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## Coding Properties of 7-Methylguanine

Abstract. *The abnormal base, 7-methylguanine, has been introduced into copolymers of uridylic and guanylic acids by methylation under controlled conditions. The resulting methylated copolymers have a markedly decreased template activity for polypeptide synthesis in vitro due to steric effects of the 7-methyl groups. Contrary to expectation, these polymers do not permit incorporation of amino acids whose codons contain the bases uracil and adenine.*

The mutagenic effects of alkylating agents have been reviewed recently (*1*, *2*); the methylating agents, methyl methanesulfonate and dimethyl sulfate, have caused mutations (*2*) in several organisms and in TMV RNA (*3*). Since these agents methylate guanine in both RNA and DNA, it has been proposed (*4*) that the mutagenic effects in DNA may result from the mispairing of 7-methylguanine with thymine during replication. This might be anticipated since the methylated nucleoside, with a *pK* of 7.2, as compared with a *pK* of 9.2 for guanosine, is partially ionized in neutral solution. Mispairing of the ionized base could occur through hydrogen bonding between the negatively charged N-1 of 7-methylguanine and N-3 of thymine, and between the amino group of 7-methylguanine and the C-2 keto group of thymine.

If similar mispairing occurred during translation, messenger RNA containing 7-methylguanine would code as if it contained adenine in place of the methylated base. This possibility can be tested by methylating synthetic polynucleotides which contain only uracil and guanine. In these polymers, uracil is alkylated with considerable difficulty (*5*) so that the effects of guanine alkylation can be studied specifically. Protein synthesis in vitro with such polymers as messenger should permit incorporation of amino acids that are normally represented by combinations of the bases uracil and adenine if 7-methylguanine mispairs as adenine.

To test this possibility, we synthesized a UG copolymer (*6*) with polynucleotide phosphorylase from *Micrococcus lysodeikticus*. Polymer was isolated after repeated extraction with phenol in the presence of Macaloid (*7*) by precipitation with three volumes of ethanol. It was then redissolved, dialyzed exhaustively in the cold against a mixture of 0.15*M* NaCl, 0.015*M* sodium citrate, 0.1*M* NaCl, and distilled water, and dried by lyophilization. Chromatography in a mixture of 95 percent ethanol and 1*M* ammonium acetate (60:40) showed only high molecular weight material.

Two separate methylations of a single UG copolymer were performed with methyl methanesulfonate in a light-scattering cell in cacodylate buffer [*p*H 7, ionic strength 0.2 (*8*)]. By following the intensity of light scattered at 90°C, it was demonstrated that there was no change in molecular weight during methylation. The methylated polymers were subsequently freed of buffer by dialysis and lyophilized to dryness.

The compositions of the polymers were determined by paper chromatography and subsequent quantitative elution after hydrolysis with HCl, and by

Table 1. Properties of UG copolymers.

| Polymer | Composition (%) | | | | $s_{20,w}$ | Mol. wt.* |
|---|---|---|---|---|---|---|
| | U | MeU | G | MeG | | |
| Original UG | 69.6 | | 30.4 | | 3.48 | $3.11 \times 10^5$ |
| Methylated UG-1 | 70.3 | | 23.3 | 6.4 | 3.47 | |
| Methylated UG-2 | 66.2 | 2.7 | 15.2 | 15.9 | 3.56 | |

* By light-scattering method.

