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Comparative studies on antibodies to poly(ADP-ribose) in rabbits and patients with systemic lupus erythematosus

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Summary. Immunochemical studies were made on the antibodies induced in rabbits against poly(ADP-ribose) and naturally-occurring antibodies in patients with systemic lupus erythematosus. Antibodies against poly(ADP-ribose) could also be induced in rabbits by oligo(ADP-ribose) associated with rat liver histones and by a complex of poly(ADP-ribose) with methylated bovine serum albumin (MBSA). The two types of antibody were inhibited to the same extent by poly(ADP-ribose). However, the antibody induced by oligo(ADP-ribose) associated with histones was inhibited by oligo(ADP-ribose) with an average chain length of 4 ADP-ribosyl units and by phosphoribosyl adenosine monophosphate (PR-AMP) but not by mono ADP-ribose, whereas that induced by poly(ADP-ribose) was practically not inhibited by these related compounds even in excess amounts.

The sera of ten cases of systemic lupus erythematosus showing high antibody activity against poly(ADP-ribose) were also examined immunochemically. It was found that the antibodies of three patients showed a similar inhibitory pattern to that of antibody induced in rabbits by oligo(ADP-ribose) associated with histones, those of three patients showed a similar pattern to that of antibody produced in rabbits by poly(ADP-ribose), and the remainder did not show either pattern. These findings suggest that oligo(ADP-ribose) associated with histones may serve as antigen to elicit naturally-occurring antibodies to poly(ADP-ribose) in patients with systemic lupus erythematosus.

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INTRODUCTION

There are several reports of antibodies to poly(ADP-ribose) in experimental animals immunized with poly(ADP-ribose) as well as in patients with systemic lupus erythematosus (SLE; Kanai, Miwa, Matsushima & Sugimura, 1974; Kanai, Miwa, Matsushima & Sugimura, 1978a; Kanai, Kawaminami, Miwa, Matsushima, Sugimura, Moroi & Yokohari, 1977). The significance of antibody to poly(ADP-ribose) has been confirmed (Okolie & Shall, 1979). Poly(ADP-ribose) is a unique biopolymer associated with chromatin and is suggested to have some regulatory roles in gene expression (Sugimura, 1973; Hilz & Stone, 1976; Hayaishi & Ueda, 1977). The existence of this biopolymer *in vivo* has been demonstrated (Kidwell & Mage, 1976; Sakura, Miwa, Tanaka, Kanai, Matsushima & Sugimura, 1977; Ferro, Minaga, Piper & Kun, 1978; Minaga, Romaschin, Kirsten & Kun, 1979). The presence of naturally-occurring antibodies to poly(ADP-ribose) in SLE patients suggests that the patients are immunized with poly(ADP-ribose) *in vivo*, but recently we found that double-stranded RNA, poly(A)poly(U) (Kanai, Sugimura & Matsushima, 1978b), could also induce specific antibodies to

poly(ADP-ribose). Therefore, RNA viruses that make double-stranded RNA during replicative stages may induce antibodies to poly(ADP-ribose) in the absence of active immunization with poly(ADP-ribose). Thus, the mechanism of formation of antibody to poly(ADP-ribose) in SLE patients may be very complicated.

Recent *in vitro* studies indicated that ADP-ribosylation of chromatin occurs mainly in histone subgroups H1 and H2b, and also suggested that non-histone proteins may undergo ADP-ribosylation (Okayama, Ueda & Hayaishi, 1978; Requelme, Burzio & Koide, 1979; Burzio, Requelme & Koide, 1979). We consider that oligo(ADP-ribose) with less than ten ADP-ribosyl units associated with chromatin should be much more stable than the free form of poly(ADP-ribose) *in vivo*, and that in the form of complexes even the oligomer should serve as a good immunogen for production of antibody to poly(ADP-ribose) *in vivo*. Based upon this assumption, we immunized rabbits with oligo(ADP-ribosyl)ated rat histone to see whether it could induce antibodies reacting with poly(ADP-ribose) and found that this was actually the case. This report mainly concerns immunochemical comparisons of antibodies induced in rabbits by oligo(ADP-ribosyl)ated histone and naturally-occurring antibodies in SLE patients. The possibility that naturally-occurring antibodies to poly(ADP-ribose) are produced by sensitization of SLE patients with oligo(ADP-ribosyl)ated histones formed *in vivo* is discussed.

