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BINDING OF DEOXYADENYLATE AND DEOXYCYTIDYLATE ANTIBODIES TO DOUBLE-STRANDED DNA

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SUMMARY : Antibodies raised against deoxyadenylate and deoxycytidylate were found to react with double stranded DNA as assessed by highly sensitive avidin-biotin microELISA. The binding was specific as it was completely inhibited by the homologous hapten. The antibodies did not react with tRNA and rRNA. These antibodies were also shown to react with supercoiled and relaxed forms of pBR322 DNA as demonstrated by gel retardation assay.

Antibodies to bases, nucleosides and nucleotides have been reported to react with ssDNA but not with dsDNA (1-3). It is generally accepted that bases are not accessible to antibodies in dsDNA. We report the presence of high titer of dsDNA-binding antibodies in antisera raised against dpA and dpC, using a highly sensitive AB microELISA. We have also studied the binding of these antibodies to plasmid DNA by gel retardation assay.

MATERIALS AND METHODS

Poly-L-lysine (MW 90,000), calf thymus DNA and deoxyribonucleotides were purchased from Sigma Chemical Co., St. Louis, USA. Avidin-HRP was from Vector Laboratories Inc., Burlingame, USA. Polyvinylchloride microtiter plates were from Becton-Dickinson, Oxnard, USA. Affinity purified goat anti-rabbit IgG was biotinylated using N-hydroxysucciniimide ester of biotin as described (4). tRNA was isolated from baker's yeast (5). rRNA (18S + 28S) was prepared from rat liver (6,7). pBR322 was isolated as described (8).

Antibodies : dpA and dpC were separately coupled to BSA by the carbodiimide method (9) and antibodies were raised in rabbits.

Abbreviations : ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; CT DNA, calf thymus DNA; AB microELISA, avidin-biotin microELISA; dpA, deoxyadenylate; dpC, deoxycytidylate; avidin-HRP, avidin-horseradish peroxidase.

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Avidin-biotin microELISA : In all cases additions were 100 µl/ml and incubations were carried out at 37°C. At the end of each incubation, plates were washed with PBS containing 0.05% Tween 20. ssDNA was prepared by boiling CT DNA solution for 10 min and chilling in ice-salt mixture. Non-specific sites were blocked by PBS containing 2% goat serum and 1 mM EDTA. The same blocking solution was used for dilution of normal rabbit serum, antisera, biotinylated goat anti-rabbit IgG and avidin-HRP. For measuring dsDNA-binding, the wells coated with native CT DNA were treated with S_1 nuclease (100 U/ml) in 50 mM Na acetate (pH 4.5) containing 50 mM NaCl, 1 mM $ZnSO_4$ and 5% glycerol for 1 h.

The microtiter plates were coated with poly-L-lysine (50 µg/ml in PBS) for 1 h following which tRNA, rRNA (10 µg/ml in PBS containing 0.2 M sodium sulphate) and native or denatured CT DNA (10 µg/ml in PBS containing 1 mM EDTA) was added and incubated for 1 h. After blocking the non-specific sites for 1 h, serially diluted antisera were added and incubated for 1 h. Biotinylated goat anti-rabbit IgG (1 µg/ml) was incubated for 30 min, followed by avidin-HRP (0.1 µg/ml) for 15 min. The substrate solution in sodium phosphate-citrate buffer (pH 5.0) containing o-phenylenediamine (1 mg/ml) and H_2O_2 (0.006%) was incubated at RT for 15 min. The reaction was terminated by addining 2 M HCl and absorbance at 490nm was measured using EIA reader. Control wells received PBS (buffer blank) or normal rabbit serum (NRS blank) in place of antiserum. The net absorbance values represent mean of triplicate determinations with SD less than 10% of the mean. For specificity analysis antiserum, diluted to give 50% of maximum binding, was preincubated with various concentrations of individual deoxyribonucleotides or DNA at RT for 30 min before adding to dsDNA coated plates. The experimental values were compared with the values obtained in absence of nucleotide/DNA during preincubation and expressed as % inhibition.