Table 2. Coding properties of the methylated UG polymers. Each milliliter of reaction mixture contained the following: tris, 0.05 mmole; KCl, 0.03 mmole; magnesium acetate, 0.013 mmole; mercaptoethanol, 0.006 mmole; ATP, 0.003 mmole; GTP, 0.0002 mmole; phosphoenolpyruvate, 0.017 mmole; pyruvate kinase, 50 μg; unlabeled amino acids, $4 \times 10^{-5}$ mmole; $C^{14}$-amino acid (specific activity, 40 μc/μmole, New England Nuclear) $2.5 \times 10^{-5}$ mmole; S-30 fraction, 6 mg of protein per milliliter; and one of the following: polyUG, 90 μg; methylated polyUG-1, 100 μg; methylated polyUG-2, 140 μg. The *p*H was 7.2 at 34°C. For each determination, 0.1 ml of the reaction mixture was used; it was incubated for 7½ minutes at 34°C before the polymer and $C^{14}$-amino acid were added. The reaction time was 40 minutes at 34°C. Samples were precipitated with 5 percent trichloroacetic acid. The mixture was heated to 90°C for 15 minutes, transferred to glass-fiber filter papers, and washed with 5 percent trichloroacetic acid, ethanol, and acetone. The radioactivity on the filter papers was then counted in a liquid scintillation counter (Nuclear Chicago).

| Amino acid | Relevant code words* | Polymer† | Incorporation | | | |
|---|---|---|---|---|---|---|
| | | | Estimated from known codons‡ | | | Experimental (%) |
| | | | 7-MeG = A (%) | 7-MeG = G (%) | 7-MeG = 0 (%) | |
| Phenylalanine | UUU | UG | 100 | 100 | 100 | 100§ |
| | | MeUG-1 | 100 | 100 | 100 | 100 |
| | | MeUG-2 | 100 | 100 | 100 | 100 |
| Valine | GUU, | UG | 62.7 | 62.7 | 62.7 | 40.9 ± 2.3¶ |
| | GUA, | MeUG-1 | 47.2 | 57.1 | 44.2 | 35.5 ± 4.5 |
| | GUG | MeUG-2 | 34.1 | 64.0 | 28.6 | 30.6 ± 3.6 |
| Glycine | GGU, | UG | 27.3 | 27.3 | 27.3 | 13.5 ± 1.7 |
| | GGA, | MeUG-1 | 15.6 | 22.3 | 14.6 | 10.9 ± 1.4 |
| | GGG | MeUG-2 | 7.8 | 27.0 | 6.5 | 6.6 ± 1.8 |
| Tyrosine | UAU | UG | | | | 0.93 ± 0.49 |
| | | MeUG-1 | 9.1 | | | .96 ± .45 |
| | | MeUG-2 | 24.1 | | | 1.84 ± .69 |
| Isoleucine | AUU | UG | | | | 0.02 ± 0.02 |
| | | MeUG-1 | 9.1 | | | .08 ± .08 |
| | | MeUG-2 | 24.1 | | | .07 ± .09 |
| Serine | AGU | UG | | | | 2.5 ± .003 |
| | | MeUG-1 | 3.0 | | | 2.6 ± .2 |
| | | MeUG-2 | 5.5 | | | 1.9 ± 1.3 |

* Codons containing U, G, and A. See Söll *et al.* (*11*). † See Table 1. ‡ Calculated from experimentally determined base ratios assuming random order of the bases. § Total incorporation of phenylalanine was about 100,000 dpm, 60,000 dpm, and 10,000 dpm for polyUG, methylated polyMeUG-1, and polyMeUG-2, respectively. Controls minus added polymers contained less than 400 dpm for all amino acids except glycine (900 dpm) and have been subtracted. ¶ Average of six independent determinations for phenylalanine, valine, and glycine and four for tyrosine, isoleucine, and serine.

Table 3. Incorporating ability of UG polymers. Conditions those of Table 2.

| Polymers added | Phenylalanine (dpm) incorporated |
|---|---|
| UG (6 $\mu$g) | 78,500 |
| UG (12 $\mu$g) | 90,100 |
| MeUG-1 (9 $\mu$g) | 55,300 |
| MeUG-2 (5 $\mu$g) | 7,630 |
| UG (6 $\mu$g) and MeUG-1 (9 $\mu$g)* | 79,400 |
| UG (6 $\mu$g) and MeUG-2 (5 $\mu$g*) | 78,600 |
| MeUG-1 (9 $\mu$g) then UG (6 $\mu$g)† | 77,500 |
| MeUG-2 (5 $\mu$g) then UG (6 $\mu$g)† | 77,600 |
| UG (6 $\mu$g) then MeUG-1 (9 $\mu$g)† | 85,300 |
| UG (6 $\mu$g) then MeUG-2 (5 $\mu$g)† | 72,100 |
| None | 370 |