MATERIALS AND METHODS

Purification and isolation of poly(ADP-ribose) and oligo(ADP-ribosyl)ated histone

Poly(ADP-ribose) was purified by the method of Sugimura and co-workers (Sugimura, Yoshimura, Miwa, Nagai & Nagao, 1971). Briefly, calf thymus nuclei were incubated with [U-^{14}C]-nicotinamide adenine dinucleotide (NAD) for 30 min at 37°. The resulting poly(ADP-ribose) was purified by treatment with pronase and nucleases and by hydroxylapatite (HA) column chromatography. Oligo(ADP-ribosyl)-ation of rat liver chromatin was achieved by incubating rat liver nuclei (Wistar, female) with [U-^{14}C] NAD by the method of Fujimura & Sugimura (1971). After 30-min incubation, oligo(ADP-ribosyl)ated histone was extracted from the incubation mixture with 0·25 N HCl and precipitated with trichloroacetic acid (20%) by the method of Okayama and co-workers (Okayama *et al.*, 1978). The purity of the histone was checked by acid-urea (0·9 N acetic acid/2·5 M urea) polyacrylamide (15%) gel electrophoresis by the method of Panyim & Chalkley (1969). Results showed that the content of non-histone protein was less than 1% (data not shown). However, our preparation of oligo(ADP-ribosyl)ated histone used as antigen contained about 85% of unmodified histone. We did not separate unmodified histone from oligo(ADP-ribosyl)ated histone because the mixture of these components is thought to be similar to that *in vivo*, where the content of oligo(ADP-ribosyl)ated histone may be much lower than that of unmodified histone. Two samples of oligo(ADP-ribosyl)ated histone were prepared for immunization. Judging from their radioactivity, they contained 8 μg and 4 μg of oligo(ADP-ribose) per mg protein, respectively. Unmodified histone was similarly extracted from rat liver nuclei without incubation with NAD and was found by acid-urea gel electrophoresis to be as pure as the oligo(ADP-ribosyl)ated histone. The average chain lengths of ADP-ribosyl units of the oligomers bound to histones were 2·2 and 1·6, respectively, as determined by the method of Tanaka and co-workers (Tanaka, Miwa, Hayashi, Kubota, Matsushima & Sugimura, 1977).

Immunization of rabbits with poly(ADP-ribose) and oligo(ADP-ribosyl)ated histone

Immune sera were obtained from New Zealand white rabbits (male, 2·5–3 kg body weight). Rabbits were immunized with poly(ADP-ribose) by the method described previously (Kanai, *et al.*, 1974). Samples of oligo(ADP-ribosyl)ated histone (2 mg) with an average chain length of 2·2 ADP-ribosyl units or of unmodified histone were dissolved in saline and emulsified with an equal volume of Freund's complete adjuvant (FCA) as for immunization with poly(ADP-ribose). The emulsion (1 ml) was injected into the foot pads of the fore legs of two rabbits. Forty days later, 500 μg of each antigen in saline without FCA was injected into the animals intradermally. Thirty days after the second injection, 2 mg of antigen emulsion prepared as for the first injection, was injected intramuscularly. Fourteen days after the third injection, blood was collected by cardiac puncture. In the case of oligo(ADP-ribosyl)ated histone with an average chain length of 1·6, twice as much antigen, was injected at each immunization step. Sera were inactivated by heating at 56° for 30 min before use.

Biochemical analysis of antigen, oligo(ADP-ribosyl)ated histone

A solution of 40 mg (8×10^4 c.p.m.) of oligo(ADP-ribosyl)ated histone used as antigen in 20 ml of 50 mM Tris-HCl buffer, pH 8·0, was digested with 20 mg of pronase E and the oligomer liberated was recovered in the aqueous layer after phenol extraction. This procedure has been reported in detail (Fujimura & Sugimura, 1971). The aqueous layer was washed with ether to remove phenol and then dialysed against 10 mM phosphate buffer, pH 6·8, in a Spectrapor membrane 3 (Mr. 3,500 cutoff, Spectrum Medical Industries Inc., Los Angeles). The recovery of radioactivity after dialysis was 75%. The dialysed fraction (4×10^4) was applied to a column of hydroxylapatite (HA, 1×6 cm) which had been equilibrated with 10 mM phosphate buffer, pH 6·8. The column was eluted stepwise with 10, 100 and 500 mM phosphate buffer, pH 6·8, and fractions of 2 ml eluate were collected at a flow rate of 15 ml/hr (Fig. 3). The average chain lengths of the fractions were determined as described above. Briefly, the fractions were treated successively with alkaline phosphatase and pronase, and the aqueous layer obtained by phenol extraction was finally treated with snake venom phosphodiesterase (PDE). Products obtained by these treatments were subjected to paper chromatography. The average chain length was calculated from the ratio of PR-AMP to 5′-adenosine monophosphate (AMP) and ribosyl adenosine monophosphate.

