APPLICATION

MALDI 2

Optimised sample preparation for the analysis of high molecular mass proteins

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Matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF-MS) has, since its introduction in 1988[1, 2], been used very successfully for the mass determination of molecules of various types. As a general rule in MALDI-ToF-MS, the mass resolution obtained ($R = M/\Delta M$) decreases with increasing mass. This is increasingly seen with biopolymers due to adducts formed from buffer components typically present. In the case of large biopolymers (M>50 kDa) the mass resolution obtained is sometimes less than 100.

The following note demonstrates how mass measurement of large proteins by MALDI has been optimised. As an example, commercially available recombinant human serum albumin has been chosen (rHSA; M = 66438 Da). The composition of this is typically as follows:

- 50 mg/ml rHSA (Calbiochem Cat. No.: 126661)
- 145 mM NaCl
- 8 mM Natriumoctanoat
- 0.0015 % TWEEN 80
- 0.003 mM/g Protein K^+

For the MALDI measurements this solution was diluted 1:100 with pure water (500 ng/µl; 10 pmol/µl)

Choice of matrix

Proteins are usually measured in MALDI-ToF-MS using the matrices:

- gentisic acid (DHB)
- α-cyano-4-hydroxycinnamic acid (CN)
- sinapinic acid (SIN)

The following spectra show the influence of α-cyano-4-hydroxycinnamic acid and sinapinic acid on the ionisation of rHSA.

The spectrum obtained with sinapinic acid shows that single charged ions predominate. In comparison α-cyano-4-hydroxycinnamic acid generates a high proportion of multiple charged ions. As a rule proteins with a molecular mass larger than 6000 Dalton are best measured with sinapinic acid as matrix.

[1] Karas M., Hillenkamp F.: Anal. Chem. 60: 2299-2301 (1988)
[2] Tanaka K. et.al.: Rapid Commun. Mass Spectrom. 2:151-153 (1988)

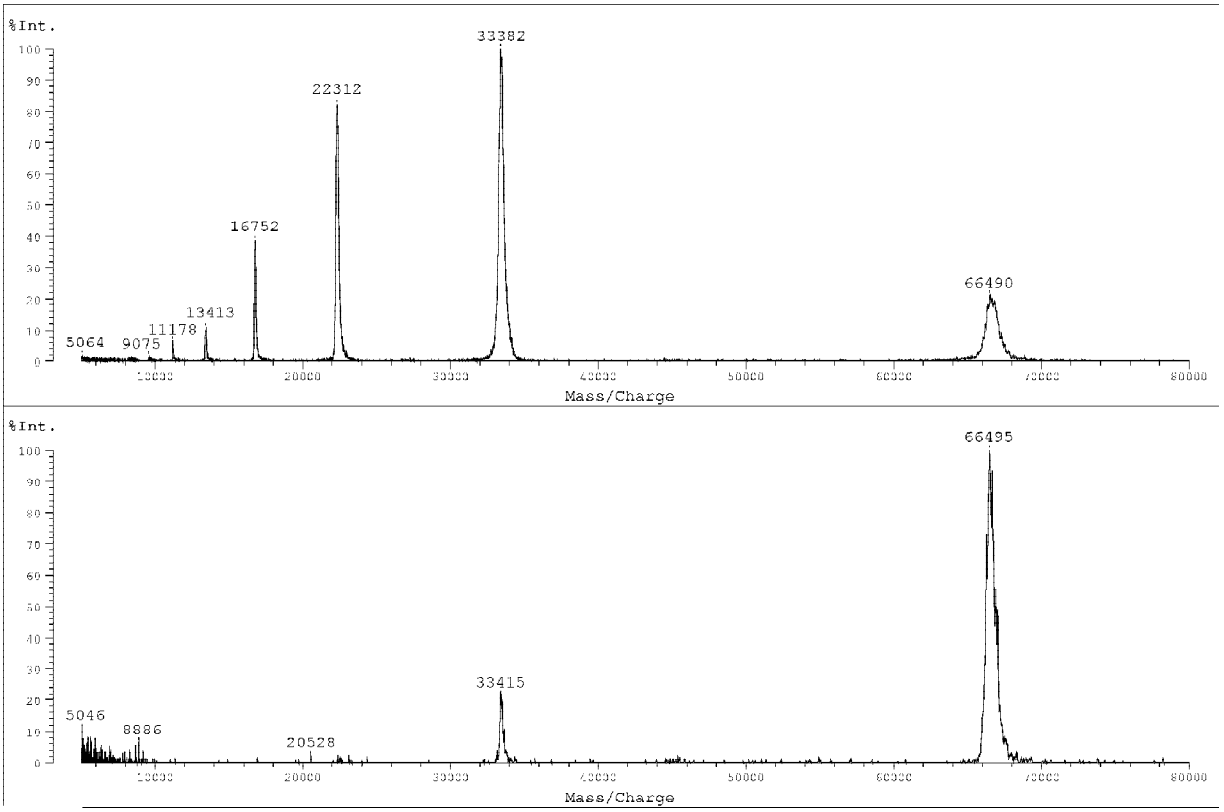

Figure 1: MALDI-MS spectra of rHSA with α-cyano-4-hydroxycinnamic acid and sinapinic acid as matrix in comparison.

Sample preparation

Sample preparation for MALDI-ToF-MS is quick and easy. The samples are normally applied to the sample holder as follows. 0.5 µl of matrix is applied followed by 0.5 µl of sample and a further 0.5 µl of matrix. The sample holder is dried in a stream of air after each step.

By the use of a suitable pretreatment of the sample before application to the sample holder it is often possible to optimise the mass measurement.

