Hepatitis B Surface Antigen (HBs Ag): tryptophan Content and Biological Activity

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SUMMARY

The tryptophan content of hepatitis B surface antigen (HBsAg) was studied by spectrophotometric titration with N-bromosuccinimide (NBS) at pH 4.0 in 8.0 M-urea. A tryptophan value of 13.9 % or 22.4 residues/mol (mol. wt. = 30000) was obtained by this method. This value coupled with 2-3 mol per cent tyrosine content obtained by amino acid composition studies accounts for the unusually high extinction coefficient of HBsAg (at 280 nm $E_{1\text{cm}}^{1\%} = 37.26$). The modification of tryptophan residues with NBS did not cause cleavage of the protein or loss of biologic activity as determined by radioimmunoassay.

Hepatitis B surface antigen (HBsAg) (HBsAg; Nomenclature, 1974) occurs as 20 nm virus-like particles in human serum and is remarkably stable to a variety of physicochemical treatments (Blumberg, Alter & Visnich, 1965; Shulman, 1970; Krugman & Giles, 1972; Zuckerman, 1972). It is particularly resistant to treatment with proteolytic enzymes such as pepsin, trypsin, chymotrypsin and pronase (Kim & Bissel, 1971; Millman, Loeb & Bayer, 1970). Purified antigen has the unusually high extinction coefficient at 280 nm of $E_{1\text{cm}}^{1\%} = 37.26$ (Vyas et al. 1972; Gerin & Vyas, 1972) indicating a high molar concentration of tyrosine and tryptophan. Continuous scanning of the purified HBsAg revealed a shoulder at 290 nm (Gerin & Vyas, 1972). Amino acid analysis has revealed only 2-3 mol percent tyrosine (Vyas et al. 1972). Tyrosine and tryptophan ratio estimated by the method of Gaitonde & Dorey (1970) did not yield values to account for the high extinction coefficient (Vyas, Rao & Ibrahim, 1972). Therefore, we have estimated the tryptophan content by spectrophotometric titration with N-bromosuccinimide (NBS) according to the method of Patchornik, Lawson & Witkop (1958) as described by Spande & Witkop (1967).

Hepatitis B antigen of subtype $a\delta$ was purified from the plasma of a healthy carrier by a combination of isopycnic banding and differential sedimentation on caesium chloride gradients (Vyas et al. 1972). The purified antigen was devoid of detectable human plasma proteins as determined by gel-diffusion analysis with antiserum to normal human serum. The antigen thus prepared was extensively dialysed against saline.

A known amount of the purified protein in saline with $E_{280} = 1.1$ was frozen and lyophilized. The sample was then dissolved in 0.5 ml of 8.0 M-urea at pH 4.0 (adjusted with acetic acid) and scanned from 250 to 400 nm on a Zeiss Spectrophotometer. To the constantly stirred 0.5 ml HBsAg solution, 10 µl samples of 10 mM-NBS solution were added at intervals of 15 min. The $E_{280}$ was recorded each time until there was no further change. The sample was scanned once again from 250 to 400 nm (Fig. 1). A decrease in $E_{280}$ was also observed in 8.0 M-urea with 0.15 M-NaCl at pH 4.0 in comparison with the spectrum of an identical amount of HBsAg in saline. Corrections for this effect and the dilution produced by addition of NBS were applied in calculating the tryptophan content. Tryptophan content revealed by this method was 13.9 % or 22.4 residues/mol of protein based on a mol. wt. of 30000 used in the calculation. The value of tryptophan derived
Fig. 1. The extinction spectra of hepatitis B surface antigen in 8.0 M-urea at pH 4.0, before and after treatment with Y-bromosuccinimide (NBS). The protein was manually scanned on a Zeiss Spectrophotometer. N-bromosuccinimide was added in lots of 10 µl at 15 min intervals. The sample was scanned once again when no further change in extinction occurred. (●—●), spectrum before NBS treatment; (○—○), spectrum after NBS treatment.

Table 1. Effect of N-bromosuccinimide (NBS) treatment on hepatitis B surface antigen*

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Radioactivity (ct/min)</th>
<th>Negative control</th>
<th>Untreated</th>
<th>NBS-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>400</td>
<td>7330</td>
<td>7324</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td></td>
<td>7240</td>
<td>7049</td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td></td>
<td>7128</td>
<td>6980</td>
<td></td>
</tr>
<tr>
<td>1:8</td>
<td></td>
<td>7100</td>
<td>6670</td>
<td></td>
</tr>
<tr>
<td>1:16</td>
<td></td>
<td>5550</td>
<td>3500</td>
<td></td>
</tr>
</tbody>
</table>

* Conditions of NBS treatment are described in the test. Untreated sample was subjected to conditions identical with the treated sample except for NBS treatment. Amount of protein was the same in treated and untreated samples. Radioimmunoassay was carried out according to instructions supplied with the kits (Abbott Laboratories, AUSTRIA™). From this investigation coupled with the previously determined tyrosine content accounted well for the molar extinction coefficient of the protein. Reduction of the protein with β-mercaptoethanol followed by alkylation according to the method previously described (Vyas, Rao & Ibrahim, 1972) did not yield increased tryptophan content on titration with NBS, indicating that most of the tryptophan residues are available for titration with NBS at pH 4.0 in 8.0 M-urea. No decrease in $E_{280}$ was observed after dialysis of the NBS treated HBsAg, indicating no detectable cleavage of protein by NBS into dialysable peptides. When twofold dilutions of HBsAg, with and without NBS treatment, were tested by radioimmunoassay (Abbott Laboratories, AUSTRIA™ P25), no significant loss of serologic activity was observed as a result of the NBS treatment (Table 1). From the
foregoing observations it can be concluded that the high extinction coefficient is accounted
for by the high tryptophan content of the protein moiety. Unusually large hydrophobic
forces due to high tryptophan content could render the protein highly resistant to a variety
of physiochemical treatment and inaccessible to enzymic cleavage. However, the serologic
activity of the HB~Ag protein does not appear to depend on the tryptophan residues.

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