* Second polymer was added less than 10 seconds after the first. † Second polymer was added 1 minute after the first.

analysis of the spectrum of the hydrolyzate. The presence of 7-methylguanine as the primary derivative was established by comparison of the ultraviolet spectrum of the eluted derivative with that of 7-methylguanine and by cochromatography with that compound. In the more heavily methylated polymer, a small amount of a substance, assumed to be 3-methyluridylic acid, was eluted from the area in the chromatogram corresponding to this compound.

Further characterization (Table 1) included sedimentation in cacodylate buffer (*p*H 7, ionic strength 0.2) in a Spinco model E ultracentrifuge equipped with absorption optics, which showed no change in $s_{20,w}$ on alkylation; determination of the molecular weight (by light scattering) of the original polymer; and an analysis of the optical density at 260 m$\mu$ as a function of temperature between 5° and 85°C. The later determination showed no evidence of appreciable secondary structure in any of the polymers.

The capacity of the original polyUG and the methylated polymers to code for various amino acids was tested to determine whether the polymers containing 7-methylguanine would permit the incorporation of amino acids whose codons contain adenine in addition to incorporation of the amino acids with uracil- and guanine-containing codons incorporated by the polyUG control. Extracts for S-30 fractions were prepared from *E. coli* K10 [strain S26R1D of Garen *et al.* (*9*)] by the procedure of Nirenberg and Matthaei (*10*) after the cells had been ruptured in a French pressure cell. None of the amino acids known to be coded for by adenine-containing codons are incorporated significantly more by the methylated than by the control polyUG (Table 2).

Thus, under the conditions of these experiments, 7-methylguanine does not appear to code like adenine, an indication either that the ionization of 7-methylguanine is not sufficient (50 percent at *p*H 7.2 if its *pK* is unaffected by incorporation in the polymer) to allow for miscoding or that the steric effect of the methyl group hinders polymer binding, base pairing, or some other recognition process taking part in polypeptide formation in vitro. The observed decrease in the percentage of incorporation of valine and glycine, relative to phenylalanine, with the increase in the 7-methylguanine content of the various polymers follows the pattern expected if 7-methylguanine does not code like guanine, rather than the pattern expected if it still coded like guanine. It appears, therefore, that 7-methylguanine renders coding triplets inactive. The suggestion made by Brooks and Lawley of mispairing of bases does not seem to operate during the translation stage of protein synthesis in vitro.

As can be seen from the overall phenylalanine incorporation (Table 2, §), 7-methylguanine does interfere with peptide synthesis, since both methylated polymers were less effective as templates than the original polyUG was. By titrating the polymers, ratios of 100:60:9 were found for the amounts of $C^{14}$-phenylalanine incorporated, in the presence of 19 unlabeled amino acids, by equivalent amounts of polyUG, methylated polyUG-1, and methylated polyUG-2, respectively. Since the melting curves for all three polymers were identical, a difference in secondary structure known to affect template activity in vitro is thereby eliminated as an explanation for the decreased activities of the methylated polymers.

