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PLASMA DESORPTION MASS SPECTROMETRY : THE STABILITY OF MOLECULAR IONS

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Resume

Les largeurs de pics et les intensités relatives des ions moléculaires mono et multi-chargés apparaissant dans les spectres de masse par plasma désorption sont utilisées comme une sonde pour la stabilité des ions moléculaires. Deux peptides qui ont des poids moléculaires et des structures tertiaires identiques mais des structures primaires et des points iso-électriques différents ont été préparés en solutions de pH divers avant d'être adsorbés sur nitrocellulose. Les largeurs de pics des ions moléculaires résultats ont été optimisées près de leurs points iso-électriques respectifs. Des études similaires ont été entreprises en ajoutant du glutathion, une matrice qui a des effets sur les spectres PDMS équivalents à la nitrocellulose.

Abstract - The peak widths and relative intensities of the singly- and multiply-charged molecular ions appearing in plasma desorption mass spectra are used as a probe for the stability of molecular ions. Two peptides, lysozyme and lactalbumin, which have similar molecular weights and tertiary structures, but different primary structures and isoelectric points, were prepared in solutions of different pH, before adsorption to nitrocellulose foils. The peak widths of the resultant molecular ion signals were minimized near their respective isoelectric points, which is consistent with reports on the stability of their tertiary structures and/or enzymatic activities near the isoelectric point. Similar studies were carried out with the addition of glutathione, a matrix which has effects on PDMS spectra similar to that of the nitrocellulose surface.

1 - INTRODUCTION

During the last several years, a number of new mass spectral ionization techniques have been developed, which employ highly energetic photon and particle beams. These include: laser desorption /1/, secondary ion mass spectrometry /2/, fast atom bombardment /3/ and plasma desorption mass spectrometry /4/. These techniques are of considerable interest to biochemists, because (despite the high primary energies) they have enabled the desorption of intact molecular ions of complex biomolecules and their subsequent mass analysis by mass spectrometry. In addition, the absence of substantial and informative gas phase fragmentation of molecules above 5000 daltons suggests that such ions are formed with unusual stability and with very little internal energy.

In the particle techniques, primary ions have been used in both the kilovolt and megavolt region. The latter were first employed for biomolecular analysis by Macfarlane and Torgerson /4/, who recorded their mass spectra using a time-of-flight analyzer. While the major coherent ion signal for large peptides is observed as the molecular ion peak, considerable fragmentation does occur in the accelerating and drift regions of the time-of-flight analyzer, but contributes primarily to the incoherent baseline and the peak width /5-8/, and is largely due to the loss of small peripheral groups /5,9,10/.

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In the PDMS technique, samples are deposited on a thin aluminum or aluminized mylar foil by electrospraying acidic solutions of the sample /11/. Heavy fragments from the spontaneous fission of Cf-252 penetrate the foil and desorb secondary ions, whose masses are measured by their flight times after acceleration. Recently, two alternative approaches to the simple electrospraying technique have been developed. These include the addition of the tripeptide glutathione to the acidic solution used to electrospray the sample /12/ and the use of nitrocellulose surfaces for adsorption of the sample /13/. What is remarkable is that they have identical effects on the mass spectra: an increase in the molecular ion signal relative to the incoherent baseline, an increase in the abundance of multiply-charged ions, and a significant decrease in the spectral peak widths /12,14,15/. In both cases, it has been suggested that such features reflect a lowering of the binding energy between sample and substrate /12,15/. Additionally, Alai, et. al. /12/ have noted the use of glutathione in the refolding of lysozyme /16/, and suggested that folded peptides are more loosely bound to the surface and are desorbed with lower internal energy. Roepstorff, et. al. /17/ have also suggested that the use of nitrocellulose surfaces or the glutathione matrix results in the desorption of "cooler" molecular ions, while Chait /18/ has shown that molecular ions of porcine insulin desorbed from nitrocellulose undergo less metastable fragmentation than those from electrospayed foils.

