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Collision efficiency in an electrospray source interfaced with a magnetic mass spectrometer

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Dedicated to Dr. Yannik Hoppilliard on the occasion of her 60th birthday.

Abstract

The collision efficiency in two distinct areas of an electrospray ion source has been compared on the basis of diagnostic ion intensities related to the voltages applied on three different lenses. The dissociation of covalent or non-covalent bonds and the shift of the charge state distribution of multiple-charged protein ions were used as indicators of the internal energy amount acquired by collisional activation. The results show that, for a given voltage difference between two successive lenses on the ion beam axis, the more efficient collision-induced dissociation processes are observed after the skimmer and not before. Although the enhancement of the fragmentation rate by increasing the skimmer voltage could be accompanied by a loss of sensitivity, this procedure should be of interest for analytical purposes. (Int J Mass Spectrom 219 (2002) 403–408)
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Keywords: Electrospray; Collisional activation

1. Introduction

Electrospray ionization (ESI) is now widely used for studying both biological macromolecules and organic compounds extracted from natural sources or produced by chemical synthesis. An interesting feature of ESI is the collisional activation of ions at the source interface thereby providing valuable structural information [1–4].

In the course of technological developments by the pioneering research laboratories and instrument manufacturers, various expressions such as "in-source collision-induced dissociation," "nozzle-skimmer fragmentation," "cone voltage fragmentation" or "orifice fragmentation" have appeared in the literature [5]. These different terminological variations reflect the general concept of using a differential potential between the first sampling lens (in this work the "sampling cone") and the skimmer lens in order to transfer some internal energy to the ions generated by electrospray. By adjusting the voltage of the sampling cone, the ion kinetic energy can be enhanced and the low-energy collisions against the residual gas present at the ion source interface increase the internal energy deposition on the ions.

In a source configuration with a double-stage differential pumping, which now seems to be the standard design, it is generally assumed that the efficient area for the collision process is in front of the skimmer lens. However, Smith et al. [6] showed that collision-induced dissociation could occur in a second region, localized between the skimmer and a focusing

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hexapole lens, and could be enhanced by increasing the skimmer voltage. In this study, a TAGA 6000E triple quadruple mass spectrometer (Sciex) was used, fitted with a modified interface where a second differential pumping stage was added. The authors' conclusion regarding this aspect of their study was based on two considerations. By increasing the voltage between the skimmer and the hexapolar lens, they noted first a decrease of the ion transmission and, secondly, a higher rate of metastable dissociation due to an increase of the average internal energy of ions.

The design of a double differential pumping stage emerged from works related to the coupling of ESI sources to magnetic mass spectrometers in order to avoid the corona discharge phenomenon [7]. It is noteworthy that in a magnetic instrument, the skimmer lens is generally used as reference for the accelerating voltage. In the case of the Micromass "pepper pot" ion source (Fig. 1), it is possible to modify the skimmer potential by adjusting the value of a potentiometer called "Ion Energy." The kinetic energy of ions between skimmer and hexapole can thus be controlled by the operator. This possibility opens the way for investigating comparatively the collision efficiencies in the standard collision area (in front of the skimmer lens) and in a second one (between skimmer and hexapole). This is the subject of the present paper.

2. Experimental

All experiments were carried out by using a Micromass ZabSpec-T tandem magnetic mass spectrometer (Manchester, UK) fitted with an electrospray "pepper pot" ion source. The voltage difference between the sampling cone and the skimmer ($V_{sc} - V_s$) ranges from 17 to 257 V and from −11 to +69 V between the skimmer and the hexapole lens ($V_s - V_h$).