Gel retardation assay : It was carried out as described (10). IgG was prepared separately from dpA and dpC antiserum by sodium sulphate fractionation (11) and used as a source of antibodies. IgG prepared similarly from normal rabbit serum served as control. Antibodies (180 µg) and pBR322 (0.48 ug) were added to a solution containing 150 mM NaCl and 1 mM EDTA and incubated for 1 h at RT and electrophoresed on 0.7% agarose in Tris-borate-EDTA (pH 7.5) for 3 h at RT. Gel was stained by ethidium bromide (0.5 ug/ml) and photographed. In controls, assay was carried out in presence or absence of 180 ug of normal IgG.

RESULTS AND DISCUSSION

The binding of dpA and dpC antibodies to dsDNA was characterised using a highly sensitive AB microELISA. Any single-stranded regions on native CT DNA were removed by S_1 nuclease. Both the antibodies were found to react with dsDNA (Fig. 1 a,b). The titers of ssDNA and dsDNA-binding populations were comparable in both dpA and dpC antisera.

Antibodies raised against bases, nucleosides and nucleotides have been shown to react with ssDNA but not with dsDNA (1-3,9,12-14). It has been suggested that the inability of these antibodies to react with dsDNA is due to the inaccessibility of bases in dsDNA. However, low but definite binding of dpG and dpC antibodies to dsDNA has been shown using nitrocellulose filter binding assay (15). Our results also indicate the inability of dpA and dpC antibodies to react with tRNA and rRNA (Fig. 1 a,b).

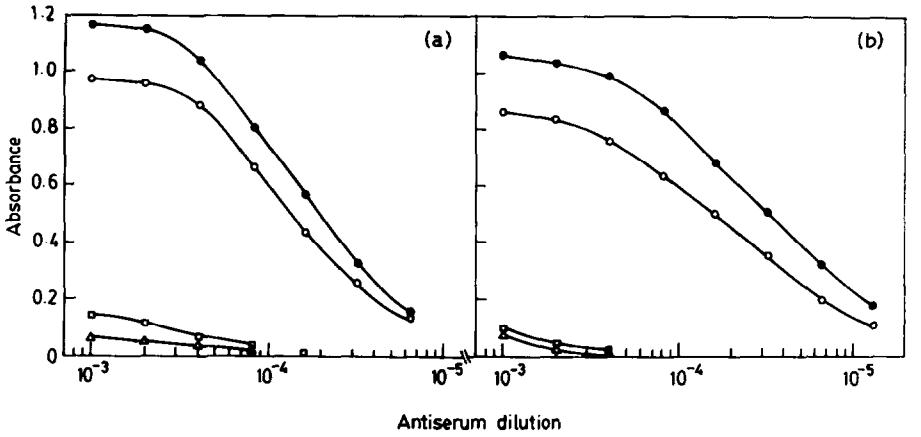


Figure 1. AB microELISA of dpA (a) and dpC (b) antisera. ssDNA (●), dsDNA (○), yeast tRNA (□) or rat liver rRNA (△) was adsorbed to poly-L-lysine coated plates and incubated with serial dilutions of antisera. Antibodies bound to nucleic acids were assayed using biotinylated goat anti-rabbit IgG and avidin-HRP. Net absorbance at 490 nm after subtracting NRS control values is plotted.

This inability was not due to RNA degradation because adenosine antibodies, under the same conditions, were found to react with tRNA, rRNA and poly $(A)^+$ RNA. Moreover, 0.2 M sodium sulphate was used to inhibit ribonuclease action (16). Since the sugar moiety of deoxyribonucleotides is not modified during coupling to carrier protein by the carbodiimide method, there is a possibility that the resultant antibodies are specific for deoxyribose sugar and hence do not react with RNA. dpC antibodies have been reported not to react with RNA (17).

The binding of antibodies to dsDNA was inhibited by the homologous hapten in a dose-dependent manner (Fig. 2 a,b). Other deoxyribonucleotides did not inhibit the binding demonstrating the nucleotide specificity of dsDNA-binding. The concentration of dpA and dpC required to completely inhibit the binding of the corresponding antibodies to dsDNA was 1×10^{-5}M and 1.5×10^{-4} M respectively. Both ssDNA and dsDNA inhibited the binding of dpA and dpC antibodies to dsDNA (Fig 3 a,b). Similarly, the binding of SLE antibodies to dsDNA has been reported to be inhibited by ssDNA as well as dsDNA (3).