The possibility that there were specific inhibitors in the preparations of methylated polymers was eliminated by experiments, such as that shown in Table 3. The addition of the methylated polymers in concentrations that would nearly saturate the ribosomes before, after, or simultaneously with the original polyUG did not affect its template activity. Decreased template activity seems, then, to be related to the methylation of the bases in the polymer. Since 7-methylguanine–containing triplets do not seem to code, the possibility remains that, if polypeptide synthesis is interrupted, smaller polypeptides will be pro-

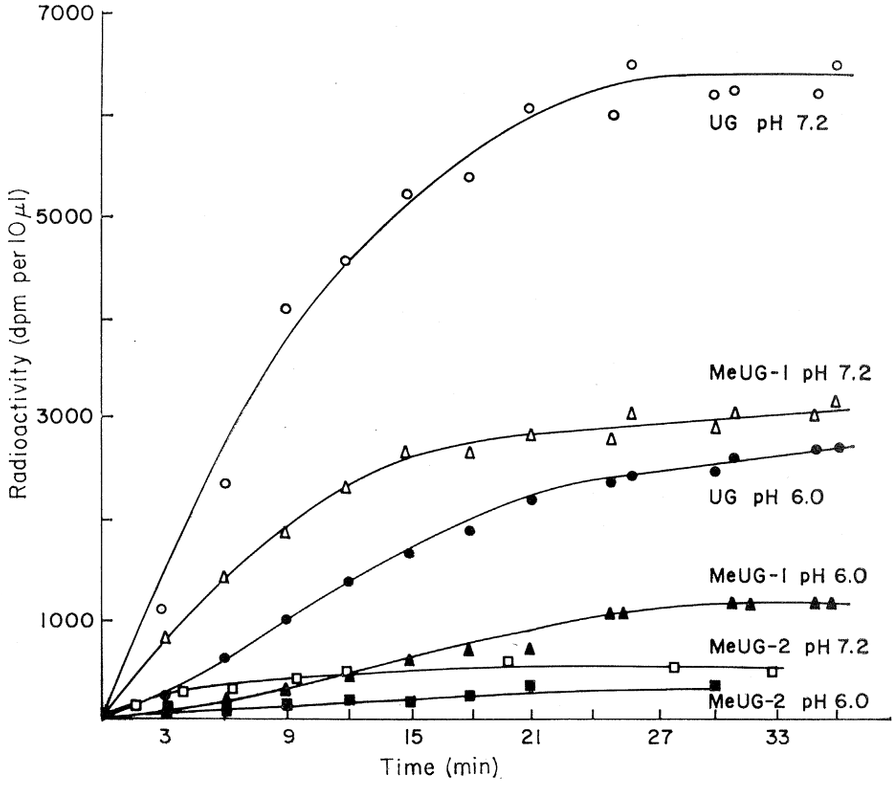


Fig. 1. Effect of *p*H on the rate of incorporation. Incorporation conditions as described for Table 2. Each point represents the total number of disintegrations per minute in a 10-$\mu$l portion of the 0.2-ml starting reaction mixture, analyzed by the filter paper method of Mans and Novelli (*12*). At *p*H 7.2: ○-○, polyUG; △-△, methylated polyUG-1; □-□, methylated polyUG-2. At *p*H 6.0: ●-●, polyUG; ▲-▲, methylated polyUG-1; ■-■, methylated polyUG-2.

duced. Considering that about 13 and 34 percent of the triplets of methylated polyUG-1 and methylated polyUG-2, respectively, contain 7-methylguanine, many of the peptides might well be too short to be precipitated by the trichloroacetic acid and hence would not be counted.

The methylation of guanosine could affect the template activity by changing its charge since, at *p*H 7.2, where all the above experiments were run, only 50 percent of the 7-methylguanosine is in the protonated form. To evaluate the effect of charge, incorporation experiments were conducted at *p*H 6.0 where the nonprotonated form of 7-methylguanosine is reduced to 6 percent (as compared with 1 percent for guanosine at *p*H 7.2).

Figure 1 shows comparisons of the kinetics of incorporation for each polymer at *p*H 7.2 (open symbols) and *p*H 6.0 (filled symbols). The overall efficiency of incorporation is reduced to about 40 percent by the lowering of *p*H, as can be seen from the polyUG control (circles). The activity of both of the methylated polymers is also reduced by about the same amount (40 to 50 percent). The decrease in the ionized form of 7-methylguanine did not increase the template activity of the methylated polymers relative to the control polyUG, suggesting that it is not the ionization but probably the presence of a sterically important 7-methyl group which hinders the activity, and that base-pairing alone is not sufficient for codon recognition during peptide synthesis in vitro.