The analysis were carried out with a solution of horse heart myoglobin (Sigma) under denaturing solvent conditions (10 μM in water/methanol/acetic acid, 49:49:1, v/v/v). This apoprotein solution was injected into the mass spectrometer at a flow rate of 5 μL min^{-1} using a syringe pump (Harvard apparatus model 22). Another sample solution was prepared by dissolving holomyoglobin in 20 mM aqueous ammonium bicarbonate buffer (10 μM, pH 7.9), the other

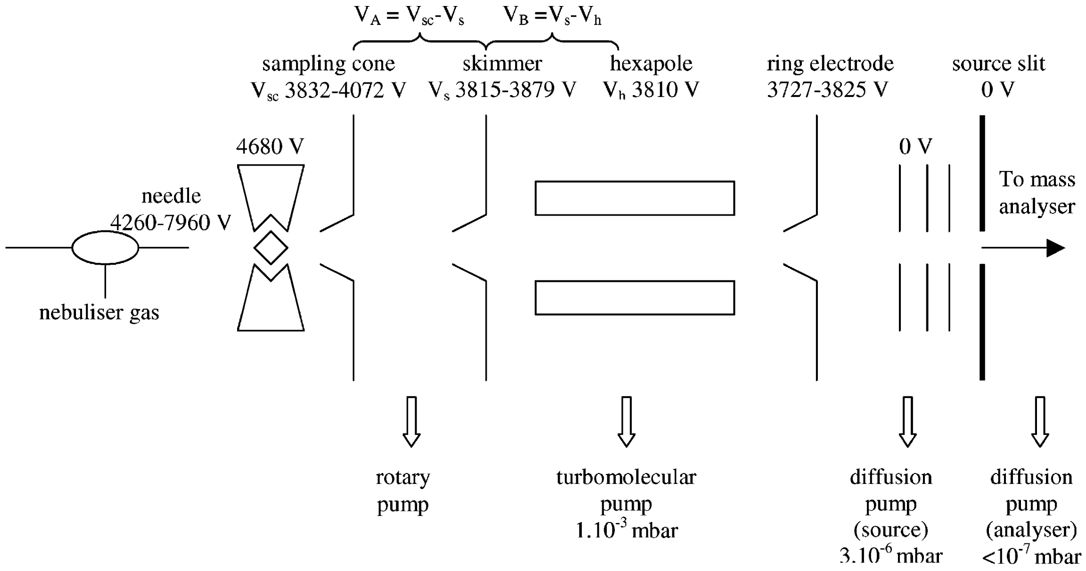

Fig. 1. Scheme of the Micromass "pepper pot" electrospray ion source fitted on a ZabSpec-T mass spectrometer (Micromass, Manchester, UK).