The binding of antibodies to dsDNA was conclusively demonstrated using pBR322 DNA in gel retardation assay. Both antibodies were shown to bind supercoiled as well as relaxed forms of pBR322 DNA (Fig. 4, lane c and e). However, the extent of binding was higher with the supercoiled form as compared to the relaxed form. Normal rabbit IgG did not bind to plasmid DNA (Fig. 4, lane b). Plasmid-antibody complexes of heterogenous

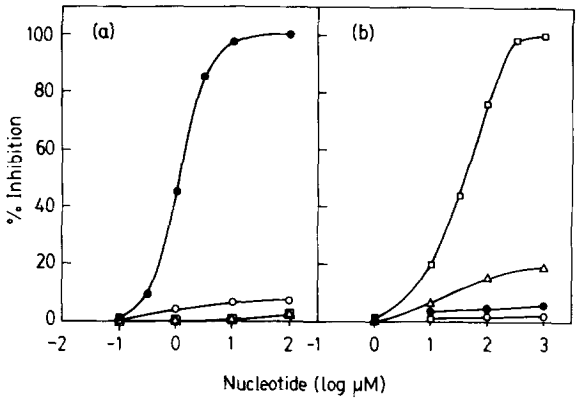
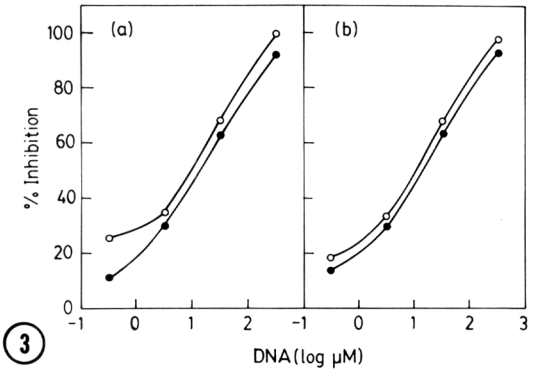


Figure 2. Hapten-specific binding of dpA and dpC antibodies to dsDNA. dpA antiserum (a) or dpC antiserum (b) diluted 1:12,000 and 1:20,000 respectively was preincubated with increasing concentrations of dpA (●), dpG (○), dpC (□) or dpT (△) for 30 min at RT. Aliquots were added to dsDNA coated plates and further steps were carried out as described in Fig. 1. The values are expressed as % inhibition. Absorbances obtained in absence of inhibitor were 0.561 ± 0.035 and 0.485 ± 0.028 for dpA and dpC antiserum respectively.



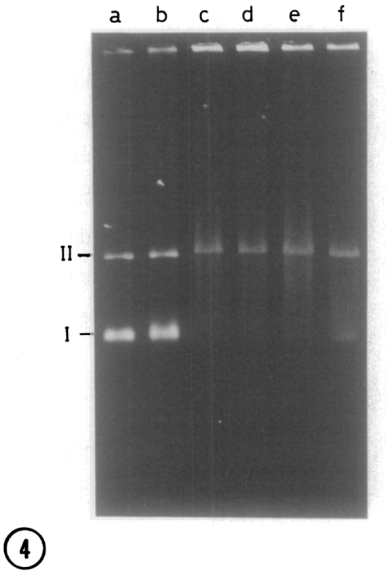


Figure 3. Inhibition of antibody binding to dsDNA by preincubation with ssDNA and dsDNA. The assay was carried out as described in Fig. 2 except that preincubation was with ssDNA (●) or dsDNA (○). Absorbances obtained in absence of inhibitor were 0.528 ± 0.031 and 0.489 ± 0.03 for dpA and dpC antiserum respectively. DNA concentration is expressed as molar concentration of nucleotide base pairs.

Figure 4. Binding of dpA and dpC antibodies to pBR322 DNA as analysed by gel retardation assay. pBR322 DNA was incubated with buffer (lane a), normal IgG (lane b), dpA antibodies (lanes c and d) or dpC antibodies (lanes e and f) for 1 h at RT and electrophoresed on 0.7% agarose. Gel was stained by ethidium bromide. Incubation mixture in case of lanes d and f contained 17 mM dpA and 34 mM dpC respectively. I, supercoiled form; II, relaxed form.

size could be seen in antibody treated samples (Fig. 4, lane c-f). High molecular weight complexes formed as a result of extensive cross-linking of plasmid DNA by antibodies could be seen at the origin. Specificity of binding was apparent as the homologous hapten, at high concentration, partially inhibited the binding of antibodies to pBR322 DNA (Fig. 4, lane d and f).

