STUDIES ON ANTI-POLY(ADENOSINE DIPHOSPHATE RIBOSE) ANTIBODY[1]

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SUMMARY

Specific antibody against poly(ADP-Rib) was produced in a rabbit by injecting poly(ADP-Rib) mixed with methylated bovine serum albumin. Under standardized conditions, 1 mg of purified anti-poly(ADP-Rib) antibody combined with 400 pmoles (4 µg) of poly(ADP-Rib) and was retained on a millipore filter. The binding of [^{14}C]poly-(ADP-Rib) was not inhibited by poly(A) or other related nucleotides.

INTRODUCTION

Poly(ADP-Rib)[2] is a biopolymer which is synthesized from NAD with poly(ADP-Rib) polymerase in cell nuclei (1). The biological function of poly(ADP-Rib) was suggested to be related to DNA polymerase activity in nuclei (2, 3, 4). There are several reports on the natural occurrence of poly(ADP-Rib) (5, 6, 7, 8) and its physicochemical characteristics have also been described (1).

Anti-poly(ADP-Rib) antibody should be useful for investigating the natural occurrence of poly(ADP-Rib). We succeeded in producing antibody against poly(ADP-Rib) in a rabbit by injecting a complex of poly(ADP-Rib) and MBSA, following the method of Plescia et al. (9). Specific precipitating antibody against poly(ADP-Rib) was produced by a single injection of the poly(ADP-Rib)-MBSA complex emulsified

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2. The abbreviations used are : poly(ADP-Rib), polymer of ADP-Rib ; ADP-Rib, adenosine diphosphate-ribose ; Ado(P)-Rib-P, 2'-(5"-phosphoribosyl)-5'-AMP ; MBSA, methylated bovine serum albumin ; PBS, phosphate buffered saline (pH 7.4).

with an equal volume of complete Freund's adjuvant into the foot pads of a rabbit. The immune complex formed between poly(ADP-Rib) and IgG containing its antibody was retained on a millipore filter of 0.22 μ pore size. The non-specific reaction between poly(ADP-Rib) and normal IgG from an untreated rabbit was negligible. This is the first report on the formation of anti-poly(ADP-Rib) antibody.

MATERIALS AND METHODS

Preparation of poly(ADP-Rib) and [^{14}C]poly(ADP-Rib). Poly(ADP-Rib) and [^{14}C]poly(ADP-Rib) were prepared by the method of Sugimura et al. (10). Poly-(ADP-Rib) was separated after incubation of calf thymus nuclei with unlabeled NAD, and [^{14}C]poly(ADP-Rib) after incubation of rat liver nuclei with NMN and [adenine-8-^{14}C]ATP. Both preparations contained less than one percent contaminating protein, as determined by the method of Lowry et al. (11). The chain length of poly(ADP-Rib) was determined by the method of Fujimura and Sugimura (12), and the molecular weight of poly(ADP-Rib) was roughly 10,000 daltons.

Production of anti-poly(ADP-Rib) antibody. MBSA was prepared by the method of Sueoka and Cheng (13). One mg of MBSA in distilled water and 660 μg of poly-(ADP-Rib) in PBS were mixed and emulsified with an equal volume of complete Freund's adjuvant. The final volume of emulsion was 1.2 ml. A male rabbit, weighing 2.5 kg, was injected at multisites in the foot pads with the emulsion using a needle with a brim. Four weeks later, the rabbit was bled by cardiac puncture. The antiserum was separated and incubated at 56°C for 30 min.

Purification of IgG from antiserum and normal rabbit serum. Serum was fraction-ated with ammonium sulfate using the method of Deutsh (14) with a slight modification. IgG in a crude γ-globulin fraction was further purified by passage through a column of Sephadex G-200, and then column chromatography on DEAE-cellulose. The IgG fraction obtained from antiserum was used as anti-poly(ADP-Rib) antibody.

Immunological assays for poly(ADP-Rib). Ouchterlony double diffusion analysis was performed on a microslide glass mounted with 0.9 % purified agar in PBS and for rapid quantitative assay a millipore filter of 0.22 μ pore size was used. Mixtures of 1 mg of IgG (160 μl) and various amounts of poly(ADP-Rib) (2.1 x 10^5 cpm/nmole) in test tubes (1 x 10 cm) were adjusted to 1 ml with PBS. The mixtures were incu-bated at 37°C for 1 hour with shaking, and then at 0°C for 1 hour. After the reaction, the wall of the test tube was scraped thoroughly with a spatula and then the reaction mixture was applied to a millipore filter, previously soaked in PBS with aspiration. The test tube was washed with 3 ml of PBS and this solution was also transferred to the millipore filter with aspiration. These procedures were carried out at 4°C. The millipore filters were dried and then the radioactivities of the immune complexes col-lected on them were determined with a liquid scintillation counter.