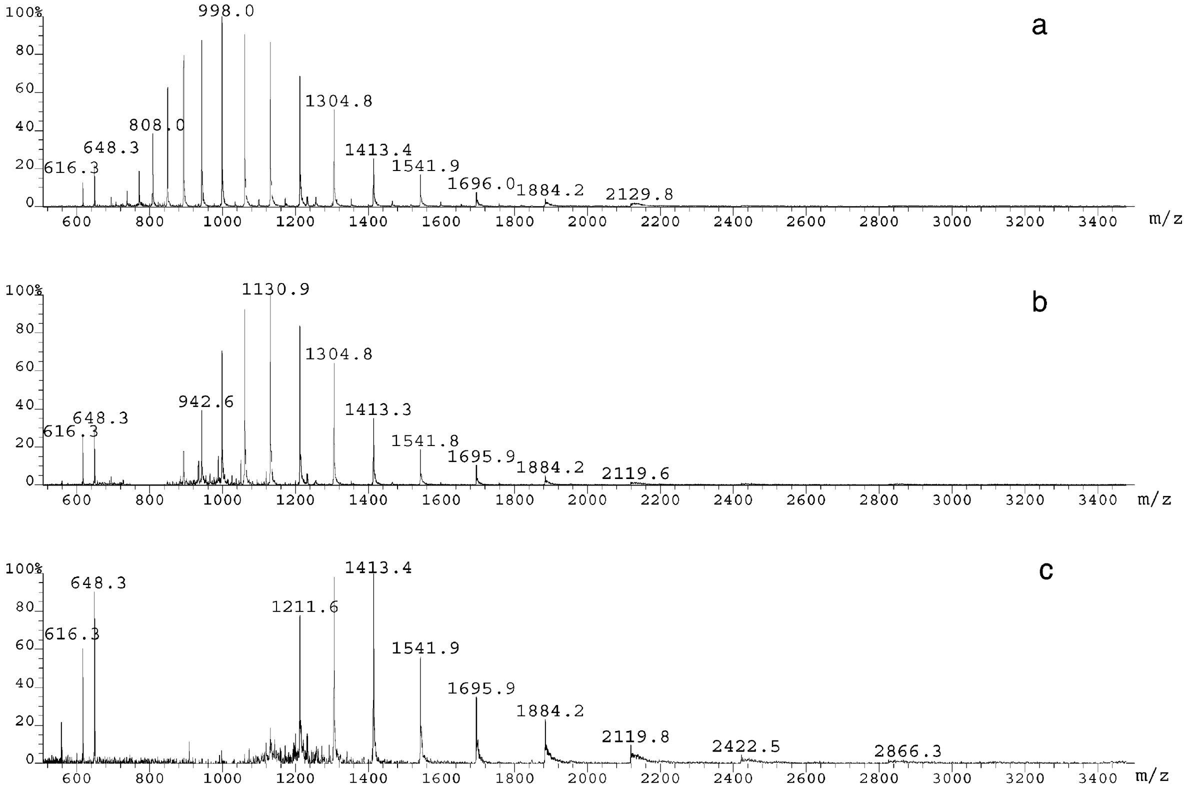

Fig. 2. . Electrospray mass spectra of myoglobin recorded at a total voltage difference V_T 70 V with (a) $V_A = 65$ V and $V_B = 5$ V, (b) $V_A = 41$ V and $V_B = 29$ V, and (c) $V_A = 17$ V and $V_B = 53$ V (denaturing solvent conditions, see Section 2).

experimental parameters being the same as for denaturing conditions.

In the following text, we will define two distinct areas within the ion source interface, the first of which (called A) being located between the sampling cone (sc) and the skimmer (s), the second zone (B) being comprised between the skimmer (s) and the hexapole (h). Two different values represent the electric fields in the two zones: $V_{sc} - V_s(V_A)$ and $V_s - V_h(V_B)$. The total voltage V_T can be defined as $V_{sc} - V_h$:

$$V_T = V_{sc} - V_h = (V_{sc} - V_s) + (V_s - V_h) = V_A + V_B$$

For a given value of V_T, several pairs (V_A;V_B) can be tuned.

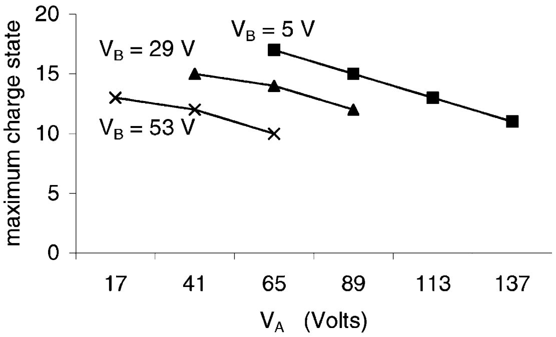

Fig. 3. Influence of the V_A voltage on the maximum charge state of denatured myoglobin with different V_B values: 5 V (■), 29 V (▲) and 53 V (×).

3. Results and discussion

The modification of the charge state distribution of a protein submitted to ESI by increasing the collision energy at the ion source interface has been previously described [8]. Recent results show that a more efficient