Antibodies to dsDNA are present in sera of some patients with SLE as well as in some strains of mice which spontaneously develop autoimmune disease (1-3). However, attempts to induce dsDNA-binding antibodies by immunisation with nucleic acids or their constituents have been unsuccessful, giving rise to the idea that it is not possible to induce these antibodies in experimental animals (2,3,18). Contrary to this view, the present study demonstrates that it is indeed possible to obtain dsDNA-binding antibodies through immunisation of experimental animals.

Though ELISA without avidin-biotin amplification has been used for assaying nucleic acid reactive antibodies (19-22), it does not appear to be as sensitive as the AB microELISA described in this paper. Nitrocellulose filter binding assay has been used very widely for studying binding of hapten-specific antibodies to nucleic acids (9,14,15,17). However, this assay has lower sensitivity and higher background, and requires individual radiolabelled nucleic acids. In addition, IgG fraction must be used in order to avoid non-specific binding of serum proteins to nucleic acids. In contrast, AB micro-ELISA has higher sensitivity and lower background, obviates the requirement of labelled nucleic acids and can be performed with antiserum. AB micro-ELISA as well as gel retardation assay should be of great help in characterising nucleic acid-reactive antibodies.

REFERENCES

1. Stollar, B.D. (1973) In Sela, M. (ed). The Antigens, Academic Press, New York, Vol. I, pp. 1-85.
2. Stollar, B.D. (1980) In Vunakins, H.V. and Langone, J.J. (eds), Methods in Enzymology, Academic Press, New York, Vol 70, pp. 70-85.
3. Stollar, B.D. (1986) CRC Crit. Rev. Biochem. 20, 1-36.
4. Guesdon, J.L., Ternynck, T. and Avrameas, S. (1979) J. Histochem. Cytochem. 27, 1131-1139.
5. Zubay, G. (1962) J. Mol. Biol. 4, 347-356.
6. Palmiter, R.D. (1974) Biochemistry 13, 3606-3615.
7. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA, 69, 1408-1412.
8. Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.
9. Humayun, M.Z. and Jacob, T.M. (1973) Biochim. Biophys. Acta 331, 41-53.
10. Garner, M.M. and Revzin, K. (1981) Nucleic Acids Res. 9, 3047-3060.
11. Kekwick, R.A. (1940) Biochem. J. 34, 1248-1256.
12. Erlanger, B.F. and Beiser, S.M. (1964) Proc. Natl. Acad. Sci. USA, 52, 68-74.

13. Drocourt, J.L. and Leng, M. (1975) Eur. J. Biochem. 56, 149-155.
14. Jayaraman, K. and Jacob, T.M. (1980) Mol. Immunol. 17, 769-775.
15. Jacob, A. and Jacob, T.M. (1982) Nucleic Acids Res. 10, 6273-6280.
16. Rosenberg, B.J., Erlanger, B.F. and Beiser, S.M. (1973) Biochemistry 12, 2191-2197.
17. Reddy, M.V. and Jacob, T.M. (1981) Curr. Sci. India, 50, 308-310.
18. Lacour, F., Nahon-Merlin, E. and Michelson, W. (1973) Curr. Top. Microbiol. Immunol. 62, 1-39.
19. Andrzejewski, Jr.C., Rauch, J., Lafer, E., Stollar, B.D. and Schwartz, R.S. (1981) J. Immunol. 126, 226-231.
20. Klotz, J.L. (1982) In Langone, J.J. and Vunakis, H.V. (eds) Methods in Enzymology, Academic Press, New York, Vol. 84, pp. 194-201.
21. Shoenfeld, Y., Rauch, J., Massicotte, H., Datta, S.K., Andre-Schwartz, J., Stollar, B.D. and Schwartz, R.S. (1983) N. Eng. J. Med. 308, 414-420.
22. Zouali, M. and Stollar, B.D. (1986) J. Immunol. Methods 90, 105-110.