Effects of Deoxyribonucleic Acid and Histone on the Number and Length of Chains of Poly(adenosine diphosphate-ribose)†

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ABSTRACT: The roles of DNA and histone in synthesis of poly(adenosine diphosphate-ribose) [poly(ADP-Rib)] by a partially purified enzyme from rat liver nuclei were studied. Both DNA and histone were found to be required for poly(ADP-Rib) synthesis. With high concentrations of histone, polyanions such as poly(U), poly(A), poly(C), poly(vinyl sulfate), heparin, or methyl dextran sulfate were also effective for the synthesis of poly(ADP-Rib), but these polyanions were all less effective than DNA. Additions of DNA and histone markedly increased both the length of poly(ADP-Rib) and the number of chains formed. When added with histone, acid-soluble oligodeoxyribonucleotide, poly(dG)·poly(dC), and poly[d(A-T)], respectively, increased the number and length of poly(ADP-Rib) chains formed. Polyanions, such as poly(U), poly(A), poly(C), and poly(vinyl sulfate), had similar effects to DNA. Chromatograms of the reaction products on hydroxylapatite and on Sephadex G-50 showed that the presence of both DNA and histone resulted in synthesis of poly(ADP-Rib) of increased chain length. Poly(ADP-Rib) of longer chain length was found after chase with NAD, DNA, and histone.

Poly(adenosine diphosphate-ribose) [poly(ADP-Rib)][1] is a polymer consisting of repeating units of adenosine diphosphate-ribose (ADP-Rib) linked by glycosidic bonds of ribosyl-(1′→2′)-ribose of adenosine (Chambon *et al.*, 1966; Reeder *et al.*, 1967; Hasegawa *et al.*, 1967). Isolated nuclei or chromatin of mammalian cells catalyze the synthesis of poly(ADP-Rib) from NAD with the release of nicotinamide (Chambon *et al.*, 1966; Nishizuka *et al.*, 1967; Fujimura *et al.*, 1967; Gill, 1972). Poly(ADP-Rib) has AMP at one terminus and is suspected to be bound covalently to histone at the other terminus (Nishizuka *et al.*, 1968, 1969; Otake *et al.*, 1969). The chain length of the polymer is from 1 to 20 units of ADP-Rib.

Yamada *et al.* (1971) and Yoshihara (1972) have shown that partially purified enzyme preparations from rat liver nuclei catalyze the formation of poly(ADP-Rib) from NAD. The enzyme requires DNA and histone for activity. The product of the reaction is a polymer with several units of ADP-Rib. The enzyme responsible for this reaction is referred to here as poly(ADP-Rib) polymerase.

This work was on the initiation of synthesis, and synthesis of poly(ADP-Rib) and on how DNA, histone, or other macromolecules are involved in these processes. This paper presents evidence that both DNA and histone have a close relationship to the number and length of poly(ADP-Rib) chains.

Materials and Methods

Radiochemicals. [*Adenine*-U-^{14}C]NAD (167 Ci/mol) was obtained from the Radiochemical Centre, Amersham. [*Adenosine*-G-^{3}H]ATP (9.53 Ci/mmol) was from New England Nuclear, Boston, Mass. [*Adenosine*-G-^{3}H]NAD was prepared from [*adenosine*-G-^{3}H]ATP and NMN using purified NAD-pyrophosphorylase (Kornberg, 1950) as described previously (Yamada *et al.*, 1971).

Chemicals. NAD, other nucleotides, lysine-rich histone of calf thymus (type III), and calf thymus DNA were obtained from Sigma Chemical Co., St. Louis, Mo. Heparin, sodium salt, was from Connaught Medical Research Laboratories, University of Toronto, Toronto; methyl dextran sulfate from Kowa Pure Chemical Co. Ltd., Tokyo; potassium poly(vinyl sulfate) from Wako Pure Chemical Co. Ltd., Tokyo; poly[d(A-T)], poly(dG)·poly(dC), poly(C), and poly(U) from Miles Laboratories, Inc., Kankakee, Ill.; poly(A) from Cal Biochem, Inc., Los Angeles, Calif.; Sephadex G-50, medium, from Pharmacia Fine Chemicals, Uppsala; hydroxylapatite, Bio-Gel HTP from Bio-Rad Laboratories, Richmond, Calif. 5′-Phosphoryl-terminated acid-soluble oligodeoxyribonucleotide was prepared by incubating calf thymus DNA with DNase I (Richardson and Kornberg, 1964). Purified tRNA mixture of *Escherichia coli* was a gift from Dr. S. Nishimura of this Institute.

