# Analysis of Glycopeptides by Fast Atom Bombardment Mass Spectrometry in Relation to their Hydrophobicity

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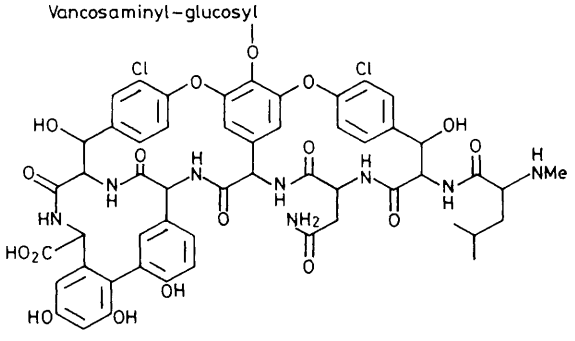
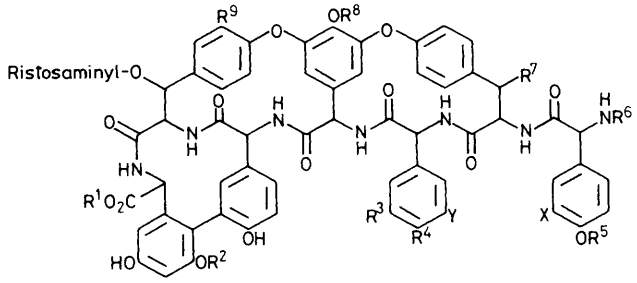
A high sugar content in a glycopeptide can result in a reduction in intensity, or suppression, of its fast atom bombardment mass spectrum; the problem can be alleviated by analysing a more hydrophobic derivative.

Fast atom bombardment mass spectrometry (f.a.b.–m.s.)[1] has proved to be extremely useful in the analysis of relatively polar and high molecular weight molecules. It has been shown that surface active molecules give a good signal response;[2,3] this is because the beam of fast xenon atoms penetrates only *ca.* 10 nm into the matrix (*e.g.* glycerol) and sputtering into the gas phase only occurs from the surface region. More recently, it has been shown that an increase in the hydrophobicity of the molecule being analysed can increase the signal response;[4,5] and more specifically, result in suppression of signals from hydrophilic peptides in the presence of hydrophobic peptides.[5] We now show that analogous considerations are important in the analysis of glycopeptides.

The glycopeptide antibiotics vancomycin (**1**), ristocetin A (**2**), actaplanin A (**3**), and β-avoparcin (**4**) were subjected to f.a.b. analysis. In each case machine conditions (8 keV xenon




(**1**) Vancomycin
(**5**) Partial aglycone: vancosamine removed
(**6**) Total aglycone




| | Ristocetin A (**2**) | Actaplanin A (**3**) | β-Avoparcin (**4**) |
|---|---|---|---|
| $R^1$ | Me | Me | H |
| $R^2$ | Mannosyl | Mannosyl | H |
| $R^3$ | OH | *O*-Mannosyl | Cl |
| $R^4$ | Me | Me | OH |
| $R^5$ | H | H | Rhamnoyl |
| $R^6$ | H | H | Me |
| $R^7$ | OH | H | *O*-Mannosyl |
| $R^8$ | Arabinosyl–mannosyl–(rhamnosyl)–glucosyl | Mannosyl–glucosyl | Ristosaminyl–glucosyl |
| $R^9$ | H | Cl | Cl |
| X, Y | coupled together through oxygen | | Both H |

(**7**) Ristocetin partial aglycone, $R^8 = R^2 = H$
(**8**) Ristocetin total aglycone: ristosaminyl also removed

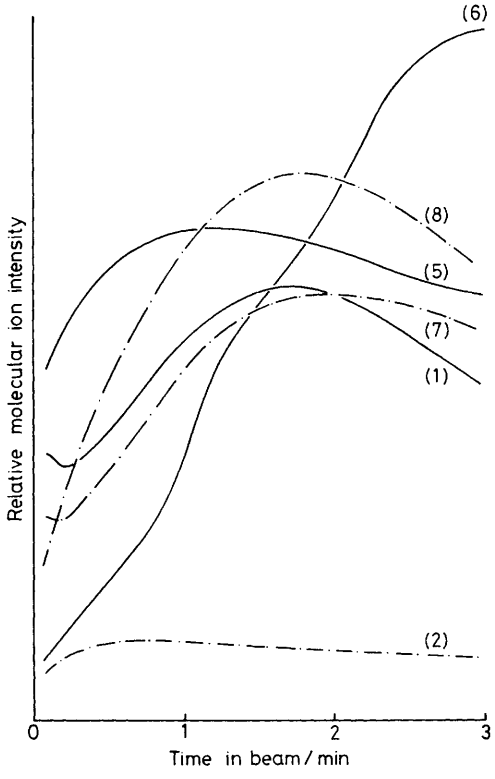



**Figure 1.** Relative abundances of $MH^+$ signals from vancomycin (**1**), ristocetin A (**2**), and some of their hydrolysis products (all 12 nmol samples in 2 μl of thioglycerol–glycerol (1 : 1) as a function of time.

atoms) and sample size [9 nmol in 2 μl of thioglycerol–glycerol (1 : 1)][6] were identical. After 2 minutes in the xenon beam, the $MH^+$ abundance from vancomycin (2 sugar units) was 8 times greater than that due to β-avoparcin (5 sugar units), and 5 times greater than that from actaplanin A (5 sugar units) and ristocetin A (6 sugar units). Based on our previous work involving peptides,[5] we propose that this effect is due to the more hydrophilic nature of the three antibiotics containing five or six sugars; there will be discrimination against such molecules occupying the surface of the matrix. To test this hypothesis, an equimolar mixture (12 nmol) of vancomycin (**1**) and ristocetin A (**2**) was analysed by f.a.b.–m.s. in the same matrix as above. During a period of 5 minutes of xenon bombardment, a signal due to $MH^+$ of ristocetin A was not observed, whereas the vancomycin $MH^+$ signal was clearly visible. We conclude that the more hydrophilic ristocetin A molecules are unable to compete effectively for the matrix surface with the more hydrophobic vancomycin molecules.

In order to verify the importance of the sugars in determining the f.a.b. signal response of the antibiotics, we have partially, and totally removed the sugars from vancomycin (**1**) and ristocetin A (**2**). The relevant data are reproduced in Figure 1. The most dramatic increase in intensity (~6 fold) was observed on going from ristocetin A (**2**) to ristocetin aglycone (**8**) (a loss of 6 sugar residues). There is a clear correlation between the number of sugars present and the relative $MH^+$ abundances.

Currently, we have no experimentally supported hypothesis to explain the relatively slow initial rise in the $MH^+$ signal due to the vancomycin aglycone (Figure 1).

In conclusion it can be seen that the presence of large numbers of sugar residues can suppress the signal intensity of glycopeptides in f.a.b.–m.s. In principle, this problem may be solved by (i) use of a more hydrophilic matrix or (ii) preparation of a derivative of suitable hydrophobicity while using the same or a similar matrix. The latter can often be achieved without much difficulty. The former is more desirable, but may be more difficult to achieve in practice; molecules with suitable low volatility and solvent power but greater hydrophilicity than glycerol do not appear to be numerous. Finally, we note that the potential of f.a.b. mass spectrometry in structural studies on glycopeptides derived from proteins is of great importance.[7] Our data indicate that, in an enzymic digest of such glycopeptides, signals due to

peptides carrying sugars may be partially or totally suppressed by more hydrophobic peptides. One solution is to study the saccharide portion *via* permethylation[8] or acetylation.[9]

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