Assay of antibody to poly(ADP-ribose) and determination of antibody specificity

Antibody against poly(ADP-ribose) was assayed with a Millipore filter using highly labelled [^{14}C]poly(ADP-ribose) (2,700 c.p.m., 4 ng), and antibody activity was expressed as antibody units per ml of serum, as described previously (Kanai *et al.*, 1978a). Highly labelled [^{14}C]poly(ADP-ribose) was obtained by incubating rat liver nuclei with nicotinamide mononucleotide (NMN) and [^{14}C]ATP(560 mCi/mmol) and was purified by the method of Sugimura and co-workers (Sugimura *et al.*, 1971). In some experiments, antibody was determined after separation of whole serum into 19s and 7s antibody on Sephadex G-200, which had been equilibrated with phosphate-buffer saline (10 mM phosphate, 0·14 M NaCl·pH 7·4) (PBS), as described previously (Kanai *et al.*, 1978a). Antibody specificity was checked by measuring inhibition of [^{14}C]poly(ADP-ribose) binding to antibody with unlabelled poly(ADP-ribose), oligo(ADP-ribose) or monomers of poly(ADP-ribose), ADP-ribose and PR-AMP as reported previously (Kanai *et al.*, 1978a,b).

Chemicals

[U-^{14}C]ATP (560 mCi/mmol) and [U-^{14}C]NAD (310 mCi/mmol) were purchased from the Radiochemical Centre, Amersham. NMN was purchased from Sigma, Chemical Co., NAD from Boehringer Mannheim. Pronase E was a product of Kaken Kagaku Co., Tokyo. *E. coli* alkaline phosphatase and PDE were purchased from Worthington Biochemical Corp. ADP-ribose was obtained from Sigma Chemical Co., and another monomer of poly(ADP-ribose), PR-AMP, was obtained by splitting poly(ADP-ribose) with PDE, and was separated by dialysis using a Spectrapor membrane (Mr. 1000 cutoff, Spectrum Medical Industries Inc., Los Angeles). Its purity was nearly 100%, as measured by paper chromatography by the method of Fujimura & Sugimura (1971). Oligo(ADP-ribose) was also obtained by splitting poly(ADP-ribose) with PDE using a shorter time of treatment, and it was separated by HA column chromatography. The average chain length of the oligomer was found to be 4. The structures of poly(ADP-ribose) and its structural components are illustrated in Fig. 4 with special reference to the relation between the enzymic degradation of poly(ADP-ribose) and its products.

RESULTS

[^{14}C]poly(ADP-ribose) binding activity and antibody class of the sera of rabbits immunized with oligo(ADP-ribosyl)ated histone

Figure 1 shows the binding of [^{14}C]poly(ADP-ribose) to the sera of rabbits immunized with oligo(ADP-ribosyl)ated histone with an average chain length of 2·2, unmodified histone, and poly(ADP-ribose) in comparison with its binding to the sera of untreated rabbits. Binding to the sera of rabbits immunized with oligo(ADP-ribosyl)ated histone was much higher than that to sera of untreated rabbits, but not so high as the binding to the sera of rabbits immunized with poly(ADP-ribose)–MBSA complexes. Similar results were obtained for oligo(ADP-ribosyl)ated histone with an average chain length of 1·6. The extent of binding of [^{14}C]poly(ADP-ribose) to the sera of rabbits immunized with oligo(ADP-ribosyl)ated histone was approximately the same as that to SLE sera, which show the highest binding to poly(ADP-ribose) known

