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CORRESPONDENCE

Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10 000 Daltons

Sir: Until recently the desorption of ions of bioorganic compounds in the mass range above 10 000 daltons seemed to be exclusively the domain of plasma desorption mass spectrometry (PDMS) (*1-4*). In 1987 Tanaka et. al. (*5*) reported laser desorption of protein molecular ions up to a mass of 34 000 daltons. Oligomers of lysozyme containing up to seven monomeric units have also been observed by this group, using a pulsed N_2 laser and a matrix of a metal powder, finely dispersed in glycerol. Fast atom bombardment or liquid secondary ion mass spectrometry (SIMS) data on compounds above 10 000 molecular weight show weak signal intensities and poor signal-to-noise (S/N) ratios. The only exception is the results reported on the analysis of small proteins in the range of 10-24000 daltons obtained with a 30-keV cesium ion source in a conventional double-focusing mass spectrometer (*6*), but sample amounts in the 10-μg range were necessary in that case.

In the following, the first results on ultraviolet laser desorption (UVLD) of bioorganic compounds in the mass range above 10 000 daltons will be reported. Strong molecular ion signals were registered by use of an organic matrix with strong absorption at the wavelength used for controlled energy deposition and soft desorption (*7*).

EXPERIMENTAL SECTION

A reflector-type time-of-flight (TOF) mass spectrometer equipped with a Q-switched quadrupled Nd-Yag-Laser (pulse length 10 ns, wavelength 266 nm) was used for ion generation and analysis. The laser was focused to a spot between 10 and 50 μm in diameter. Samples were used as supplied commercially and dissolved in water at a concentration of about 10^{-5} M. One microliter of sample solution (10^{-11} mol) was mixed with 1 μL of 10^{-3} M aqueous solution of nicotinic acid serving as the absorbing matrix. The mixture was dripped onto a metallic substrate and air-dried. The sample then covered an area of ~5 mm^2 on the substrate. Individual spectra obtained with single laser shots of about 10^8 W/cm^2 irradiance and an area of about 10^{-3} mm^2 already showed detectable signal intensities. Sum spectra as shown in Figures 1-4 are obtained by irradiating the same spot 20-50 times. Provided suitable sample preparation techniques are developed, a much smaller amount of sample would suffice to obtain the same results. A time of 2-5 min, needed to acccumulate 20-50 single spectra, was limited by the laser repetition frequency of 1 Hz and by the PC data processing time. The LAMMA 1000 instrument used was designed for microprobe analysis of inorganic and organic compounds in the low mass range. It operates at an ion kinetic energy of 3 keV and the postacceleration potential was limited to a maximum of 9 kV. The ion velocity of lysozyme (molecular weight 14 306), e.g. at the conversion electrode, amounts to a value of only 1.1×10^4 m/s and is even lower for the higher mass compounds. Note that a minimum velocity of ca. 1.7×10^4 m/s was determined for insulin ion detection in PDMS (*2*). Signals were registered by a Biomation transient recorder with an aquisition memory of only 2048 channels resulting in a very low time resolution in the high mass range (for details see results). Currently this also limits the accuracy of mass calibration, which is done with the sodium and matrix signals in the low mass range. Thus all results have to be understood as a documentation of the general feasibility of the technique for the generation of high molecular mass ions.

RESULTS AND DISCUSSION

Spectra of four proteins are reported here: lysozyme (from chicken egg white, molecular weight 14 306), β-lactoglobulin A (from bovine milk, molecular weight 18 277), porcine trypsin (molecular weight 23 463), and albumin (bovine, approximate molecular weight 67 000) (all molecular weights are averages). Comparable results have been obtained for a variety of other proteins.

Figure 1 shows the laser desorption (LD) spectrum of lysozyme. Without time delay the mass range at an analog to digital time resolution of 200 ns/channel extends from 0 to ~16 000 daltons. For higher masses a time delay or decreased time resolution had to be used. In the low mass range up to 1000 daltons, strong signals of nicotinic acid matrix ions and possibly also analyte fragment ion signals are observed. Besides the dominating peak of the molecular ion, a dimer and doubly charged molecular ion are clearly detected as well as a $3M^{2+}$ signal. The same holds for β-lactoglobulin A (Figure 2). The geometrically determined mass centroids of the peaks are indicated at the mass axis of the spectrum. For porcine trypsin (Figure 3) a time per channel of 500 ns was used. The upper trace in this figure shows the total registered mass range of 0-100000 daltons. Again the background signal of the matrix below 1000 daltons is seen. The lower trace shows a section of the upper one as indicated by the arrows. Again molecular ions are found as M^+, $2M^+$, and M^{2+}. Figure 4 shows the LD-spectrum of bovine albumin with a molecular weight of about 67 000. Molecular ion signals are still obvious though the signal-to-noise ratio has declined compared to 20 000 daltons range. The absolute flight time for the bovine albumin

