The Specific Reduction of a and d Antigenic Determinants after Periodate Treatment of Hepatitis B Surface Antigen (HBsAg)

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SUMMARY

HBsAg antigenicity was found to be sensitive to periodate treatment. Antigenic determinants a and d were especially sensitive, losing almost all of their activity, while determinants r and w were found to be quite stable. Also, it is now possible to prepare the monospecific antibodies, anti-r and anti-w, by the use of this new, simpler procedure.

Hepatitis B surface antigens (HBsAg) share a group-specific determinant a, which itself may contain several subspecificities. Two subtype determinant systems behave in an allelic manner, d/y and r/w (Le Bouvier, 1971; Bancroft, Mundon & Russell, 1972), so four subtypes can be recognized, i.e. adr, adw, ayr and ayw.

It is also known that the titre of HBsAg is reduced by some chemical treatments. The loss of HBsAg antigenicity by reduction with dithiothreitol (DTT) and full recovery by re-oxidation was first reported by Sukeno et al. (1972). Also Vyas, Rao & Ibrahim (1972) and Dressman et al. (1973) reported a loss in HBsAg antigenicity by DTT reduction. Later Imai et al. (1974a) succeeded in demonstrating the capacity of reduced HBsAg to react with human antibodies. This resulted in the discovery of the Re antigen. Burrell et al. (1973) reported a loss of more than 90% antigenicity after treatment with periodate but did not refer to any differences that occurred between the subtypes. No clear-cut evidence has been produced on the conformational aspects of subtype antigenicity. In the present paper the susceptibility of subtype antigenicity to periodate treatment is reported.

The purification of both HBsAg subtypes, adr and adw, were performed by a method previously reported by us (Shiraishi et al. 1977). Highly purified HBsAg (20 nm) of each subtype with a reversed passive haemagglutination (RPHA; Schuurs & Kačaki, 1974) titre of 1:64000 were obtained from 5 l of plasma donated by HBsAg-positive blood donors of both subtypes. It was shown that the preparations did not contain any human serum components by complement fixation tests with rabbit anti-human serum antibodies (Behring-Werke AG, Marburg-Lahn, Germany).

The antigenicity of HBsAg was monitored both by immunoelectrosyneresis and single radial immunodiffusion in the purification process and by RPHA at the time of periodate treatment. The passive haemagglutination inhibition test (Imai et al. 1974b) was used for the respective determination of subtypes a, d, r and w before and after periodate treatment. A polystyrene V-bottom microtitre plate was used for the serial dilutions of the test samples. A specified PHA buffer prepared by adding 10 g of sucrose and 10 ml of normal rabbit serum to 1 l of 0.01 M-phosphate buffer containing 0.15 M-NaCl, pH 7.2 (PBS), was used in this procedure. To each well was added 25 µl of PHA buffer containing 2 haemagglutination units of the monospecific antisera directed against the respective antigenic determinant to be tested. The plate was agitated for 30 s with a Micro-mixer and incubated to allow adsorption at 37 °C for 2 h. Then 25 µl of a 1% target cell suspension was added to each well, mixed, and incubated at 24 °C for 1 h to measure the residual PHA
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Fig. 1. (a) Effect of periodate treatment at 37 °C for 240 min on HBsAg antigenicities: ●, adr; ○, adw. (b) Effect of 0.05 M-periodate treatment at 37 °C on each antigenic determinant: ○, a determinant; ●, d determinant; ▲, r determinant; ■, w determinant.

titre of the respective monospecific antisera. The target cells were prepared by coating SRBC with HBsAg bearing adr and adw subtypes. The plates were observed, and the inhibition of haemagglutination was determined: haemagglutination indicated the absence of the antigenic determinant in the test sample, whereas no haemagglutination indicated its presence. For an antigen-positive sample, the highest dilution giving complete inhibition of haemagglutination was determined and the antigenic titre of sample was expressed as the reciprocal of this dilution. For the immunodiffusion test, 1.0 % agarose A-37 (L'Industrie Biologique Française, France), dissolved in 0.01 M-Veronal buffer (pH 8.6), was used.