Enzymes. Phosphodiesterase of snake venom and crystalline DNase I were from Worthington Biochemical Corp., Freehold, N. J. The phosphodiesterase was purified further by passage through a column of Dowex 50-X8 (sodium form) to remove 5′-nucleotidase (Keller, 1964). Purified Pronase of *Streptomyces griseus* was a gift from Dr. Nomoto of Seikagaku Kogyo Co., Tokyo.

Enzyme Preparation. Poly(ADP-Rib) polymerase was partially purified from rat liver nuclei, essentially as described previously (Yamada *et al.*, 1971). The enzyme preparation at step 4 had a specific activity of 8.7–15.0 units/mg of protein, where 1 unit is equivalent to 1 nmol of ADP-Rib incorporated into the acid-insoluble fraction in 1 min under defined conditions. The enzyme preparation was stored in small portions for several months at −80° without significant loss of the activity.

Assay for Poly(ADP-Rib) Synthesis. The reaction mixture (0.125 ml) contained 12.5 μmol of Tris-HCl (pH 8.0), 10 μg of calf thymus DNA, 1.0 μmol of $MgCl_2$, 0.05 μmol of dithiothreitol, 2.0 μg of calf thymus lysine-rich histone, 7.5 nmol of [*adenosine*-^{3}H]NAD (2.16×10^5 cpm/nmol), and poly(ADP-Rib) polymerase as indicated. After 3 min at 37° a 100-μl

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[1] Abbreviations used are: ADP-Rib, adenosine diphosphate-ribose; poly(ADP-Rib), polymer of ADP-Rib; Ado(P)-Rib-P, 2′-(5′′-phosphoribosyl)-5′-AMP. All other abbreviations are as listed in *Biochemistry* 5, 1445 (1966), and 9, 4022 (1970).

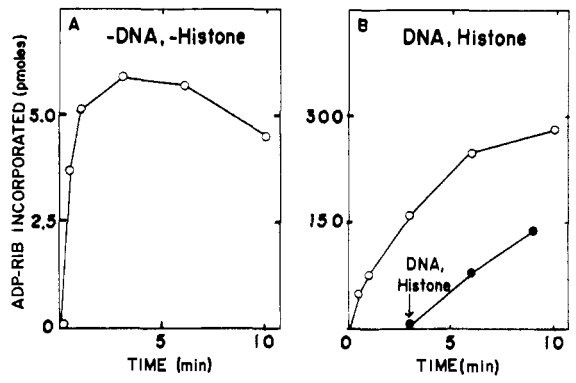

FIGURE 1: Time courses of poly(ADP-Rib) formation with and without histone and DNA. (A) The volume of the reaction mixture was three times that described in Materials and Methods, and DNA and lysine-rich histone were omitted. Enzyme protein (25.5 μg) was used. Aliquots (50 μl) were removed at intervals to follow poly(ADP-Rib) synthesis. (B) DNA (24.0 μg) and lysine-rich histone (6.0 μg) were added to the reaction mixture described in part A. All other conditions were as described in part A: (○) additions at time zero; (●) additions after 3 min.

aliquot of the reaction mixture was applied to a Whatman glass fiber filter, GF/C. The amount of acid-insoluble material on the filter was determined by the method of Bollum (1966). The acid-insoluble radioactivity on the filter was counted in a Beckman liquid scintillation spectrometer.

Isolation of Poly(ADP-Rib). Poly(ADP-Rib) was prepared as described previously (Matsubara *et al.*, 1970) with a slight modification. At the end of the reaction 2 volumes of cold ethanol and 0.12 volume of 5 M acetate buffer (pH 5.0) were added to the reaction mixture. The precipitate was washed successively with 66% ethanol–0.2 M acetate buffer (pH 5.0), ethanol, and ether. Then it was dissolved in 0.02 M Tris-HCl buffer (pH 7.4) and digested for 1 hr at 37° with Pronase (40 μg/ml). The reaction mixture was extracted with aqueous phenol (90%, w/w) to remove the protein, and then with ether.

Estimation of the Number and Length of Poly(ADP-Rib) Chains. Complete digestion of poly(ADP-Rib) with venom phosphodiesterase resulted in formation of 5′-AMP and Ado-(P)-Rib-P, so the number of chains of poly(ADP-Rib) was estimated from the amount of 5′-AMP liberated in this way. The average chain length of poly(ADP-Rib) was estimated from the value of 1 plus the ratio of Ado(P)-Rib-P to 5′-AMP (Yamada *et al.*, 1971). The reaction mixture for the digestion contained 2 μmol of Tris-HCl (pH 7.4), 10–400 pmol of poly-(ADP-Rib) as ADP-Rib units, 1 μmol of $MgCl_2$, 0.01 μmol of 5′-AMP, and 4.5 units of venom phosphodiesterase in a total volume of 100 μl. After incubating the mixture at 37° for 30 min, a further 4.5 units of excess phosphodiesterase was added and the mixture was incubated further for 60 min. Carrier 5′-AMP and ADP-Rib were added and the mixture was applied to Toyo filter paper no. 51A and developed for 21 hr with a system of isobutyric acid–ammonium hydroxide–water (66:1:33, v/v). Then the paper was cut into 1.5 cm length and radioactivity was measured in a liquid scintillation spectrometer.

