Induction of streptolysin formation by biosynthetic polyribonucleotides

The polyribonucleotides synthesized by polynucleotide phosphorylase[1-3] from nucleoside-5′-diphosphates have been shown to possess the structure of RNA*. Since poly AGUC, the synthetic polynucleotide containing adenylic, guanylic, uridylic, and cytidylic acid residues, is indistinguishable from natural RNA[4-6], it was desirable to ascertain whether this compound and other biosynthetic polynucleotides are endowed with a biological activity characteristic of natural ribonucleic acids.

An interesting biological effect of RNA was disclosed by OKAMOTO's finding[7] that yeast RNA stimulated the synthesis of streptolysin (streptolysin S) by growing hemolytic streptococci. HOSOYA *et al.*[8] and BERNHEIMER[9] found later that RNA also promoted streptolysin synthesis by resting cell suspensions. Further studies showed that the activity of RNA was markedly increased after exhaustive digestion with pancreatic ribonuclease[8, 13], the non-dialyzable residue being particularly active. The activity was lost after prolonged acid or alkaline hydrolysis.

The effect of synthetic polynucleotides on streptolysin synthesis was studied using both the resting[12] and growing[11] cell techniques. The polynucleotides were prepared with polynucleotide phosphorylase of *Azotobacter vinelandii* as previously described[3]. Poly AGUC(1:1:1:1) was prepared from an equimolar mixture of ADP, GDP, UDP, and CDP and gave the following base analysis (adenine taken as 10):(adenine, 10, guanine, 11.3; uracil, 6.4; cytosine, 5.9). Poly AGUC(1:0.5:1:1) was prepared from equimolar concentrations of ADP, UDP, and CDP, and half the molar concentration of GDP; its base analysis was: adenine, 10; guanine, 6.2; uracil, 6.8; cytosine 6.8 Poly AU (containing both adenylic and uridylic acid residues) was prepared from an, equimolar mixture of ADP and UDP and poly AC (containing both adenylic and cytidylic acid residues) from an equimolar mixture of ADP and CDP. Poly GUC (containing guanylic, uridylic, and cytidylic acid residues) was prepared from equimolar concentrations of UDP and CDP and half the molar concentration of GDP; its base analysis was (guanine taken as 10): guanine, 10.0; uracil, 10.5; cytosine, 11.4. The base analyses were carried out on perchloric acid hydrolysates[14] by the method of WYATT[15].

Comparison of Table I and Fig. 1 shows that the results obtained by the growing and resting cell methods were in excellent agreement. Poly AGUC (1:1:1:1) was more active than yeast RNA and was quite active without prior ribonuclease digestion (Table I), while poly AGUC (1:0.5:1:1) had but negligible activity and poly AU and poly AC (Table I) were inactive, at the levels tested, even after ribonuclease digestion. Poly GUC was inactive, but after ribonuclease digestion it had demonstrable activity in both resting and growing cell systems. The sodium salt of GDP in concentrations up to 800 μg/ml and the sodium salt of GMP in concentrations up to 1280 μg/ml failed to induce formation of streptolysin in both systems. Poly A (polynucleotide containing only adenylic acid residues) was inactive in unhydrolyzed form, after alkaline hydrolysis and after hydrolysis with snake venom phosphodiesterase.

The base composition of poly AGUC (1:1:1:1) is similar to that of *Azotobacter* RNA[6] and, as now seen, this synthetic RNA exhibits a biological activity characteristic of natural RNA. The possibility of modifying the base composition of biosynthetic polynucleotides and of preparing polymers containing only one base or any given combination of bases disclosed an important aspect of the effect of RNA and its ribonuclease "core" on streptolysin synthesis, namely that the active structure must be an oligonucleotide relatively rich in guanylic acid residues. This is apparent from (a) the high activity of the ribonuclease "core" of RNA, (b) the inactivity of polynucleotides containing no guanylic acid, and (c) the negligible activity of polynucleotides

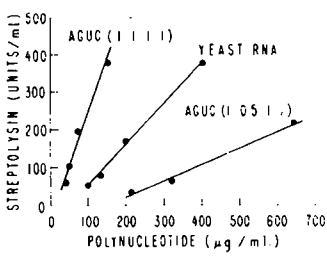

Fig. 1. Effect of ribonuclease digestion products of polyribonucleotides on streptolysin synthesis by growing cells of *Streptococcus pyogenes*. To 1.5 ml neopeptone–infusion broth, containing 0.01 % sodium thioglycolate, were added 0.2 ml test solution and 0.3 ml of a 16 h broth culture of *S. pyogenes*, strain C20 3S. After incubating 8 h at 37°, the cultures were refrigerated until the next day when they were titrated for hemolytic activity (streptolysin S). The ribonuclease digestions and streptolysin assays were carried out as previously described[9, 11]. The yeast RNA was a commercial product (Schwarz Laboratories, Inc.).