Determination of the binding capacity of anti-poly(ADP-Rib) with poly(ADP-Rib). Mixtures of 1 μg of [^{14}C]poly(ADP-Rib) (2.1 x 10^4 cpm) and various amounts of

unlabeled poly(ADP-Rib) were incubated with 1 mg of IgG and radioactivity was determined as described above.

Inhibition test. Based on the above experiment, 30 μg or more of ADP-Rib, Ado-(P)-Rib-P or related nucleotides were added as inhibitors to samples of 4 μg of [^{14}C]-poly(ADP-Rib) (2.1 x 10^4 cpm), and after incubation with 1 mg of IgG, radioactivity was determined as described above.

Chemicals. [Adenine-8-^{14}C]ATP was purchased from Schwarz/Mann. NAD and NMN were donated from Kyowa Hakko Co., Tokyo. Calf thymus DNA and ADP-Rib were purchased from Sigma Chemical Co., poly(G), poly(C) and poly(U) from Miles Laboratory, Inc., poly(A) from Calbiochem, oligo$(A)_{10}$ and $(A)_5$ from Boehringer Mannheim, Mannheim (Germany), and yeast RNA from Worthington Biochemical Corp. Ado(P)-Rib-P was isolated from poly(ADP-Rib) by the method of Shima et al. (15). Bovine serum albumin was obtained from Sigma Chemical Co. Complete Freund's adjuvant and purified agar were from Difco.

RESULTS AND DISCUSSION

Ouchterloney double diffusion analysis of anti-poly(ADP-Rib) antibody. Fig. 1 shows the results of Ouchterloney double diffusion analysis of poly(ADP-Rib), poly(A) and ADP-Rib with anti-poly(ADP-Rib) antibody. Poly(ADP-Rib) gave a single precipitation line against anti-poly(ADP-Rib) antibody, but poly(A) and ADP-Rib gave no precipitation line. This shows the specificity of the antibody.

Immunological assay of poly(ADP-Rib). Fig. 2 shows the results of immunological assay for poly(ADP-Rib) using a millipore filter. Within a fairly wide range of concentrations of poly(ADP-Rib), a linear relationship was obtained between the amount of poly(ADP-Rib) and the radioactivity retained on the millipore filter. More than 90 % of the radioactivity was recovered on the filter with up to 200 pmoles (2 μg) of poly-(ADP-Rib) per 1 mg of antibody.

Binding capacity of antibody to poly(ADP-Rib). Based upon these results, various amounts of unlabeled poly(ADP-Rib) were added to 100 pmoles (1 μg) of [^{14}C]poly-(ADP-Rib) with a specific activity of 2.1 x 10^5 cpm/nmole, and then incubated with 1 mg of antibody, as described in the Materials and Methods. On addition of more than 3 μg of unlabeled poly(ADP-Rib) to the reaction mixture, the radioactivity on the millipore filter decreased logarithmically, as shown in Fig. 3. This means that the

for binding of half the original radioactivity. Accordingly, 30 μg or more of ADP-Rib, Ado(P)-Rib-P and related anologs were used in tests on inhibition of binding of poly-(ADP-Rib) with antibody. It was found that related polynucleotides did not change the binding of [^{14}C]poly(ADP-Rib) at all, as shown in Table 1. These results again confirm the specificity of the anti-poly(ADP-Rib) antibody.

The time for induction of the precipitating antibody against poly(ADP-Rib) in a rabbit by our immunization method was relatively short, compared to the time required for induction of antibody against nucleic acids. The antibody activity against poly-(ADP-Rib) was found in IgG and in this experiment, IgG was used throughout. Non-specific binding of [^{14}C]poly(ADP-Rib) with IgG from an untreated rabbit was not observed, as shown in Fig. 2. The antigenic determinant is unknown, but may involve repetitions of ribose-ribose or pyrophosphoric bond, because neither poly(A) nor ADP-Rib inhibited the binding of [^{14}C]poly(ADP-Rib) to antibody.

Anti-poly(ADP-Rib) antibody should be very useful for survey of the natural occurrence of poly(ADP-Rib). It is still uncertain whether an oligomer of ADP-Rib can react with anti-poly(ADP-Rib). The present paper describes a rapid and simple immunological assay for poly(ADP-Rib) using a millipore filter and the specificity of the antibody. We are now attempting to establish a radioimmunoassay for poly(ADP-Rib).

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