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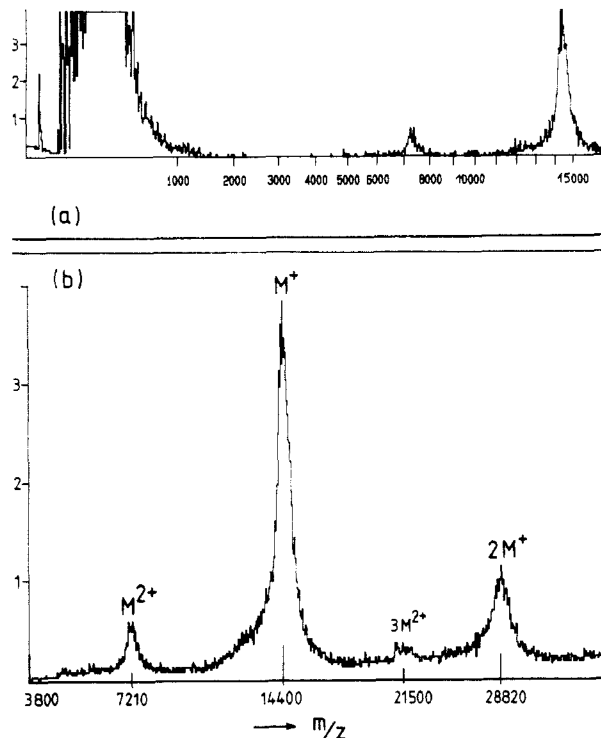

Figure 1. Matrix-UVLD spectrum of lysozyme (from chicken egg): (a) total registered mass range, 25 accumulated individual spectra; (b) mass range of molecular ion region, 50 accumulated individual spectra.

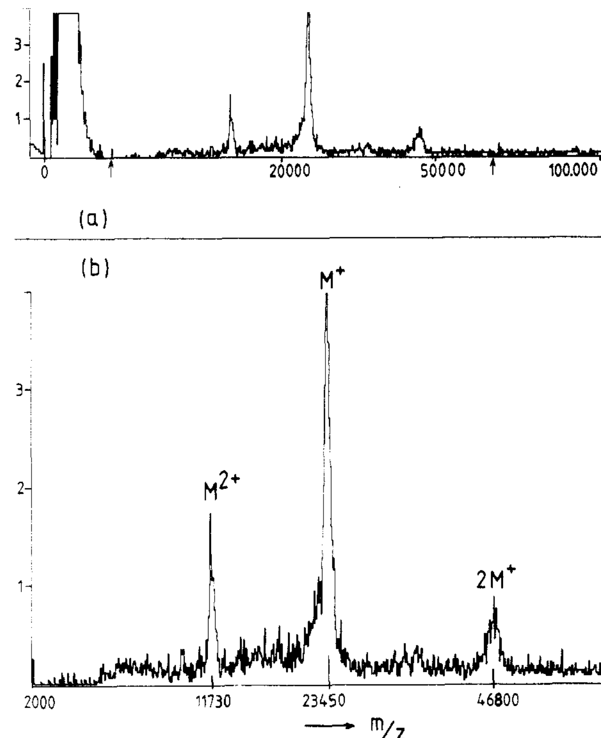

Figure 3. Matrix-UVLD spectrum of porcine trypsin, 50 accumulated individual spectra: (a) mass range 0–100000 daltons; (b) mass range of molecular ion region.