Hydroxylapatite Column Chromatography of Poly(ADP-Rib). Short-chain poly(ADP-Rib) was separated from longer chain poly(ADP-Rib) by hydroxylapatite column chromatography as described previously (Sugimura *et al.*, 1971).

Results

Time Course of the Reaction. Figure 1 shows the time course of the incorporation of ADP-Rib from NAD into the acid-insoluble fraction in the absence and presence of added DNA and lysine-rich histone. The amount of incorporation of ADP-Rib in the absence of DNA and lysine-rich histone increased rapidly, reaching a plateau in 3 min and later decreasing gradually (Figure 1A). The amount of incorporation of ADP-Rib on addition of both DNA and lysine-rich histone was proportional to the incubation time for 6 min and then increased more slowly for 30 min (Figure 1B). The amount of incorporation of ADP-Rib in 3 min without added DNA and lysine-rich histone was less than 3% of that on their addition. The rate of incorporation of ADP-Rib was similar when DNA and histone were added after 3 min to that when they were present initially (Figure 1B).

Effect of Lysine-Rich Histone. Figure 2 shows that increasing concentrations of lysine-rich histone have different effects on the incorporation of ADP-Rib in the presence of DNA or polyanions such as poly(U), poly(A), poly(C), poly(vinyl sulfate), methyl dextran sulfate, or heparin. On increasing the amount of lysine-rich histone in the presence of 10 μg of DNA, the amount of incorporation of ADP-Rib increased and reached a maximum with 2.0 μg of lysine-rich histone in the reaction mixture (Figure 2A). Increasing the concentrations of lysine-rich histone in the presence of polyanions, such as poly(U), poly(A), poly(C), poly(vinyl sulfate), methyl dextran sulfate, or heparin, resulted in gradual increase in the amount of incorporation of ADP-Rib. With 50 μg of lysine-rich histone (Figure 2A,B), incorporation with all the polyanions was similar to that with DNA. The amount of the incorporation of ADP-Rib with 2.0 μg of lysine-rich histone in the presence of the various polyanions was less than 6% of that in the presence of added DNA (Figure 2A,B). Increasing concentrations of lysine-rich histone above 10 μg in the absence of DNA and polyanion caused only a slight increase in the incorporation of ADP-Rib (Figure 2A). This increase caused by adding lysine-rich histone alone seems to be due to the DNA present in lysine-rich histone preparation, since lysine-rich histone contained 0.5–1.1% DNA. Lysine-rich histone could not be replaced by bovine serum albumin (Figure 2A).

Thus these results show that DNA in the presence of lysine-rich histone is more effective for the incorporation of ADP-Rib than polyanions such as poly(A), poly(U), poly(C), methyl dextran sulfate, heparin, or poly(vinyl sulfate).

Effects of DNA and Polyanion. As shown in Figure 3, upon increasing the amounts of DNA, poly(A), or methyl dextran sulfate in the presence of 2 μg (Figure 3A) or 20 μg (Figure 3B) of lysine-rich histone, the incorporation of ADP-Rib by DNA increased and was clearly distinguished from that caused by poly(A) or methyl dextran sulfate. With 2 μg of lysine-rich histone in the reaction mixture, the enzyme activity was proportional to the amount of DNA with up to 2 μg of DNA and reached a maximum and plateau with 5 μg of DNA, whereas addition of poly(A) or methyl dextran sulfate caused very little increase. The enzyme activity with the polyanions was less than 10% of that with DNA (Figure 3A). With 20 μg of lysine-rich histone, the enzyme activity was enhanced by adding 5 μg of DNA, poly(A), or methyl dextran sulfate. The enzyme activity was markedly reduced by addition of more than 10 μg of methyl dextran sulfate, but not by addition of above this concentration of DNA or poly(A) (Figure 3B).

