---	---	---
	Serine	---	---	---	---	3.3	0.7

*Values given refer to net incorporation (in mμmoles/mg ribosomal protein) after subtraction of small blank incorporation without polynucleotide.