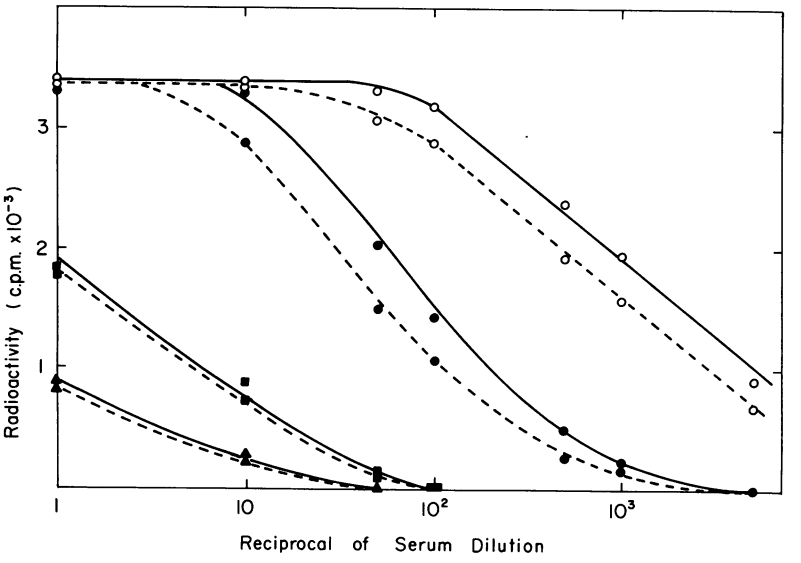

Figure 1. [^{14}C]poly(ADP-ribose) binding to the sera of rabbits immunized with various kinds of antigens and to the sera of untreated rabbits. Conditions were as described in the text. (○) sera of rabbits immunized with poly(ADP-ribose) complexed with MBSA; (●) sera of rabbits immunized with oligo(ADP-ribosyl)ated histone with an average chain length of 2·2; (■) sera of rabbits immunized with unmodified histone; (▲) sera of untreated rabbits.

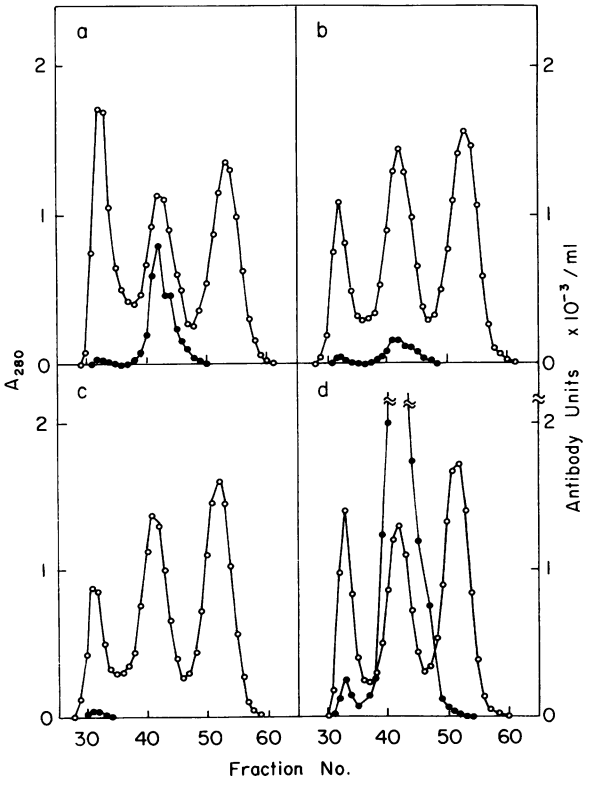

Figure 2. Profile of elution from Sephadex G-200 of antibody to poly(ADP-ribose) in the various rabbit sera, shown in Fig. 1. Antiserum and control serum were diluted two-fold with PBS, and 1·5 ml of diluted serum was applied to a column of Sephadex G-200 (1·2 × 84 cm), which had been equilibrated with PBS. The antibody titre against poly(ADP-ribose) was determined by the Millipore filter method, and was expressed as antibody units/ml of fraction. The first, second and third peaks represent 19S and 7S antibody and albumin, respectively. a and b, rabbits A and B immunized with oligo(ADP-ribosyl)ated histone with an average chain length of 2·2; c, rabbit C immunized with unmodified histone, d, rabbit D immunized with poly(ADP-ribose). (○) A_{280}; (●) antibody titre.

so far (data not shown). The binding of [^{14}C]poly(ADP-ribose) to the sera of rabbits immunized with unmodified rat histone was slightly higher than that to the sera of untreated rabbits, but this binding may have been due to antibody formed to naturally-occurring oligo(ADP-ribosyl)ated histone in the unmodified histone preparation used as antigen.

The antisera obtained by the various immunization procedures described in the Materials and Methods were applied to a column of Sephadex G-200. Figure 2 shows the profiles of the antibody titres against poly(ADP-ribose) in the fractions eluted from the column. Figures 2a and 2b show the antibody profiles of rabbits A and B, respectively, immunized with oligo(ADP-ribosyl)ated histone. Both rabbits pro-

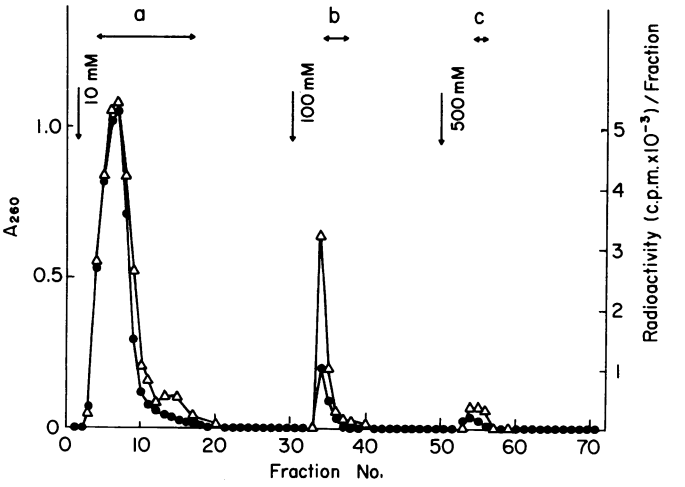

Figure 3. Analysis of antigen, oligo(ADP-ribosyl)ated histone with an average chain length of 2·2, by hydroxylapatite column chromatography (HA). The methods for preparation of the sample, which had been subjected to chromatography on HA, and for determination of the chain length of each fraction eluted from the column are described in the text. (△) radioactivity; (●) A_{260}.