Effects of Various DNAs and Lysine-Rich Histone on the Number and Length of Poly(ADP-Rib) Chains. The results described above and in a previous paper (Yamada *et al.*, 1971) show that the reaction for poly(ADP-Rib) synthesis depends on added DNA and lysine-rich histone. Next experiments

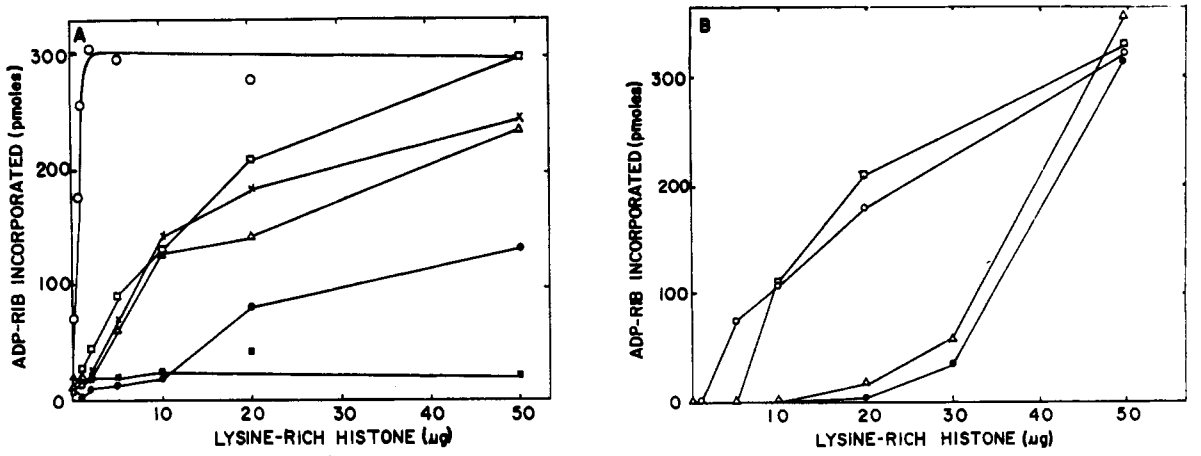

FIGURE 2: Effects of lysine-rich histone on the reactions with various polyanions. (A) The reaction conditions were as described under Materials and Methods. Reaction mixtures (0.125 ml) contained the amount of lysine-rich histone indicated, 10 μg of DNA or polyanions as indicated, and 7.2 μg of the enzyme protein: (●) without DNA and polyanions; (○) DNA; (□) poly(U); (■) poly(U) and bovine serum albumin in place of lysine-rich histone; (△) poly(A); (×) poly(C). The reaction period was 3 min. (B) All other conditions were as described in part A: (○) 1.0 μg of methyl dextran sulfate; (●) 10 μg of methyl dextran sulfate; (△) 10 μg of heparin; (□) 3 μg of poly(vinyl sulfate).

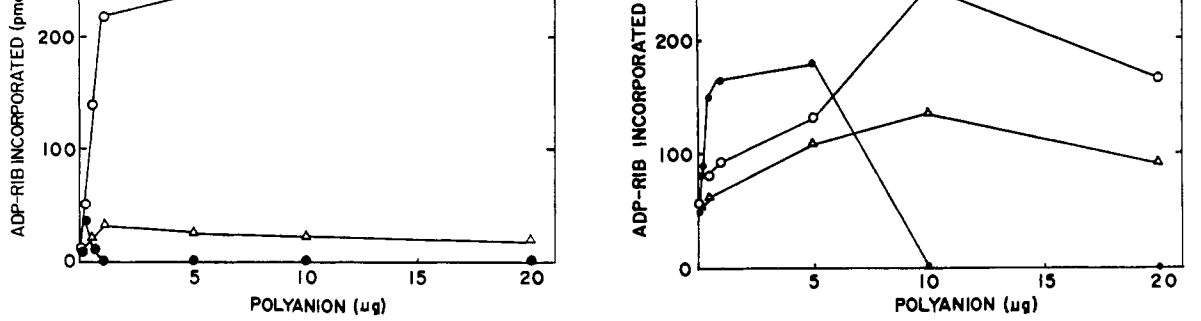

FIGURE 3: Effects of DNA and polyanions on the reaction with 2 μg and 20 μg of lysine-rich histone. (A) Reaction conditions were as described in Materials and Methods. The reaction mixture (0.125 ml) contained various amounts of DNA, poly(A), or methyl dextran sulfate as indicated, 2.0 μg of lysine-rich histone, and 7.2 μg of enzyme protein: (○) DNA; (●) methyl dextran sulfate; (△) poly(A). The reaction time was 3 min. (B) Lysine-rich histone (20 μg) was added to the reaction mixture. All other conditions as described in part A: (○) DNA; (●) methyl dextran sulfate; (△) poly(A).

