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POLYPHENYLALANINE SYNTHESIS DIRECTED BY
BROMINATED POLYURIDYLIC ACID¹

David A. Smith

Biomedical Research Group, Los Alamos Scientific Laboratory
University of California, Los Alamos, New Mexico

Brominated poly U when mixed with equimolar amounts of poly A has a decreased hypochromicity compared to poly U-poly A mixtures (Yu and Zamecnik, 1964). This has been interpreted to mean that bromination of uracil residues weakens the hydrogen-bonding potential for adenine residues. This report demonstrates that brominated poly U is at least as active as poly U in stimulating polyphenylalanine formation in a cell-free protein synthesizing system (Nirenberg and Matthaei, 1961).

EXPERIMENTAL METHODS AND RESULTS

Bromination of poly U (obtained from Miles Chemical Company, Elkhart, Indiana) was performed under nearly the same conditions as used by Yu and Zamecnik (1963, 1964).

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5-Bromo-6-hydroxy-5,6-dihydrouracil residues are the main product when bromination is carried out in this manner.

Poly U was dissolved in 0.01 M sodium acetate, pH 6.3, to a monomer concentration of 12.4 mM. Various amounts of 12.4 mM aqueous bromine were added with stirring to aliquots, and the solutions were diluted to a final concentration of 6.2 mM.

In some cases, 12.4 mM poly U was mixed with an equal volume of 25 mM bromine. In all cases, bromination was carried out in an ice bath. The presence of sodium acetate as a buffer prevented a drastic fall in pH during bromination but had no effect on the amount of bromine taken up by the polymer as judged by the decrease in absorbancy at 259 m μ .

Poly U brominated in sodium acetate was tested for its ability to stimulate polyphenylalanine formation. Table I shows the results of a typical experiment. In nearly all experiments poly U brominated in sodium acetate stimulated more phenylalanine incorporation than did poly U itself. The only exceptions were two cases (out of a total of 15 duplicate incubations) where a brominated poly U stimulated phenylalanine incorporation to the same extent as poly U. The reason for this stimulation is not clear. Bromide ions in the concentrations found in the brominated poly U solutions have little effect on phenylalanine incorporation. It may be that the brominated poly U is resistant to nucleases.

TABLE I. Effect of Brominated Poly U on Phenylalanine Polymerization

Polymer	A ₂₅₉ as % of Poly U	Phenylalanine Incorporated (pmoles)
Poly U		172
Brominated Poly U Preparations:		
0.25	82	269
0.50	61	250
0.75	42	230
1.00	21	236
2.00	8	0

The incubations were carried out essentially as described by Nirenberg (1963). 0.4 mg E. coli sRNA, 1.24 μ moles (as mononucleotide) of poly U or brominated poly U, and 1.4 mg S-30 protein were added to each tube. The incubation volume was 0.55 ml. Incubations were continued for 40 minutes at 37° and analyzed by the method of Mans and Novelli (1960). Incorporation was corrected for the amount of phenylalanine incorporated in the absence of polynucleotide. Brominated poly U preparations are referred to by the molar ratio of bromine to nucleotide used in their preparation.

Another striking feature of Table I is that poly U treated with an equimolar amount of bromine (with 21% of the original absorbance at 259 $m\mu$) greatly stimulates phenylalanine incorporation, while poly U treated with twice as much bromine is completely inactive. In the presence of excess bromine other, probably dibrominated, products are formed (Yu and Zamecnik, 1963; Wang, 1957).

Experiments were done in which poly U was brominated in distilled water rather than in sodium acetate. In this case (as shown in Fig. 1), poly U partially lost its ability to

Fig. 1. Phenylalanine incorporation as stimulated by brominated poly U preparations. (a) Poly U brominated in 0.01 M sodium acetate, pH 6.3; (b) poly U brominated in distilled water and tested one day after bromination; and (c) same as (b) except tested four days after preparation of the brominated poly U solutions.

stimulate phenylalanine uptake. The magnitude of the loss was proportional to the time elapsed since the bromination was performed and also to the amount of bromine taken up and hence to the acidity. Saturation of the pyrimidine ring makes the glycosyl bond labile to acid (Levene and LaForge, 1912; Green and Cohen, 1957), and loss of brominated uracil residues is likely to be responsible for the loss in ability to stimulate polyphenylalanine formation. The amount of brominated poly U added to the incubation mixtures did not change their pH. The hypochromicity produced when these brominated poly U solutions were mixed with poly A also decreased with time.

Polynucleotides altered so as to prevent hydrogen bonding with complementary bases are inactive in amino acid-incorporating systems (Ludlum et al., 1964; Wahba et al., 1963), while many other polynucleotide analogues do stimulate amino acid polymerization (Grunberg-Manago and Michelson, 1964). It can be concluded from the data presented here that the 5,6 double bond of uracil is not required for poly U to retain messenger activity.

The ability of brominated poly U to form complexes with poly A and the characteristics of this binding are currently under investigation.

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