duced 7S antibody with a little 19S antibody. The antibody profile of rabbit A resembled that of rabbit D immunized with poly(ADP-ribose)-MBSA complexes (Fig. 2d), except that the amount of 7S antibody was less. The slight [^{14}C]poly(ADP-ribose) binding of sera of rabbits immunized with unmodified histone was confined to the 19S γ-globulin fraction, as shown in Fig. 2C, and was similar to the binding of the sera from untreated rabbits (data not shown). The Sephadex-150 elution profiles of antibody to poly(ADP-ribose) of the sera of SLE patients showed that the amount of 7S antibody usually exceeded the amount of 19S antibody (Kanai *et al.*, 1977).

Purity and characterization of oligo(ADP-ribosyl)ated histone

The purities of oligo(ADP-ribosyl)ated histones with average chain lengths of 2·2 and 1·6 were checked by acid-urea polyacrylamide gel electrophoresis in parallel with unmodified histone, as described in the Materials and Methods. Staining the gel with Amido black 10B showed that these fractions were not contaminated with non-histone protein, and that the electrophoretic mobilities of histone subgroups, H1, H2a, H2b, H3 and H4 in oligo(ADP-ribosyl)ated histone and unmodified histone were similar (data not shown). Next, the chain length of ADP-ribosyl units of oligomer or possible polymer associated with histone was examined as described in the Materials and Methods. When oligomers or possible polymers derived from oligo(ADP-ribosyl)ated histone with an average chain length of 2·2 were applied to an HA column, 89, 10 and 1% of the total radioactivity was found in fraction a, b, and c, respectively, as shown in Fig. 3. The average chain lengths of these fractions were less than 2, 4 and 8, respectively. Similar results were obtained for oligo(ADP-ribosyl)ated histone with an average chain length of 1·6. Therefore, it seems

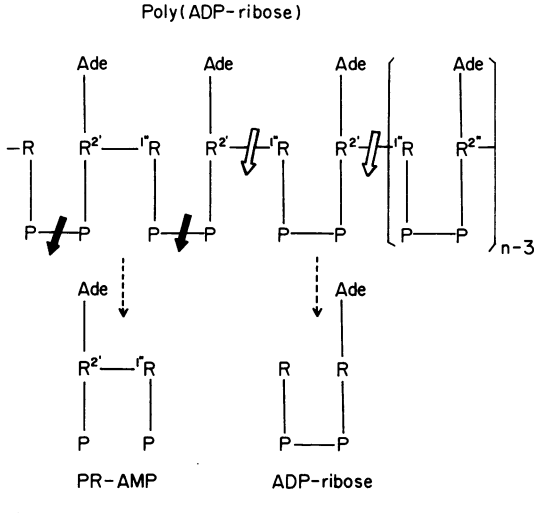

Figure 4. Structure and components of poly(ADP-ribose) with special reference to enzymic degradation of poly(ADP-ribose). Ade, adenine; R, D-ribose; P, phosphate; ⇒, Cleavage site for glycohydrolase; ⇒, Cleavage site for PDE.