The methyl group might hinder the base-pairing process, binding of the template to ribosomes, or perhaps other enzymatic reactions related to codon recognition and peptide formation. The results in Table 3 suggest that the methylated polymers may not bind to ribosomes as well as polyUG does. Under conditions of limiting ribosomes, the addition of methylated polyUG-1 or methylated polyUG-2 before the addition of polyUG does not inhibit the overall template activity of polyUG. In other experiments the system could be incubated with methylated polyUG-2 up to 10 minutes without decreasing total disintegrations measured after the later addition of polyUG. Either the polyUG can displace a previously weakly bound methylated polymer or the methylated polymers bind very slowly. The decreased activity of the methylated polymers might then be related to this weak binding property.

In the foregoing experiments we have shown the steric effect of the added methyl group of 7-methylguanine on the mechanism of translation in vitro. This result suggests further that the mutagenic effects of chemical methylation observed in vivo might be the result of altered base-pairing during polynucleotide transcription.

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## Chains of Particles in Shear Flow

Abstract. *Interesting physical models of threads, rouleaux of red blood cells, and other flexible structures (including macromolecules) have been prepared with chains of spheres and discs. When suspended in a viscous liquid undergoing shear flow, the aggregates rotate as nearly rigid bodies, even though they have no tensile strength or stiffness until shear rates high enough to cause bending and then breaking are reached. By adding a second liquid phase which causes a meniscus to bridge adjacent particles, sufficient tensile strength can be provided to cause bending without breakage.*

Spheres suspended in a dielectric liquid attract one another in an electric field and eventually arrange themselves as linear chains aligned in the direction of the field (*1*). Since the spheres do not necessarily touch one another, the aggregates thus formed may be regarded as threadlike particles with neither tensile strength nor stiffness. Nevertheless, they rotate and remain straight in Couette (shear) flow after the electric field is removed (Fig. 1).

From a consideration of the lubrication equations for spheres in close proximity, we have demonstrated theoretically that when the spheres are in contact, the aggregate should rotate as a rigid body, without relative rotation of the spheres and without bending, in a spherical elliptical orbit similar to that predicted by Jeffery for a rigid prolate spheroid (*2*). However, when there are small gaps between the spheres, the chain length should vary periodically between a minimum when the chain is oriented at right angles to the direction of the shear flow and a maximum when parallel to it.

We have confirmed these predictions with tiny (0.05 cm in diameter) metal-coated polystyrene spheres suspended in oils of the same density in which chains of as many as 20 spheres were formed by applying a 60-cy/sec alternating field of 2 kv/cm. Quantitative measurements were made with chains containing from 2 and 10 spheres at velocity gradients ($G$) up to 2 $sec^{-1}$ established in a Couette apparatus consisting of two counter-rotating cylinders (*3*). At high $G$'s the chains buckle in the quadrant in which the spheres are being pushed together and generally break in two at the same position in the chain in the suc-

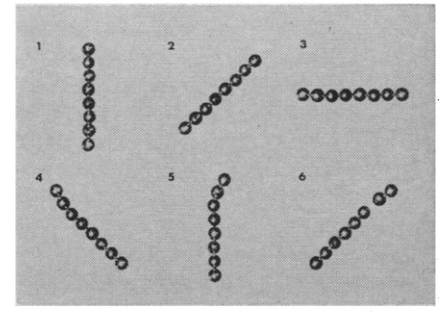


Fig. 1. A chain of eight spheres formed by applying an electric field in the vertical direction (1). With the electric field off, and the shear field on, the chain rotates clockwise (2, 3), buckles under axial compression (4, 5), and finally breaks under tension (6).