were made to see whether the increase in the incorporation of ADP-Rib by these macromolecules is due to additional poly(ADP-Rib) formation or lengthening of existing chains or both. Additions of DNA, DNA and lysine-rich histone, poly[d(A-T)] and lysine-rich histone, poly(dG)·poly(dC), and lysine-rich histone, or acid-soluble oligodeoxyribonucleotide and lysine-rich histone to the reaction mixture, increased the chain number of the poly(ADP-Rib) formed 7, 16, 22, 15, or 6 times, respectively (Table I), and also increased the average chain length of the polymer from 2.2 in the control to 6.6, 9.4, 7.3, 6.5, or 5.1, respectively (Table I). Therefore, the presence of DNA and lysine-rich histone increased both the number and length of poly(ADP-Rib) chains formed.

Effects of Polyanions and Lysine-Rich Histone on the Number and Length of Poly(ADP-Rib) Chains. Table II shows that both the number and length of polymer chains formed increased on additions of polyanions and lysine-rich histone. Additions of poly(U), poly(C), poly(A), or poly(vinyl sulfate) to medium containing lysine-rich histone increased the chain number of poly(ADP-Rib) formed 10, 9, 8, or 6 times, respectively, over the control (Table II) and also increased the chain length of the polymer from 2 in the control, to 11.9, 8.3, 6.5, or 8.5 respectively (Table II).

Chain Elongation of Poly(ADP-Rib) on Additions of DNA and Lysine-Rich Histone. As shown in previous section, addition of DNA and lysine-rich histone increased the average chain length of the polymer about 4-fold. As shown below, the reaction products formed without DNA and lysine-rich histone grew to a long-chain polymer.

Poly(ADP-Rib) species of various chain lengths can be separated on a hydroxylapatite column (Sugimura *et al.*, 1971). Figure 4 shows chromatograms of the reaction products on a hydroxylapatite column. With the reaction products formed in the presence of DNA and lysine-rich histone, the major peak containing more than 70% of the total radioactivity was eluted in tubes 90–140 with 0.35 M sodium phosphate buffer (pH 6.8) (Figure 4A). However, without DNA and lysine-rich histone no significant radioactivity was found in the fractions eluted in tubes 90–140 (Figure 4B), and most of the radioactivity was found in the breakthrough fractions and the fractions in tubes 20–90. Next, the reaction was carried out for 2 min without DNA and lysine-rich histone and then 100-fold excess of unlabeled NAD and DNA and lysine-rich histone were added and the reaction was continued for a further 3 min. On chromatography of the reaction products most of the radioactivity was eluted in tubes 90–130 and the peak fraction was eluted with 0.35 M sodium phosphate (Figure 4C).

Reaction products of large molecular size can also be sep-

TABLE I: Effects of the Various DNAs and Lysine-Rich Histone on the Number and Length of Poly(ADP-Rib) Chains.[a]

Addition	Poly-(ADP-Rib) Isolated (pmol)	Digestion Products of Poly(ADP-Rib)		Av Chain Length [1 + (*a*/*b*)]
		Ado(P)-Rib-P (*a*) (pmol)	5′-AMP (*b*) (pmol)	
None	9.3	3.4	2.8	2.2
DNA	136.6	109.6	19.5	6.6
DNA, histone	462.3	382.4	45.7	9.4
Poly[d(A-T)], histone	490.9	393.8	62.3	7.3
Poly(dG)·poly(dC), histone	298.6	235.8	42.9	6.5
Acid-soluble oligodeoxyribonucleotide, histone	93.7	68.0	16.5	5.1

[a] The conditions for poly(ADP-Rib) synthesis were as described under Materials and Methods. The reaction mixture (0.125 ml) contained 7.2 nmol of [*adenine*-U-^{14}C]NAD (3.15 × 10^5 cpm/nmol) and 17.0 μg of the enzyme protein. Where indicated 8.3 μg of DNA, 7.4 μg of poly [d(A-T)], 9.0 μg of poly(dG)·poly(dC), or 8.1 μg of acid-soluble oligodeoxyribonucleotide and 2.0 μg of lysine-rich histone (histone) were added to the reaction mixture. The reaction was carried out for 3 min at 37°. The methods for isolation of the reaction products, digestion with venom phosphodiesterase, and the separation of the digestion mixture by paper chromatography were as described under Materials and Methods.