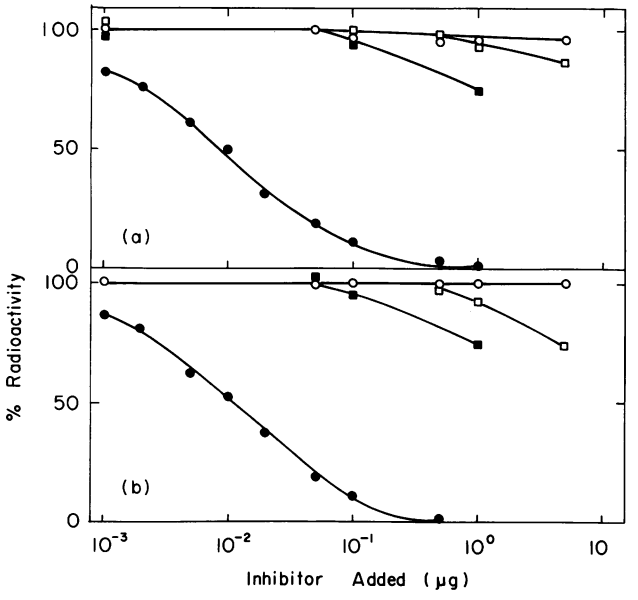

Figure 5. Inhibition by poly(ADP-ribose) and related compounds of [^{14}C]-poly(ADP-ribose) binding of antisera of rabbits immunized with oligo(ADP-ribosyl)ated histone, with an average chain length of 2·2. The sera were adjusted to a dilution binding 80% of the radioactivity added as [^{14}C]poly(ADP-ribose) (4 ng, 2700 c.p.m.) in the absence of inhibitors; i.e. 10 μl of serum at 1:20 dilution. This binding was expressed as 100%. (a) and (b) show results with sera from different rabbits. (●) poly(ADP-ribose); (■) oligo(ADP-ribose); (○) ADP-ribose; (□) PR-AMP.

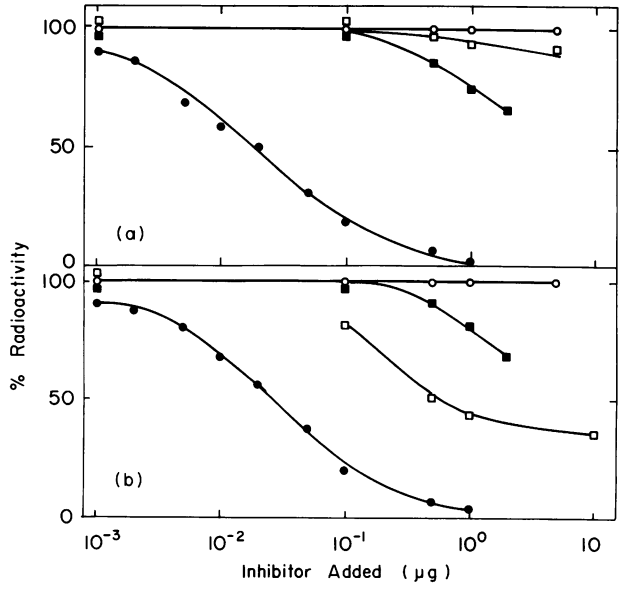

Figure 6. Inhibition by poly(ADP-ribose) and related compounds of [^{14}C]-poly(ADP-ribose) binding of the antisera of rabbits immunized with oligo(ADP-ribosyl)ated histone, with an average chain length of 1·6. The sera were adjusted to a dilution binding 70% of the radioactivity added as [^{14}C]poly(ADP-ribose) (4 ng, 2700 c.p.m.) in the absence of inhibitors; i.e. 10 μl of serum at 1:20 dilution. This binding was expressed as 100%. (a) and (b) show results with sera of different rabbits. (●) poly(ADP-ribose); (■) oligo(ADP-ribose); (○) ADP-ribose; (□) PR-AMP.

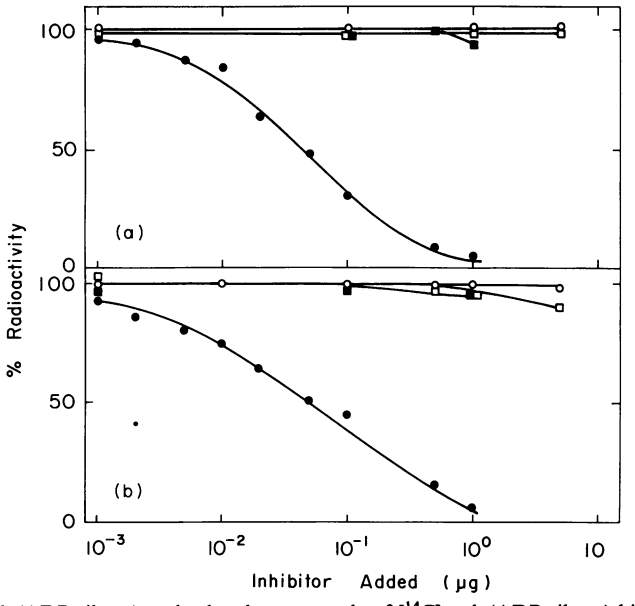

Figure 7. Inhibition by poly(ADP-ribose) and related compounds of [^{14}C]-poly(ADP-ribose) binding of the sera of rabbits immunized with poly(ADP-ribose)-MBSA complexes. The sera were adjusted to bind 80% of the radioactivity added as [^{14}C]poly(ADP-ribose) (4 ng, 2700 c.p.m.) in the absence of inhibitors; i.e. 10 μl of sera at 1:200 dilution. The binding was expressed as 100%. (a) and (b) show results with sera from different rabbits. (●) poly(ADP-ribose); (■) oligo(ADP-ribose); (○) ADP-ribose; (□) PR-AMP.

improbable that antibody reacting with poly(ADP-ribose) was elicited by fraction c, even if it bound to histone, because fraction c of both samples contained only 1% and 5% of the total ADP-ribosyl moiety of the antigen, i.e. 0·16 and 0·8 μg, respectively.