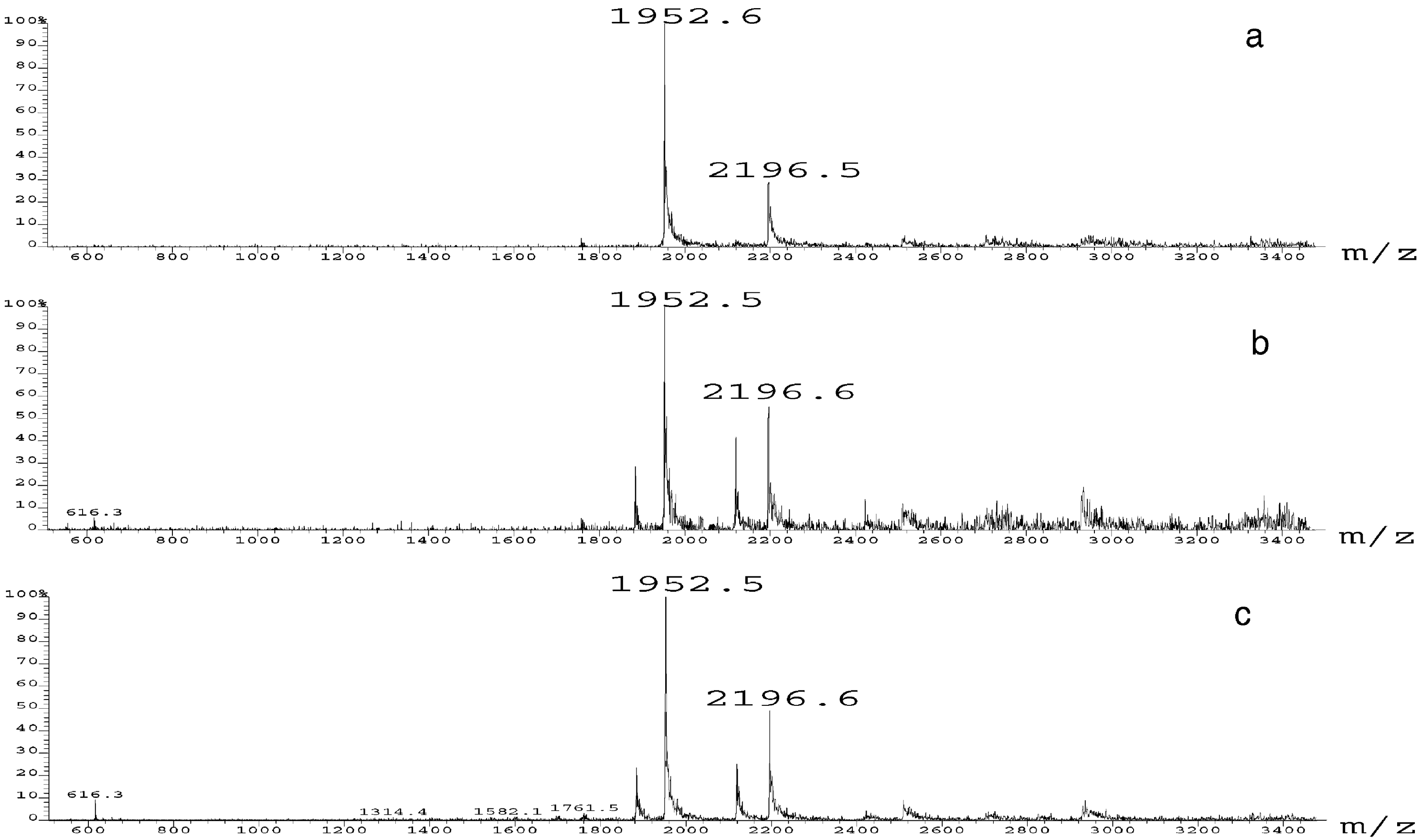

Fig. 4. Electrospray mass spectra of holomyoglobin recorded at different V_A and V_B voltages: (a) $V_A = 65\,V$, $V_B = 5\,V$, (b) $V_A = 41\,V$, $V_B = 29\,V$, and (c) $V_A = 113\,V$, $V_B = 5\,V$ (non-denaturing solvent conditions, see Section 2).

transfer of internal energy to the electrosprayed ions, leading to dissociation of protein/proton complexes, is involved in the charge state reduction process [9]. Thus, the charge state values could be used for investigating the collision efficiency at an electrospray source interface.