The periodate treatment was carried out as follows. The purified HBsAg (20 to 100 μg) in PBS were treated with varying final concentrations of sodium periodate, 0.1 M, 0.05 M and 0.01 M. The reactions were performed in a water bath at 37 °C for 0, 30, 90 and 240 min, and stopped by the addition of 60 % sucrose solution. The solutions were immediately dialysed at 4 °C against PBS containing 0.1 % sucrose, and then assayed for HBsAg activity.

Subtypes adr and adw incubated in sodium periodate at 37 °C for 4 h were used to immunize 2 groups of guinea pigs, respectively. The preparations were emulsified with an equal volume of Freund's complete adjuvant (RW 606-1, Iatron Laboratories, Tokyo, Japan), and injected into the foot pads of adult guinea pigs (about 500 g). Portions of 10 to 50 μg of protein were used per animal per inoculation. One month later the animals were boosted with the same amount of antigen, and exsanguinated 8 days later. The serum was absorbed with a HBsAg-negative normal human serum pool rendered insoluble by cross-linking with glutaraldehyde as described by Avrameas & Ternynck (1969).

The results in Fig. 1(a) show that the higher the concentration of periodate, the lower the
Fig. 2. Immunodiffusion reactions of human anti-HBs with native HBsAg and NaIO₄-treated HBsAg.

The sensitivity of each antigenic determinant to 0.05 M-periodate was measured after incubation for 0, 30, 90 and 240 min (Fig. 1 b). Whereas the determinants a and d are very sensitive to periodate treatment, the determinants r and w are quite stable. There appears to be very little difference in sensitivity between r and w. Literal consideration suggests that among the various determinants of HBsAg, a is the major determinant, d is present in much smaller amounts and there is even less of w and r. However, the exact ratio of the respective determinants on the surface of HBsAg particles has not yet been thoroughly investigated.

The results of the immunodiffusion tests suggested a confirmation of these experimental results. The periodate treated HBsAg tested against human anti-HBs (adw) showed partial identity with the native HBsAg (Fig. 2).

Our next experiment was conducted to demonstrate the specific reactivity of w and r of the guinea pig sera obtained by immunization with periodate treated adw or adr antigens.
The PHA tests showed that when adw- or adr-coated SRBC were used as the antigens, specific reactivity was found in the respective sera. An absorption experiment with soluble adr and adw antigen was conducted. When anti-adw guinea pig serum (PHA titre 1:256) was absorbed with adr (37 °C for 1 h and 4 °C overnight) and tested with adw-coated SRBC, no decrease in titre could be detected. However, when this serum was absorbed with adw, complete loss of titre against adw-coated SRBC was found. Thus, a new procedure to obtain monospecific w or r serum by using periodate treated HBsAg had been established.

The fact that the periodate treatment lowers the titre of the HBsAg antigenicity suggests that it might act on the carbohydrate chain. Much data has been collected with regard to the presence of the carbohydrates contained within HBsAg (Chairez et al. 1973; Neurath, Prince & Lippin, 1973; Neurath et al. 1975; Shiraishi et al. 1977). However it is not clear how these carbohydrates really relate to the subtype antigenicities, a, d, r and w. Our results showing that the antigenicity of only subtypes a and d was remarkably reduced by periodate treatment are in agreement with the suggestion that the carbohydrates are necessary for the serological activities of HBsAg (Burrell et al. 1973). Recently, Burrell et al. (1976) reported that material of mol. wt. 5000 to 15000, which has high group-specific determinant a activity, contains carbohydrate. The stabilities of determinants r and w to the periodate treatment suggest that these do not have the carbohydrate chain associated with the antigenic sites and so are relatively resistant to periodate oxidation.

An alternative explanation might be the action of periodate on the tryptophan residue. It was reported that the tryptophan concentration in HBsAg is especially high, 13.9 % (Rao & Vyas, 1974) and 6.4 μmol per 100 μmol amino acid (Sukeno, Shiraishi & Ishida, 1975). It is also known that periodate acts readily with tryptophan, methionine and tyrosine in proteins (Atassi, 1967). In the light of this, the fact that periodate might interact with the tryptophan present in HBsAg should be considered.

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Department of Bacteriology, H. Shiraishi
Tohoku University School of Medicine, R. Shiraichi
Seiryomachi Sendai, Japan N. Ishida
Virology Division, T. Sekine
National Cancer Center Research Institute,
Tsukiji, Chuo-ku,
Tokyo, Japan

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