Specificity of antibodies induced by oligo(ADP-ribosyl)ated histone and by poly(ADP-ribose)

Poly(ADP-ribose) strongly inhibited even antibody induced by oligo(ADP-ribosyl)ated histone with an average chain length of 2·2 or 1·6 ADP-ribosyl units (Figs 5 and 6). Similar results were obtained with antibody produced by poly(ADP-ribose)-MBSA complexes (Fig. 7). However, oligomer with 4 ADP-ribosyl units and PR-AMP also inhibited the antibody induced by these two oligo(ADP-ribosyl)ated histones, the antibody induced by oligo(ADP-ribosyl)ated histone with an average chain length of 1·6 being inhibited more strongly by PR-AMP. These compounds did not inhibit the antibody induced by poly(ADP-ribose)-MBSA complexes. Mono ADP-ribose did not inhibit either antibody, and other related compounds, such as single- and double-stranded DNA, single- and double-stranded RNA, and 3′-AMP and 5′-AMP also did not act as inhibitors (data not shown).

Table 1. Patterns of inhibition of [^{14}C]Poly(ADP-ribose) binding to the sera of SLE patients

Patient	% Inhibition by ADPR	PR-AMP	Oligo(ADPR)	Poly(ADPR)
R49	0	0	0	94
H22	4	68	37	96
K31	7	0	0	98
K37	0	16	7	40
K80	0	3	5	94
K33	0	52	30	95
I64	12	17	30	87
L32	6	16	13	79
L29	7	8	10	92
K92	0	3	22	92

The sera used in the inhibition experiment showed 70%–80% binding of the radioactivity added as [^{14}C]poly(ADP-ribose) (4 ng, 2700 c.p.m.) in the absence of inhibitors. This radioactivity is expressed as 100%. The amount of the inhibitor poly(ADP-ribose) was 1 μg, and the amounts of the other inhibitors oligo(ADP-ribose) and PR-AMP were 5 μg; i.e. 250 and 1250 times respectively, the amount of [^{14}C]poly(ADP-ribose).

Heterogeneity in the specificity of naturally-occurring antibodies to poly(ADP-ribose) in SLE patients

The specificity of the antibodies in the sera of ten cases of SLE, with high antibody titres against poly(ADP-ribose), were studied in the same way as the antibodies induced in rabbits. In nine SLE patients, the serum was preferentially inhibited by poly(ADP-ribose), as shown in Table 1. The antibodies in the sera of three patients (H22, K33 and I64) were inhibited by both the oligomer and PR-AMP, and the specificity of these SLE antibodies was similar to that of antibodies induced in rabbits by oligo(ADP-ribosyl)ated histone, as shown in Fig. 5 and 6. The antibodies in the sera of three other SLE patients (R49, K31 and K80) were completely inhibited by poly(ADP-ribose) only and their specificity resembled that of rabbit antibodies against poly(ADP-ribose), as shown in Fig. 7. The antibodies in the other SLE patients seemed to differ from both types of rabbit antibodies.

DISCUSSION

This work showed that poly(ADP-ribose) reactive antibody could be induced by oligo(ADP-ribose) bound to histone. We reported previously that poly(ADP-ribose) has strong antigenicity, inducing its specific antibody in rabbits and mice (Kanai *et al.*, 1974; Kanai *et al.*, 1978a). The antibodies induced by oligo(ADP-ribosyl)ated histone and by poly(ADP-ribose) showed similar specificity for poly(ADP-ribose), although not for its structural components, PR-AMP and oligo(ADP-ribose) other than ADP-ribose monomer. Naturally-occurring antibodies to poly(ADP-ribose) in SLE patients were strongly inhibited by poly(ADP-ribose), and those in some patients were also inhibited by poly(A)poly(U) duplex (Kanai *et al.*, 1977). However, the cross-reactions of the naturally-occurring antibodies with oligo(ADP-ribose) and PR-AMP have not been fully examined yet. Since oligo(ADP-ribosyl)ation of histone has been observed both *in vitro* and *in vivo* (Okayama *et al.*, 1978; Requelme *et al.*, 1979; Minaga *et al.*, 1979), it seems possible that histone bound oligo(ADP-ribose) may serve as an antigen to induce antibody in SLE patients. As shown in this paper, the oligomer of ADP-ribose and PR-AMP, a structural component of the oligomer, strongly inhibited the antibody to poly(ADP-ribose) in some cases of SLE. Moreover, the oligomer and PR-AMP clearly inhibited the antibody induced in rabbits by oligo(ADP-ribosyl)-ated histone. These results suggest that some cases of SLE are actively immunized with oligo(ADP-ribose) bound to histone or other nuclear proteins, with an average oligomer chain length of about 2. We are now studying the effect of oligo(ADP-ribosyl)ation of histone on the antigenicity. Results suggest that the effect depends on the chain length of ADP-ribose bound to histone (unpublished data).