TABLE II: Effects of Polyanions and Lysine-Rich Histone on the Number and Length of Poly(ADP-Rib) Chains.[a]

Addition	Poly-(ADP-Rib) Isolated (pmol)	Digestion Products of Poly(ADP-Rib)		Av Chain Length [1 + (*a*/*b*)]
		Ado(P)-Rib-P (*a*) (pmol)	5′-AMP (*b*) (pmol)	
None	7.4	2.4	2.7	1.9
Histone	45.0	26.3	7.2	4.7
DNA, histone	459.9	396.8	36.4	11.9
Poly(U), histone	260.5	188.0	25.6	8.3
Poly(C), histone	234.8	179.3	24.2	8.4
Poly(A), histone	145.6	110.3	21.6	6.1
Poly(vinyl sulfate), histone	137.8	114.9	15.3	8.5

[a] The conditions for poly(ADP-Rib) synthesis were as described under Materials and Methods. The reaction mixture contained 7.2 nmol of [*adenine*-U-^{14}C]NAD (3.15 × 10^5 cpm/nmol) and 7.2 μg of enzyme protein. Where indicated, 10 μg of poly(A), 10 μg of poly(C), or 3 μg of poly(vinyl sulfate) and 20 μg of lysine-rich histone (histone) were added to the reaction mixture. The reaction was carried out for 5 min. The methods for isolation of the reaction products, digestion with venom phosphodiesterase, and separation of the digest were as described under Materials and Methods.

arated from small ones by gel filtration on Sephadex G-50 (Figure 5). Figure 5A shows the chromatogram of the reaction products synthesized in the presence of DNA and lysine-rich histone on Sephadex G-50. More than 70% of the radioactivity of the reaction products was found in the void volume with Blue Dextran as marker and only 8% of the radioactivity was found in fractions containing NAD, added as a low molecular weight marker. However, when the reaction product synthesized in the absence of both DNA and lysine-rich histone was applied to the same column of Sephadex G-50, less than 20% of the radioactivity was found in the void volume, and 57% of the radioactivity was recovered in the fractions containing marker NAD (Figure 5B). Next reaction mixture without DNA or lysine-rich histone was incubated for 2 min, and then unlabeled NAD, DNA, and lysine-rich histone were added, and the mixture was incubated for another 3 min. Gel filtration of the reaction products after the chase gave a distinctly different pattern (Figure 5C), namely, most of the radioactivity was found in the void volume and only a little with NAD.

The chromatograms of the reaction products of the chase experiments on a hydroxylapatite column and Sephadex G-50 show that the chain length of poly(ADP-Rib) was extended by the presence of DNA and lysine-rich histone.

Discussion

Previously, we reported that DNA is required for poly(ADP-Rib) synthesis by a partially purified preparation of poly(ADP-Rib) polymerase, but that polyanions such as poly(A), poly(U), poly(C), heparin, poly(vinyl sulfate), or methyl dextran sulfate are not effective (Yamada *et al.*, 1971). However, the present findings show that polyanions such as poly(U), poly(C), poly(A), methyl dextran sulfate, heparin, or poly(vinyl sulfate), like DNA, are effective for poly(ADP-Rib) synthesis by this enzyme preparation. The discrepancy between the effects of various polyanions on poly(ADP-Rib) synthesis is due to the amount of lysine-rich histone added. The polyanions tested did not cause any stimulation of poly(ADP-Rib) synthesis with an amount of lysine-rich histone which was sufficient and optimal for poly(ADP-Rib) formation with added DNA. However, with a high concentration of lysine-rich histone, polyanions were also effective for poly(ADP-Rib) synthesis, although addition of DNA was 25-fold more effective than addition of polyanions. These findings suggest that the DNA–histone complex is more easily accessible to the enzyme than the polyanion–histone complex. Spelsberg *et al.* (1970) showed that histone can form complexes with DNA and RNA but the latter complex is less stable than the DNA–histone complex.

We studied the role of added DNA and lysine-rich histone in the reconstructed system for poly(ADP-Rib) formation. Results showed clearly that the number of poly(ADP-Rib) chains was increased 6- to 22-fold over the control by additions of various DNAs, such as calf thymus DNA, poly[d(A-T)], poly(dG)·poly(dC), or acid-soluble oligodeoxyribonucleotide in the presence of lysine-rich histone. The polyanions tested, such as poly(A), poly(U), poly(C), and poly(vinyl sulfate), in conjunction with lysine-rich histone had similar effects on the number of (ADP-Rib) chains formed. Increase in the number of polymer chains may be related to increase in formation of the DNA–histone–enzyme complex, and this complex may be responsible for initiation of poly-