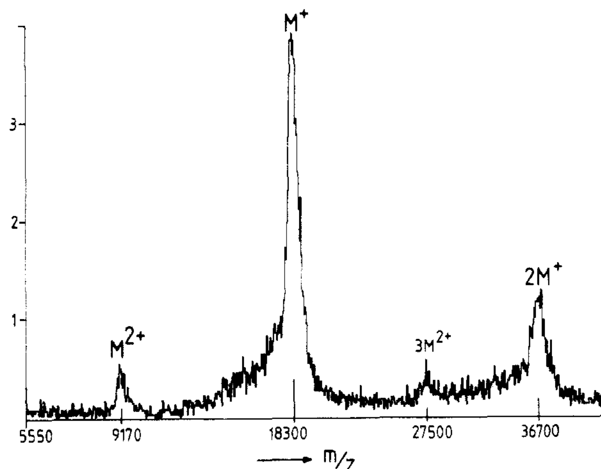

Figure 2. Matrix-UVLD spectrum of β-lactoglobulin A, mass range of molecular ion region, 50 accumulated individual spectra.

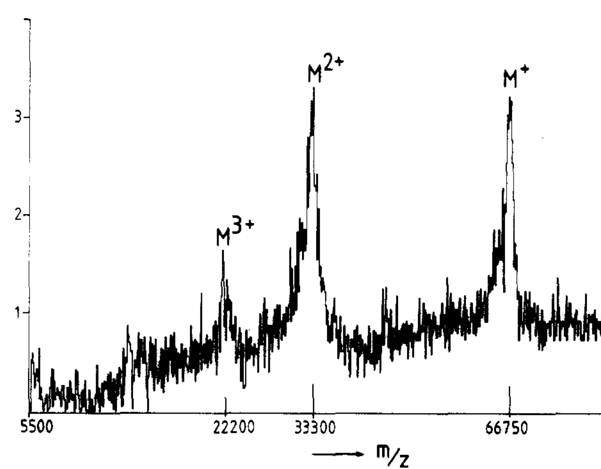

Figure 4. Matrix-UVLD spectrum of bovine albumin, mass range of molecular ion region, 100 accumulated individual spectra.

molecular ion already amounts to 0.8 ms. For bovine albumin, 100 single spectra were accumulated. These results clearly document the ability of LD to produce ions in this mass range so far inaccessible even by PDMS.

It is also worth noting that in all spectra multiply charged ions are observed. Multiple charges have so far never been reported for laser desorption ionization. They are quite common in PDMS of high mass proteins and presumably reflect the potential of these large molecules to remain stable even with several charge centers rather than a peculiarity of the excitation mechanism.

The width (full width at half maximum) of the molecular ion signal (600 daltons in the case of trypsin) is in all cases much larger than that expected from the mass resolution of the instrument of approximately 600 as measured in the low mass range. A resolution of the molecular ion signal that is expected to show both protonated and cationized species is not possible, but this is at least partly due to the low time resolution of the transient recorder (one channel of 200 ns is equivalent to 15 daltons at 14000 daltons, and one channel of 500 ns to 80 daltons at 67000 daltons). The large width may also originate from so far unknown effects in the ion–electron conversion at the low ion velocity. An improved detector system is expected to yield much better results. This expectation is supported by the fact that the transition from the commonly used 6 to 9 kV postacceleration resulted in a 10-fold improvement in signal-to-noise ratio.

Another unique feature of the matrix-UVLD technique as compared to PDMS or liquid-SIMS ion generation in the high mass region is a very low (chemical) noise level. Besides the molecular ion signals, ions are only registered in the mass range below about 1000 daltons. The matix ion intensities are only about 10–50 times higher than the sample ion signals.

CONCLUSION

The results reported demonstrate the ability of matrix-UVLD to generate a large number of intact molecular ions as well as dimers and doubly charged molecular ions of pro-

teins in the mass range above 10 000 daltons, stable at least up to times of about a millisecond. Singly charged molecular ions were in all cases the base peak of the analyte signal; no fragment ions were observed in the mass range above 1000 daltons. Additionally, multimers of the molecular ions and doubly charged molecular ions were detected improving the molecular ion detection and increasing the molecular weight determination accuracy. Also, a remarkable sensitivity is demonstrated. A detection limit in the subnanogram range for total sample mass needed for a sum spectrum appears to be realistic. All these features, no doubt, can still be optimized as discussed above. Though the general applicability of UVLD still needs to be shown by the successful desorption of a larger variety of different compounds, matrix-UVLD promises to be able to extend the accessible range for mass spectrometry of nonvolatile bioorganic compounds considerably with the added advantages of low sample consumption, ease of preparation and short measurement time.

