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Specificity of Anti-Nucleoside Antibodies*

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The method of conjugation of a nucleoside or related compound to a carrier protein may have a significant effect on the specificity of the antibodies elicited. It is demonstrated, by means of the membrane-filtration assay, that anti-isopentenyladenosine antibodies produced by the 'periodate procedure' are much more reactive with the periodate-oxidized form of the nucleoside than with the parent compound. In addition, the simplicity and specificity of the assay used suggests its use as a sensitive radioimmunoassay for this multifunctional nucleoside.

Nucleic acid-reactive antibodies are of interest not only from the primary immunological point of view, but also because of their potential use in the areas of nucleic acid structure–function relationships and nucleic acid–protein recognition (Humayun & Jacob, 1973). Of the numerous methods used to elicit such antibodies, the 'periodate procedure' of Erlanger & Beiser (1964) has been the most extensively used for preparing anti-ribonucleoside and anti-ribonucleotide antibodies. In spite of the wide exploitation of this versatile method, the possible effect of the large sugar modification (introduced during the preparation of the immunogen) on the specificity of the resulting antibodies has not so far been given adequate attention. In a study of anti-deoxyadenylate antibodies produced by the method of Halloran & Parker (1966), we have shown that the sugar moiety of the nucleotide plays an important role in recognition by the antibodies (Humayun & Jacob, 1973). In the present communication, we give the results of a specificity analysis of antibodies against N^6-(Δ^2-isopentenyl)adenosine by the sensitive membrane-binding assay, showing the marked effect of 'periodate treatment' of the nucleoside on the specificity of the antibodies, as has been predicted (Humayun & Jacob, 1973).

Materials and methods

All nucleosides were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. Bovine serum albumin was a product of Miles Laboratories, Inc., Kankakee, Ill., U.S.A. Anti-isopentenyladenosine antibodies were raised in two rabbits by injecting an isopentenyladenosine–bovine albumin conjugate prepared by the 'periodate procedure' of Erlanger & Beiser (1964) and in a manner similar to that of Hacker *et al.* (1972). Injection and bleeding schedules, quantitative precipitin reactions, immunodiffusion in agar, measurement of proteins, nucleosides etc. and the membrane-filtration assay were described earlier (Humayun & Jacob, 1973). In the filtration assay, after the usual steps, an additional wash step with 5ml of buffer [0.01M-Tris–HCl (pH7.5)–0.14M-NaCl–0.02% NaN_3] was introduced. Periodate oxidation and borohydride reduction of nucleosides were done by the method of Khym (1963), the final products being purified by paper chromatography (Humayun & Jacob, 1973). dAMP–bovine albumin and dGMP–bovine albumin conjugates were prepared as described by Humayun & Jacob (1973). [^{3}H]Isopentenyladenosine was prepared by following the general procedures for nucleosides given by Randerath & Randerath (1969) which involve the periodate oxidation of the ribose moiety of nucleosides followed by [^{3}H]borohydride reduction. The particular preparation of [^{3}H]isopentenyladenosine used here had a specific radioactivity of 2000c.p.m./pmol under the experimental conditions (uncorrected for counting efficiency and other sources of error).

Results

Fig. 1 shows a representative quantitative precipitin curve given by an early antiserum. Analysis of the antiserum by immunodiffusion in agar showed that in spite of a very strong reaction with isopentenyladenosine–bovine albumin conjugate, no reaction was noticeable with bovine albumin itself (cf. Humayun & Jacob, 1973). However, this was not a constant phenomenon, as later bleedings, even from the same animal, showed some cross-reactivity with bovine albumin. The antibodies were able to cross-react with bovine albumin conjugates of dAMP and dGMP, although to a much weaker extent.

The specific binding of [^{3}H]isopentenyladenosine by an antiserum as measured by the membrane assay is given in Fig. 2(*a*) (inset). Normal rabbit serum did not bind any radioactivity above the blank.

* This is no. 3 of the series entitled 'Immunological studies on nucleic acids and their components'; no. 2 is by Humayun & Jacob (1974).

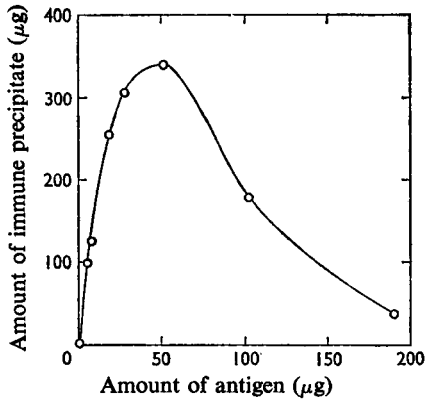

Fig. 1. *Quantitative precipitin reaction of an anti-isopentenyladenosine serum with isopentenyladenosine–bovine albumin conjugate*

Samples (25 μl) of the antiserum were used in a total volume of 0.5 ml.