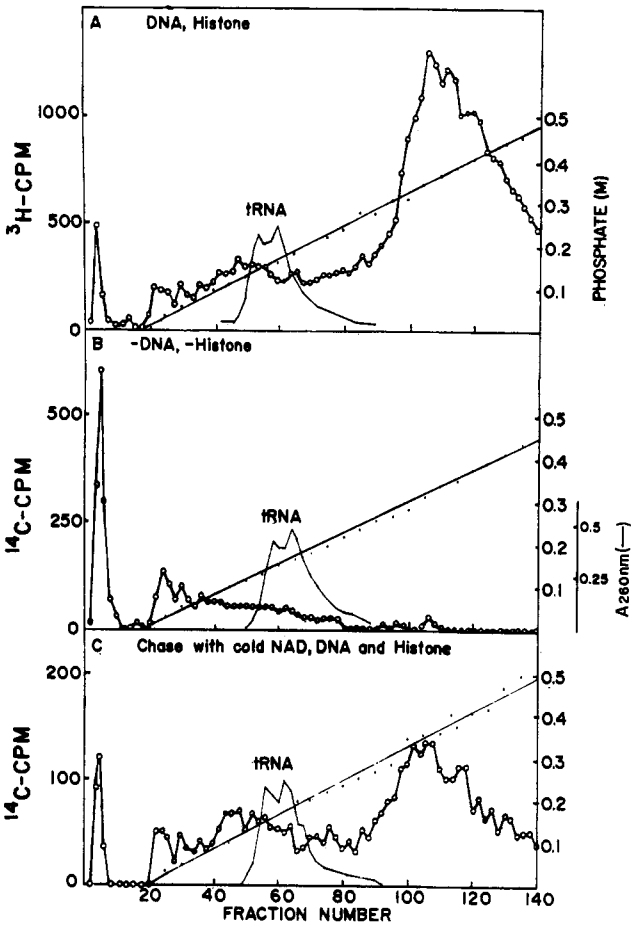

FIGURE 4: Hydroxylapatite column chromatography of the reaction products before and after chase with cold NAD, DNA, and lysine-rich histone. The reaction products were isolated as described under Materials and Methods. The products were fractionated on a hydroxylapatite column (1 × 3 cm) previously equilibrated with 1 mM sodium phosphate buffer (pH 6.8). The column was washed with 10 ml of 1 mM sodium phosphate buffer (pH 6.8) and eluted with a linear gradient of increasing phosphate concentration obtained by placing 60 ml of 1 mM phosphate buffer in the mixing chamber and 60 ml of 0.5 M sodium phosphate buffer (pH 6.8) in the reservoir. Fractions of 1 ml were collected at a flow rate of 24 ml/hr. tRNA mixture of *E. coli* (—) was included as an internal marker. (○) Radioactivity of reaction products. (A) Reaction conditions were as described under Materials and Methods. The reaction mixture (0.125 ml) contained 10 μg of DNA, 20 μg of lysine-rich histone, 8.26 nmol of [*adenosine*-^{3}H]NAD (7.43 × 10^5 cpm/nmol), and 7.2 μg of enzyme protein. The reaction period was 5 min. One-sixth of the reaction products were subjected to chromatography. (B) Reaction conditions were as described under Materials and Methods except that DNA and lysine-rich histone were omitted. The reaction mixture (0.250 ml) contained 14.4 nmol of [*adenine*-U-^{14}C]NAD (3.15 × 10^5 cpm/nmol) and 14.4 μg of enzyme protein. The reaction period was 2 min. (C) The reaction mixture was as described in part B. After incubation for 2 min, a 100-fold excess of unlabled NAD, 20 μg of DNA, and 40 μg of lysine-rich histone were added and the reaction mixture was incubated for a further 3 min.