In this study, two peptides: bovine milk \( \alpha \)-lactalbumin and chicken egg white lysozyme, with similar molecular weights (14,176 and 14,307 amu respectively), similar tertiary structures, but different isoelectric points (\( \text{pI} = 5 \) and 11 respectively), are compared. In both cases, the isoelectric point can be correlated with biological activity and stable conformations. Davies and coworkers /19/ have studied the enzymatic and lytic activity of lysozyme as a function of the ionic strength of solutions of the peptide at different pH. At low ionic strength, maximum enzymatic activity occurs at a pH of 9.2, while lytic activity is very high above pH 10. Saint-Blancard, et. al. /20/ have confirmed the lytic activity at high pH, and suggested that the isoelectric point represents a very stable conformational structure. For lactalbumin, Hanssens et. al. /21/ have used fluorescence measurements to determine that maximum stabilization of conformation occurs in the range of pH 5-4, near the isoelectric point. Thus, we examined both the abundances of multiply charged ions and the peak widths in the mass spectra of lysozyme and lactalbumin, which were prepared in solutions of different pH prior to adsorption on nitrocellulose. Additionally, since the peptide glutathione has also been reported as a refolding reagent /16/, we measured abundances and peak widths as well following the addition of increasing amounts of glutathione to the sample solution.

2 - EXPERIMENTAL METHODS

Mass spectral measurements were made using a BIO-ION Nordic (Uppsala, Sweden) BIN 10K plasma desorption mass spectrometer, equipped with a 10 uCi source of Cf-252. Data were acquired on a PDP 11/73 microcomputer, with a time resolution of 1 ns/channel. Chicken egg white lysozyme and bovine milk \( \alpha \)-lactalbumin were purchased from Sigma Co. In the first experiment samples were dissolved in aqueous solutions of acetic acid and ammonium hydroxide at different concentrations to achieve a range of pH from 1 to 11, deposited on nitrocellulose-coated foils, and analyzed after 6-8 hours. In the second experiment, 100 ug of peptide was dissolved in solutions of glacial acetic acid containing various concentrations of glutathione, and then electrospayed onto aluminized mylar foils.

3 - RESULTS AND DISCUSSION

Figure 1 shows the peak widths of the singly-, doubly- and triply-charged molecular ions of lactalbumin deposited on nitrocellulose as a function of pH. Near the isoelectric point (\( \text{pI} = 5 \)) the peak widths reach a minimum, while the molecular ion intensities and the ratio of higher charged species (data not shown) reach a maximum. Figure 2 shows the results from a similar experiment with lysozyme (\( \text{pI} = 11 \)) where the minimum peak widths are achieved at very high pH.

When lysozyme and lactalbumin are "titrated" with glutathione (data not shown) molecular ion intensities and charge states increase, while peak widths decrease until the molar ratio (glutathione/peptide) is about 20 to 30, after which these parameters remain fairly constant /22/. In both cases, a very clear "endpoint" is observed. The decreased peak widths for lysozyme (70-80ns) and lactalbumin (100-120ns) are comparable to the minima in the nitrocellulose experiments.
Figure 1. Peak widths of the singly-, doubly- and triply-charged molecular ions of lactalbumin as a function of pH.

Figure 2. Peak widths of the singly-, doubly- and triply-charged molecular ions of lysozyme as a function of pH.
4 - CONCLUSIONS

Interactions between peptides and nitrocellulose are hydrophobic /23,24/, with the hydrophobic surfaces located primarily inside the native protein /25/. The binding of peptides to nitrocellulose increases with time, which is reflected in increasing peak widths in the mass spectra when the sample is analyzed several hours after adsorption. In this case, the 6-8 hour "incubation" period serves to "amplify" the effects of the pH of the solution before the peptide was adsorbed. Since the effects of pH on the conformational structure of these two proteins has been well documented /19-21/, it is clear that the peak widths reflect the tertiary structure. In the case of the nitrocellulose surface, partial unfolding at extreme pH may accelerate exposure of the hydrophobic residues to the surface and increase binding. On the other hand, the glutathione matrix, we suspect, serves to preserve the native structures through the electrospray process, and thus achieves the same effect.

5 - ACKNOWLEDGEMENTS

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