The induction of poly(ADP-ribose) reactive antibody observed in the work was not due to contaminating poly(ADP-ribose) with more than 20 ADP-ribosyl units in the antigen, because biochemical analysis showed that the antigen consisted mainly of oligomer of less than 2 ADP-ribosyl units and that only 1%–5% of all the oligomers liberated from oligo(ADP-ribosyl)ated histone had a chain length of 8 units.

The oligo(ADP-ribosyl) moiety of the antigen used in the present experiments is a hapten with a molecular weight of about 1100 daltons, and so it is unlikely to elicit antibody without a carrier moiety. Moreover, the titre of antibody obtained by immunizing mice with poly(ADP-ribose) alone was reported to be one fiftieth of that obtained by immunizing them with poly(ADP-ribose) complexed with MBSA (Kanai *et al.*, 1978a). In the present work, the histone moiety seemed to be essential for production of antibody reacting with poly(ADP-ribose). Thus, the induction of antibody reacting with poly(ADP-ribose) by oligo(ADP-ribosyl)ated histone suggests that oligo(ADP-ribosyl)-ation of chromatin *in vivo* is closely related with the occurrence of antibody to poly(ADP-ribose) in SLE patients.

Poly(ADP-ribose) reactive antibody induced by oligo(ADP-ribosyl)ated histone retained more than 80% of the total [^{14}C]poly(ADP-ribose) even after extensive absorption with glutaraldehyde polymerized oligo(ADP-ribosyl)ated histone (data not shown). The mechanism of induction of poly(ADP-ribose) reactive antibody is unknown. However, 'heteroclitic antibody', which reacts with antigens other than the immunizing antigen, has been reported (Mäkelä, 1965, Walters & Wigzell, 1972; Merchant & Inman, 1977). Antibody that specifically reacts with poly(ADP-ribose) other than oligo(ADP-ribose) may be more similar to 'heteroclitic antibody' than the antibody induced by poly(A)Poly(U), as we reported previously (Kanai *et al.*, 1978b), because the immunogen used in the present experiments also contained the structural components of poly(ADP-ribose), i.e. ADP-ribose and PR-AMP. Antibody induced by PR-AMP cova-

lently coupled to bovine serum albumin also partly reacted with [^{14}C]poly(ADP-ribose), but [^{14}C]poly(ADP-ribose) binding to the antibody was preferentially inhibited by PR-AMP, and was inhibited less by poly(ADP-ribose) (Sakura, Miwa, Kanai, Matsushima & Sugimura, 1978). Therefore, the mechanism of production of antibody reacting with poly(ADP-ribose) by oligo(ADP-ribosyl)ated histone seems to be different from that of antibody production by PR-AMP.

The observed difference in the specificities of poly(ADP-ribose) reactive antibodies induced in rabbits by oligo(ADP-ribosyl)ated histone and by complexes of poly(ADP-ribose) and MBSA suggests a difference in the antigen-recognizing units of the two antibodies: the former may recognize the tertial structure of poly(ADP-ribose), although this has not yet been demonstrated, and the latter may in part recognize the structural units of poly(ADP-ribose).

The difference in the specificities of poly(ADP-ribose) reactive antibodies in SLE patients demonstrated in the work could be explained in the same way as the difference in specificities of poly(ADP-ribose) reactive antibodies induced in rabbits. It has been found that the antibodies to native DNA in different SLE patients were directed to different lengths of DNA base-pairs varying from twenty to 1200 (Papalian, Lafer, Wong & Stollar, 1980). However, although specific antibodies to native DNA are frequently found in SLE patients (Tan, Schur, Carr & Kunkel, 1966; Schur & Sandson, 1968; Hughes, Cohen & Christian, 1971; Koffler, Carr. Agnello, Thoburn & Kunkel, 1971), their induction in experimental animals has not yet been achieved. Further studies on naturally-occurring and experimentally-induced antibodies to poly(ADP-ribose) should provide useful information on the *in vivo* antigen–antibody system in SLE patients.

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