Reduction of Azo-group-containing Peptides in Fast Atom Bombardment Mass Spectrometry

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Azo-group-containing peptides in glycerol are reduced on bombardment with a xenon beam generated in a discharge source but not on bombardment with thermionically produced Cs^+ ions; it is suggested that the reduction is due to short-wavelength radiation from the discharge source.

In fast atom bombardment (f.a.b.) mass spectrometry of organic compounds chemical reactions such as hydrogenation, dehalogenation, and transamidation have been reported to be induced by the incident particle beam.[1—5] In these experiments the samples were dissolved in glycerol or another suitable solvent (matrix) and exposed to a xenon beam of several keV, produced by a discharge source. Among such 'artefact' reactions, reduction of disulphide bonds and azo groups has been observed, leading to the formation of $(M + nH)^+$ ions with $n \geq 2$. It has been shown recently for some organic azo dyes that such reduction effects are weak or absent in secondary ion mass spectra obtained from solid samples.[6] In this communication we present results showing that the reduction of azo groups in f.a.b. mass spectrometry is specifically related to properties of the incident particle source.

A pronounced reduction effect was observed in f.a.b. spectra of azo-group-containing peptide derivatives. For example, the spectra of Pz-Pro-Leu-Gly-Pro-Arg (Pz = 4-phenylazobenzyloxycarbonyl) showed a strong increase in abundance of the $(M + 3H)^+$ ion and a decrease in that of the $(M + H)^+$ ion as a function of time during bombardment of a glycerol solution with a xenon particle beam produced in a saddle-field discharge source.[7] The time dependence of the intensity ratio $I(M + 3H)^+/I(M + H)^+$ (Figure 1) shows that the reduction proceeds gradually with bombardment time and thus can be attributed to a chemical conversion in the bulk of

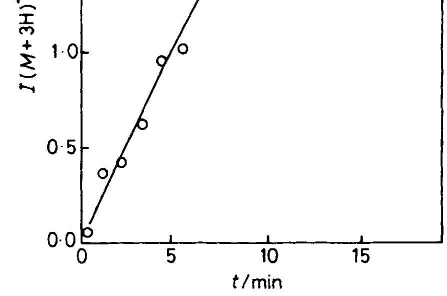

Figure 1. Time dependence of the intensity ratio $I(M + 3H)^+/I(M + H)^+$ of Pz-Pro-Leu-Gly-Pro-Arg during Xe-f.a.b. mass spectrometry of a glycerol solution. The xenon particle beam (current density ≃5 μA cm^{-2}) was produced in a saddle-field discharge source operated at 6 kV. Further experimental details are reported in ref. 8.

the sample solution only and not to the effect of single events in the surface layer. Accordingly, the yellow colour of the liquid layer gradually faded. The same result was obtained for several Pz–peptide derivatives. In addition to the reduction, it was found that during bombardment the molecular ion signals disappeared into the chemical noise, although a (colourless) liquid layer containing glycerol was still left on the probe surface,[8] indicating a strong depletion of the peptide in the remaining solution.

By contrast, in experiments applying a thermionically generated incident 5 keV Cs^+ particle beam[9] of about the same current density at the target as in the experiments with xenon, no change in the isotope patterns of the molecular ion signal, *i.e.* no reduction of the azo groups of the peptides, was observed during prolonged bombardment, either in the positive or in the negative secondary ion emission mode. Accordingly, a plot of the intensity ratio $I(M + 3H)^+/I(M + H)^+$ as function of time was a straight line at zero level. Furthermore, the molecular ion signal could be observed up to complete consumption of the glycerol matrix.

This surprising result strongly indicates a specific difference in radiation chemistry caused by the two incident particle beams. The reduction in the bulk of the solution observed with xenon is most probably not the effect of electrons generated in the collision cascade, because the penetration depth of low-energy electrons is very small and they should be trapped in solution within a few nm. An effect due to electrons supplied from outside onto the layer surface can be excluded because the reduction by xenon bombardment was found to be independent of the potential applied to the target, *i.e.* it was also observed under conditions of negative ion emission.

We believe that the reduction and the subsequent decomposition of the peptides are caused by irradiation of the sample solution with short-wavelength u.v. and *X*-rays under bombardment conditions. Radiation sources are the saddle-field discharge source and the xenon atom beam composed of neutral species, ions in various charge states,[10] and excited particles. *X*-Ray emission, for example, is possible as a result of electron capture of multiply charged ions impinging on the target. The penetration depth of the short-wavelength radiation should be in the range of about a hundred to several thousand nm, and thus would be consistent with the observed time course of the reduction in the bulk of the solution.

The radiative properties of incident particle discharge sources have not yet been evaluated. The resulting radiation chemistry may be particularly important for the use of f.a.b. in analytical applications. The question as to whether reported f.a.b.-induced 'artefact' reactions are the result of such effects should therefore be investigated by comparison of mass spectra obtained with xenon discharge *versus* those obtained with Cs^+ thermionic sources.

We are grateful to the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung und Technologie for financial support.

Received, 10th April 1987; Com. 475

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