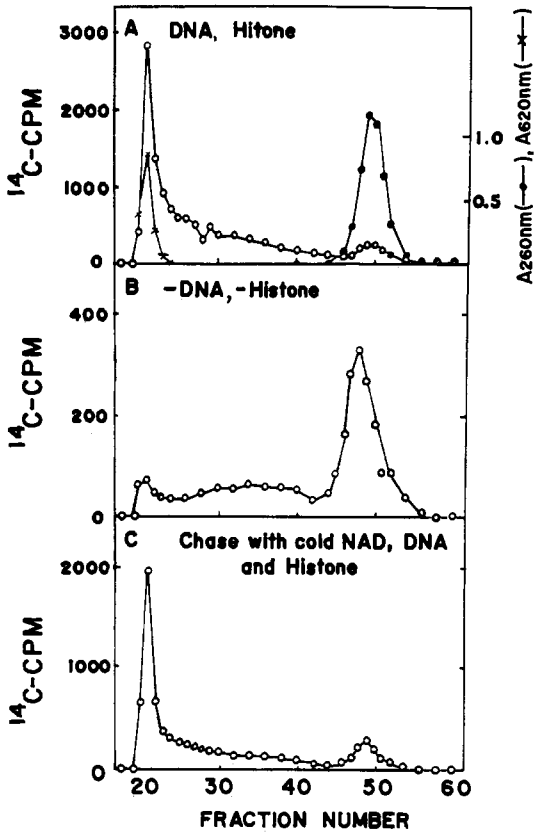

FIGURE 5: Sephadex G-50 gel filtration of the reaction products before and after chase with cold NAD, DNA, and lysine-rich histone. The reaction products were isolated as described under Materials and Methods and fractionated on a Sephadex G-50 column (1 × 110 cm). The column had been equilibrated with 10 mM Tris-HCl (pH 8.0)–1 M NaCl and was eluted with the same buffer. Fractions of 2.0 ml were collected at a flow rate of 28 ml/hr at room temperature. Blue Dextran (×) and NAD (●) included as markers. (○) Radioactivity of reaction products. (A) Reaction conditions were as described under Materials and Methods. The reaction mixture (0.125 ml) contained 10 μg of DNA, 2.0 μg of lysine-rich histone, 7.2 nmol of [*adenine*-μ-^{14}C]NAD (3.15 × 10^5 cpm/nmol), and 7.2 μg of enzyme protein. The reaction time was 5 min. One-sixth of the products were subjected to chromatography. (B) Reaction conditions were as described under Materials and Methods, except that DNA and lysine-rich histone were omitted. The reaction mixture (0.250 ml) contained 14.4 nmol of [*adenine*-U-^{14}C]NAD (3.15 × 10^5 cpm/nmol) and 14.4 μg of the enzyme protein and the reaction period was 2 min. (C) Reaction conditions were as described in part B. After incubation for 2 min, a 100-fold excess of unlabeled NAD, 20 μg of DNA, and 4.0 μg of lysine-rich histone were added and the mixture was incubated for a further 3 min.

(ADP-Rib) synthesis and chain elongation. Chemical determination of the average length of polymer chains demonstrated unequivocally that the presence of DNAs or polyanions with lysine-rich histone is essential for the synthesis of long chains of poly(ADP-Rib). Additions of various DNAs and various polyanions with lysine-rich histone increased the average chain length of the polymer from 2 to 11. However, the average length of the polymer chain was not directly correlated to the size of DNA used or the polyanions used because in the presence of acid-soluble oligodeoxyribonucleotide [less than 15 nucleotides in length (Cleaver and Boyer, 1972)] the average chain length was 5, while in the presence of calf thymus DNA (more than 3 × 10^3 nucleotides in length) it was 9. Omission of either DNA or lysine-rich histone reduced the average length and the number of poly(ADP-Rib) chains. These facts show that both DNA and lysine-rich histone are essential for increase in the number and length of the poly(ADP-Rib) chains. Nishizuka *et al.* (1969) reported that pretreatment of a chromatin with DNase I significantly reduced the average chain length of the polymer, but did not affect the number of the chains of the polymer. This discrepancy about the number of the polymer chains formed with the chromatin system and with the reconstructed system requires investigation. Chase experiments with a large amount of unlabeled NAD and both DNA and lysine-rich histone showed that the reaction products formed without DNA and lysine-rich

histone were elongated to longer polymer chains. On chasing, a new peak of radioactivity of the reaction products appeared at a position corresponding to long chains of poly(ADP-Rib) on chromatography on a hydroxylapatite column or Sephadex G-50. However, the stoichiometric relationship between the longer molecules of the polymer and the shorter molecules could not be determined accurately because the radioactivity of the reaction products obtained after the chase was 1.5–2 times more than that before the chase. The differences in the amount of the radioactive poly(ADP-Rib) formed before and after the chase may be partly because some of the enzyme-bound NAD or an unstable enzyme-bound intermediate was not recovered during isolation of the reaction products. The mechanisms of initiation of poly(ADP-Rib) synthesis and chain elongation remain to be elucidated.

It has been suggested that at one terminus poly(ADP-Rib) is bound covalently to nuclear proteins (Nishizuka *et al.*, 1968, 1969; Otake *et al.*, 1969). Poly(ADP-Rib) synthesized by a partially purified enzyme appears to be associated with protein components (unpublished data). It has also been reported that poly(ADP-Rib) polymerase is tightly associated with a chromatin or nucleoprotein complex (Ueda *et al.*, 1968; Gill, 1972). Our results suggest that both DNA and histone are essential for initiation of poly(ADP-Rib) synthesis and elongation of chains.

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