Fig. 2(*b*) shows the effect of various nucleosides and their derivatives on the specific binding of [^{3}H]isopentenyladenosine by anti-isopentenyladenosine antibodies. The following points are readily discerned from the inhibition analysis. (a) The antibodies were highly specific for isopentenyladenosine. Of the four ribonucleosides tested, only adenosine showed some inhibition, although weak, whereas the others had no effect even at the highest concentration tested (cf. Hacker *et al.*, 1972). (b) Even though the antibodies showed a marked specificity for isopentenyladenosine, its trialcohol derivative (prepared by periodate oxidation followed by borohydride reduction of isopentenyladenosine) was a much more effective inhibitor. On the basis of 50% inhibition, the latter was 1000-fold more reactive. Although the inhibition by adenosine increased significantly after periodate treatment, it was not as pronounced as for isopentenyladenosine.

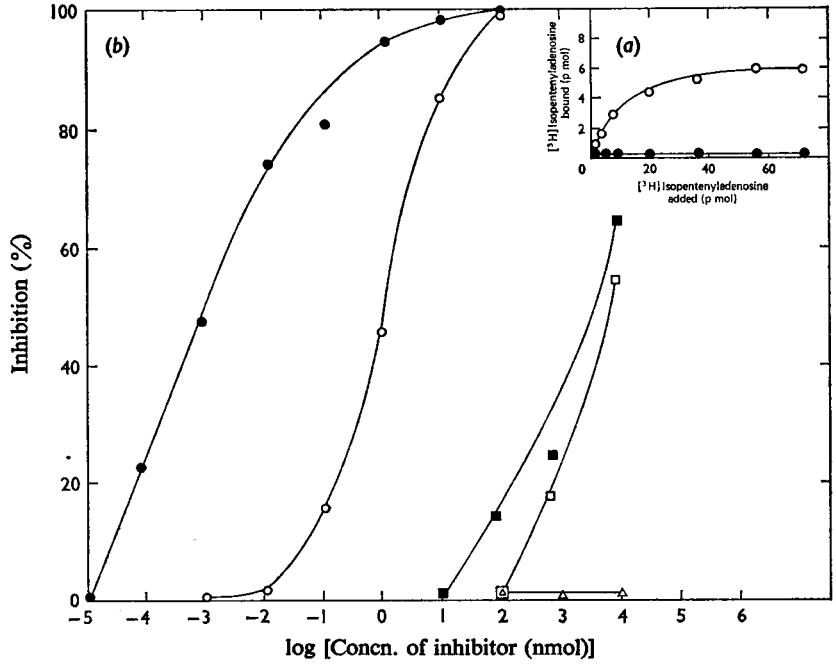

Fig. 2. *Specificity of anti-isopentenyladenosine antibodies*

(*a*) Specific binding of [^{3}H]isopentenyladenosine by an anti-isopentenyladenosine serum. The reaction mixtures contained 0.1 ml of a 1:500 diluted serum and 0.2 ml of Tris–HCl buffered saline, pH 7.5, containing the appropriate amount of [^{3}H]isopentenyladenosine. Blanks had no serum. Each value is the average of two experiments. ○, Anti-isopentenyladenosine serum; ●, normal rabbit serum. (*b*) Inhibition analysis of anti-isopentenyladenosine antibodies. The reaction mixtures contained 0.1 ml of 1:500 diluted serum, 0.1 ml of Tris–HCl buffered saline, pH 7.5, containing 3 pmol (6000 c.p.m.) of [^{3}H]isopentenyladenosine and 0.1 ml of inhibitor in buffer. Control (no inhibitor) bound 1.55 pmol (3100 c.p.m.) above a normal serum (1:500) blank, which bound 150 c.p.m. Each value represents the average of two experiments. ○, Isopentenyladenosine; □, adenosine; △, guanosine, cytidine, uridine; ●, 'periodate-treated' isopentenyladenosine; ■, 'periodate-treated' adenosine.

Discussion

Although the 'periodate procedure' has been widely used in preparing nucleic acid-reactive antibodies, the fact that the primary antibody specificity may be directed against the modified form of the hapten has not been given sufficient attention in the literature. From the results given in this communication, it is clear that the antibodies are primarily directed against the sugar-modified form of isopentenyladenosine rather than the nucleoside itself. It may be borne in mind that isopentenyladenosine is a hypermodified nucleoside, with the bulky isopentenyl substituent at the 6-amino group predictably playing a large determinant role in the antibody specificity. In spite of this the antibodies are able to distinguish clearly between the intact nucleoside and the periodate-oxidized form.

Finally, the inhibition analysis (Fig. 2*b*) suggests that this system can be used as a highly sensitive radioimmunoassay for isopentenyladenosine, and that the sensitivity of the assay will be greatly enhanced by giving suitable periodate treatment to the test material. A simple and sensitive assay for isopentenyladenosine is likely to be very useful in studies involving this interesting tRNA rare nucleoside and cytokinin, which is also reported to have a variety of other biological functions (see, e.g., Hall, 1970; Gallo *et al.*, 1972; Tritsch, 1973; Robins & Trip, 1973).

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