The mass spectrum of a solution of denatured myoglobin recorded with an optimal ion transmission corresponded to a pair (65;5) (Fig. 2a). The charge state distribution was relatively wide (10+ to 23+) and centered on the *m/z* 998 value (17+). In the lower mass part of the spectrum, the peak at *m/z* 616 was attributed to the charged heme species along with a methanol adduct ion at *m/z* 648. The mass spectrum displayed in Fig. 2b was obtained with the same V_T value as in Fig. 2a (i.e., 70 V) but with a different couple (41;29). Under such conditions, the distribution was shifted toward lower charge states (9+ to 19+) and was centered on the *m/z* 1131 ion peak (15+). The same trend was observed by reducing the V_A value to 17 V and enhancing V_B to 53 V (Fig. 2c).

In order to obtain a mass spectrum identical to that shown in Fig. 2b with a V_B value of 5 V (as in Fig. 2a), an increase of the V_A value to 89 V was necessary, thus leading to a total voltage V_T of 94 V. This result indicated that a higher collision efficiency was obtained in the second collision area than in the first one with no significant sensitivity loss. This phenomenon was observed over a wide range of sampling cone potential even though it tended to be reduced for the highest V_A values.

The mean charge state of apomyoglobin vs. V_A is shown in Fig. 3 for three different values of V_B. The curves illustrate the need of higher electric fields in the sampling cone-skimmer region than in the skimmer-hexapole zone for obtaining identical results.

A similar observation was made by looking at other diagnostic ions, the dissociation of covalent bonds or the separation of non-covalent complexes being also indicative of an increase of the precursor ion internal energy.

For example, an ion peak at *m/z* 557 corresponding to the loss of an acetyl radical (59 *u*) from the heme cation appeared for the pair (17;53) with a relative intensity of 20% (Fig. 2c). By setting the V_B value at 5 V, the same result was achieved for the couple (113;5) (not shown). In other words, an identical fragmentation rate was attained by reducing V_B by 48 V and increasing V_A by 96 V.

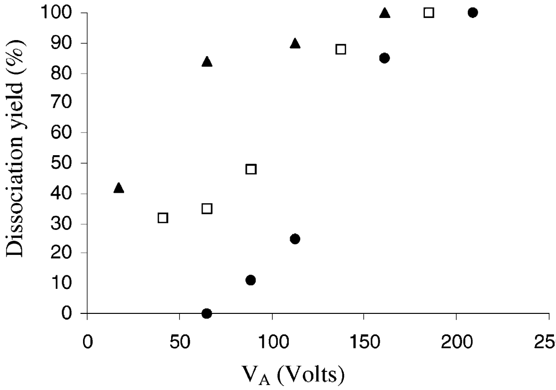

Fig. 5. Influence of the V_A voltage on the dissociation yield of holomyoglobin with different V_B values: 5 V (●), 29 V (☐), and 53 V (▲).

In the case of the holomyoglobin solution, the optimal transmission conditions were found for a couple (65;5) (Fig. 4a) which was incidentally the same as for the previous study. A significant dissociation of the protein/heme complex occurred for the couple (41;29) (Fig. 4b) as revealed by the presence of the apoprotein form (ion peaks at *m/z* 2119 and *m/z* 1884). A similar spectrum, with a fixed V_B value of 5 V, was recorded by setting V_A at 113 V (Fig. 4c). The relationship between the dissociation yield of holomyoglobin and the V_A value is illustrated in Fig. 5 for three different V_B values.

4. Conclusion

The results presented here demonstrate that a higher collision efficiency can be obtained after the skimmer than between the sampling cone and the skimmer. In order to promote the collisional activation of ions with such an ion source (a Micromass “pepper pot” ion source installed on a magnetic analyzer) an increase of the skimmer voltage before adjusting the sampling cone voltage appears as the better method. Although the enhancement of the fragmentation rate under such conditions could be accompanied by a loss of sensitivity, the analytical interest of this procedure for structure elucidation of organic molecules is real. It can be also applied with success for improving the desolvation efficiency of proteins electrosprayed under non-denaturing conditions.

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