* Abbreviations: ribonucleic acid, RNA; 5′-diphosphates (pyrophosphates) of adenosine, guanosine, uridine, and cytidine, ADP, GDP, CDP, and UDP; guanosine-5′-monophosphate, GMP.

TABLE I

EFFECT OF POLYRIBONUCLEOTIDES AND THEIR RIBONUCLEASE DIGESTION PRODUCTS ON STREPTOLYSIN SYNTHESIS BY RESTING CELLS OF *Streptococcus pyogenes*

Polynucleotide	*Concentration in assay (μg/ml)*	*Time of ribonuclease digestion (h)*	*Streptolysin (units/ml)*
Yeast RNA	400	0*	10
	40	2	140
	40	4	171
AGUC (1:1:1:1)	400	0*	226
	40	2	447
	40	4	491
AGUC (1:0.5:1:1)	400	0*	0
	40	2	33
	40	4	39
AU	400	0*	0
	100	4	0
AC	400	0*	0
	100	4	0

The polynucleotides were dissolved in 0.05 *M* tris (hydroxymethyl)aminomethane buffer, pH 7.5. 1.0 mg aliquots were digested at 37° with 0.05 mg crystalline pancreatic ribonuclease (Worthington), in a final volume of 1.0 ml for 2 and 4 h. Controls were similarly incubated for 4 h but without ribonuclease. After incubation, the samples were lyophilized, the residues dissolved in 0.033 *M* phosphate buffer, pH 7.0, containing 0.077 *M* NaCl, and suitable aliquots used for the streptolysin S assay. This was carried out as previously described[12] using *S. pyogenes*, strain S8. The yeast RNA (A:G:U:C = 10:11.2:9.8:7.9) was prepared from brewer's yeast by the method of CLARK AND SCHRYVER[16] and exhaustively dialyzed against distilled water before use.

* Control, incubated 4 h at 37° without ribonuclease.

with a low guanylic acid content. With regard to the last point, it is of interest that ribonuclease-digested yeast, wheat germ, tobacco leaf and streptococcal RNA were about equally active in stimulating streptolysin formation, whereas tobacco mosaic virus (TMV) RNA was inactive under the same conditions[17]. In contrast to other ribonucleic acids, TMV RNA contains less guanine than adenine and the same is true of its ribonuclease "core"[18]. The importance of a high guanylic acid content is also borne out by experiments* now under way with fractions derived from ribonuclease digested yeast RNA.

*Laboratory of Biochemistry, Department of Chemistry**, Faculty of Science, Nagoya University, Nagoya (Japan)* — KENTARO TANAKA, FUJIO EGAMI

Medical Section, Institute of Low Temperature Science, Hokkaido University, Sapporo (Japan) — TAKAYOSHI HAYASHI

*Department of Microbiology***, New York University College of Medicine, New York, N.Y. (U.S.A.)* — JEANETTE E. WINTER, ALAN W. BERNHEIMER

Department of Biochemistry§, New York University College of Medicine, New York, N.Y. (U.S.A.) — SANAE MII, PRISCILLA J. ORTIZ, SEVERO OCHOA

* K. TANAKA and T. HAYASHI, unpublished.

** Aided by a grant from the Scientific Research Fund of the Ministry of Education of Japan.

*** Aided by grants from the Life Insurance Medical Research Fund and the Masonic Foundation for Medical Research and Human Welfare.

§ Aided by grants from the National Institute of Arthritis and Metabolic Diseases (Grant A-529) and the National Cancer Institute (Grant C-2784) of the National Institutes of Health, United States Public Health Service, the American Cancer Society (recommended by the Committee on Growth, National Research Council), the Rockefeller Foundation, and by a contract (N6onr 279, T.O.6) between the Office of Naval Research and New York University College of Medicine.