Registry No. Lysozyme, 9001-63-2; trypsin, 9002-07-7.

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Michael Karas*
Franz Hillenkamp

Institute of Medical Physics
University of Münster
Hüfferstrasse 68
D-4400 Münster
Federal Republic of Germany

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Enhancement of Uphill Transport by a Double Carrier Membrane System

Sir: In spite of its obvious potential usefulness, two major problems for analytical applications of uphill transport membrane phenomena are (1) lower efficiency of energy conversion and (2) slower rate of transport, compared to the events occurring at biological cell membranes. It has often been noticed that thinner membranes are required to accelerate the transport rate in artificial systems. Besides extremely thin lipid bilayers for biological cell membranes, another fundamental difference between artificial organic membranes and biological cell membranes is the involvement of Na^+,K^+-ATPase for the majority of active transport in the latter systems. The essential chemistry of Na^+,K^+-ATPase is the generation of Na^+ gradient and its maintenance using the energy provided by the hydrolysis of ATP (*1*, *2*). For example, active transport of glucose is known to be driven by concentration gradient of Na^+ ions together with a carrier protein for glucose. Accumulation of the cotransported Na^+ ions in the intracellular compartment results in a drop of the driving force (concentration gradients of Na^+ ions) for pumping up glucose. However, this is restored by a Na^+,K^+-ATPase enzyme that pumps Na^+ ions from the intracellular compartment back to the extracellular one. By this action of the ATPase, high efficiency of active transport of glucose is maintained. Therefore, it seems very interesting to simulate this unique function of ATPase for enhancing efficiency of uphill transport of ions and molecules of interest by artificial membrane systems.

In the present correspondence, we report a preliminary study on double carrier membrane systems that mimic, in principle, the function of ATPase, although chemical compounds involved are totally different. It will be shown that the transport of ions with a double carrier membrane system is facilitated more efficiently than the conventional symport system.

EXPERIMENTAL SECTION

Reagents. Dicyclohexyl-18-crown-6 (99% content) and tri-*n*-octylamine (Pure Reagent grade) were obtained from Aldrich Co. (Milwaukee, WI) and Wako Co. (Tokyo, Japan), respectively. *o*-Nitrophenyl octyl ether (NPOE) was obtained from Dojin Chemical Laboratories (Kumamoto, Japan). Picric acid (Kanto Co., Tokyo, Japan) and lithium hydroxide (Wako Co.) were both of G.R. grade. A solution of lithium picrate was prepared by neutralizing picric acid solution with lithium hydroxide solution. Buffer solutions of pH 3.8 were prepared with lithium acetate and hydrochloric acid solutions. Other reagents used were all of G.R. grade. Milli-Q (Millipore, Bedford, MA) water was used.

Apparatus. The cell used for transport experiments is made of glass and has two compartments of different volumes (Figure 1): one for a feed solution (50 mL) and the other for receiving (1 mL). A Seiko E&I metal furnace flameless atomic absorption spectrometer (AAS) SAS 727 (Tokyo, Japan) was used for determining K^+ ions. A Shimadzu spectrophotometer UV-240 (Kyoto, Japan) was used for determination of picrate ions. A TOA glass electrode pH meter HM-60S (Tokyo, Japan) was used for pH measurements.

Preparation of Liquid Membranes and Transport Experiments. A Sumitomo Denko (Osaka, Japan) Fluoropore membrane filter, type FP-010 (pore size, 0.10 μm; thickness, 80 μm; diameter, 47 mm), is cut into two equal pieces. One piece of the membrane filter is impregnated with 2.5 mM dicyclohexyl-18-crown-6 (DC18C6)–dichlorobenzene (DB) solution and the other with a 2.5 mM tri-*n*-octylamine (TOA)–NPOE solution. A pair of the carrier-impregnated liquid membranes (DC18C6 and TOA membranes) thus prepared are placed side by side separately to cover two of each window at the exit of the feed compartment, which is then fastened tightly with the receiving part of the cell using a strong clip (Figure 1). The respective feed (50 mL) and receiving (1 mL) solutions are provided into each compartment of the transport cell. The feed solution is stirred mechanically with a stirrer (the receiving solution was not stirred). For determination of K^+ ions transported, a very small volume (usually

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