[1] M. GRUNBERG-MANAGO AND S. OCHOA, *J. Am. Chem. Soc.*, 77 (1955) 3165.
[2] M. GRUNBERG-MANAGO, P. J. ORTIZ AND S. OCHOA, *Science*, 122 (1955) 907.
[3] M. GRUNBERG-MANAGO, P. J. ORTIZ AND S. OCHOA, *Biochim. Biophys. Acta*, 20 (1956) 269.
[4] S. OCHOA, *Federation Proc.*, 15 (1956) 832.
[5] S. OCHOA AND L. A. HEPPEL, in W. D. MCELROY AND B. GLASS, *The Chemical Basis of Heredity*, The Johns Hopkins Press, Baltimore, 1957, p. 615.
[6] S. OCHOA, S. MII, M. C. SCHNEIDER, R. M. S. SMELLIE, R. C. WARNER AND P. J. ORTIZ, *Federation Proc.*, 16 (1957) 228.
[7] H. OKAMOTO, *Japan. J. Med. Sci., IV, Pharmacol.*, 12 (1939) 167.
[8] S. HOSOYA, T. HAYASHI, Y. HOMMA, F. EGAMI, M. SHIMOMURA AND Y. YAGI, *Japan. J. Exptl. Med.*, 20 (1949) 27.
[9] A. W. BERNHEIMER, *J. Exptl. Med.*, 90 (1949) 373.
[10] R. ITO, *Japan. J. Exptl. Med.*, 13 (1940) 85.
[11] A. W. BERNHEIMER AND M. RODBART, *J. Exptl. Med.*, 88 (1948) 149.
[12] K. TANAKA, S. MAEKAWA, T. HAYASHI AND Y. KUROIWA, *J. Biochem. (Tokyo)*, 43 (1956) 827.
[13] K. TANAKA, S. MAEKAWA AND T. HAYASHI, *J. Biochem. (Tokyo)*, 43 (1956) 833.
[14] A. MARSHAK AND H. J. VOGEL, *J. Biol. Chem.*, 189 (1951) 597.
[15] G. R. WYATT, *Biochem. J.*, 48 (1951) 584.
[16] G. CLARKE AND S. B. SCHRYVER, *Biochem. J.*, 11 (1917) 319.
[17] A. W. BERNHEIMER, in W. D. MCELROY AND B. GLASS, *Phosphorus Metabolism*, Vol. 2, The Johns Hopkins Press, Baltimore, 1952, p. 358.
[18] K. K. REDDI AND C. A. KNIGHT, *J. Biol. Chem.*, 221 (1956) 629.

Received July 5th, 1957

Formation of non-phage-antigenic protein in *E. coli* infected with T2 phage*

It has been found that the integrity of the infecting phage deoxypentosenucleic acid (DNA) is required for the successful initiation of the syntheses of both phage-specific protein (phage antigen)[1] and DNA. Recently, a possibility that the parental phage DNA controls the formations of phage antigen and DNA *indirectly* through a substance other than DNA has been suggested[1,2]. If the control by the parental DNA is really indirect, there must be a formation of a new substance or substances immediately after the infection but before the appearances of phage DNA and antigenic protein. The present note describes some of our experimental results on a protein formed just after infection which are in favour of the hypothesis of indirect control by the parental DNA.

The formation of protein in *Escherichia coli*, strain B(H), infected with bacteriophage T2r⁺ was studied in the lysates at different times of phage development. T2-antigenic protein is defined here as that protein which can be precipitated specifically with anti-T2 rabbit serum[1]. The result of a typical experiment is presented in Fig. 1. It is clear that there is a very active protein synthesis immediately after infection, confirming the results of previous workers[3], and that the protein formed before 10 min after infection does not contain T2-antigenic protein. The rate of the formation of this non-phage-antigenic protein is high for the first 10 min and then gradually tapers off.

It was found that the non-phage-antigenic protein is not a precursor protein of phage antigen, because most of the ^{35}S (more than 90 %) in non-phage-antigenic protein fraction, which had been labeled by feeding ^{35}S only during the first 8 min after infection, could not move into the phage-antigenic protein fraction even after 60 min incubation in ^{35}S-free medium.

The next question is whether it is a normal bacterial protein or not. The following evidences suggest that it is not bacterial. (1) The fact that the infection with T2 phage can provoke a synthesis of the non-antigenic protein in ultraviolet light (UV) irradiated *E. coli*, in which a synthesis of bacterial protein is largely suppressed (see Fig. 2). (2) A similar observation that the adenine-requiring mutant of *E. coli* can form the non-antigenic protein immediately after infection even in a medium without adenine, while it can hardly synthesize protein in this medium if it is not infected (I. WATANABE AND Y. KIHO, unpublished). From these results, it can be considered that the non-antigenic protein is different from the bacterial protein which can be produced in uninfected normal coli. (3) The fact that protein synthesis is prerequisite to the formation of new phage DNA after infection[4]. This fact indicates the presence of a new kind of protein in the

* Supported in part by a grant from the Scientific Research Fund of the Ministry of Education