Figure 2 shows the comparison of three spectra of rHSA acquired with varying pretreatments of the sample.

- without pretreatment
- after methanol/chloroform precipitation[3]
- after dialysis with pure water

With the methanol/chloroform precipitation[3] described it is possible to remove lipids and detergents from the sample. Simultaneously one can concentrate the sample. The dialysis with pure water causes a dilution of the sample and removes all low molecular mass components from the sample (depending on choice of the diaphragm).

[3] Wessel D., Flügge U.I.: A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Anal. Biochem. 138: 141-143 (1984)

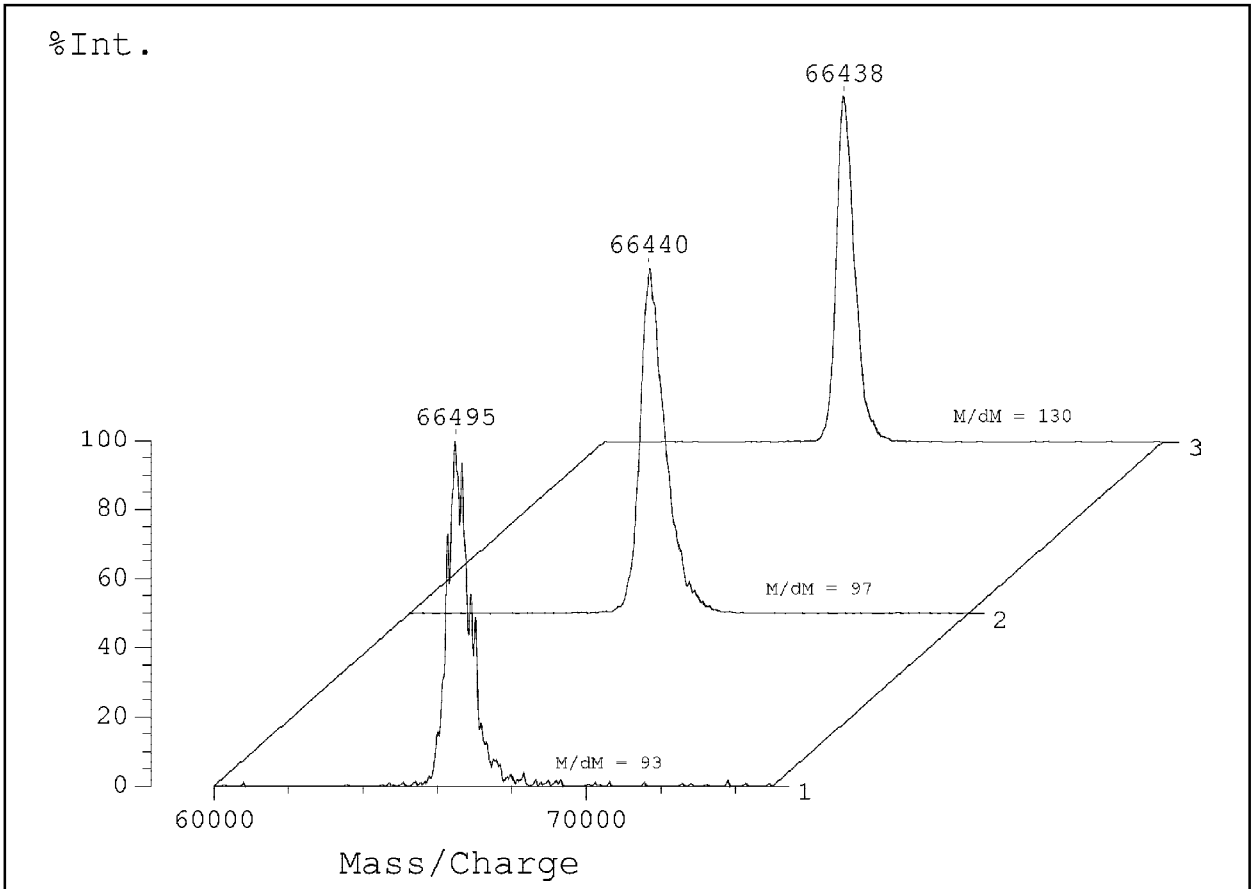

Figure 2: comparison of the spectra of rHSA
1. without pretreatment
2. after precipitation[3]
3. after dialysis of the sample

The methanol/chloroform precipitation provides no significant improvement of the mass resolution. However in the case of dialysis with pure water, an increase of the resolution from R = 93 to R = 130 has been achieved.

Experimental method for dialysis

A membrane (Millipore VSWP 02500) was placed on a surface of pure water. 10 µl of the sample were applied to the surface of the membrane and incubated for several hours. Finally the sample was applied to the sample holder in the usual way.

Measurements

Figure 3 shows the spectrum of a protein with a molecular mass of 280 kDa. Before measuring, the sample was dialysed as described above for two hours. Without this sample pretreatment it was not possible to obtain any results for this protein.

It is also possible to determine single components out of a complex mixture with MALDI-ToF-MS. For example, it is possible to determine immunoglobulins in a sample of serum. In figure 4 the spectrum of a serum sample of an (secondary) immunised mouse is shown. The only pretreatment of the serum before MALDI-ToF-MS was a dialysis for two hours using the method described above.

Summary

As has been shown it is possible to obtain a significant improvement in MALDI-Spectra by the means of a simple pretreatment procedure. With the methods described the sample can be manipulated and measured on a micro-scale. Furthermore with MALDI-ToF-MS it is not only possible to measure single substances but, as has been demonstrated, even complex mixtures can be analysed.

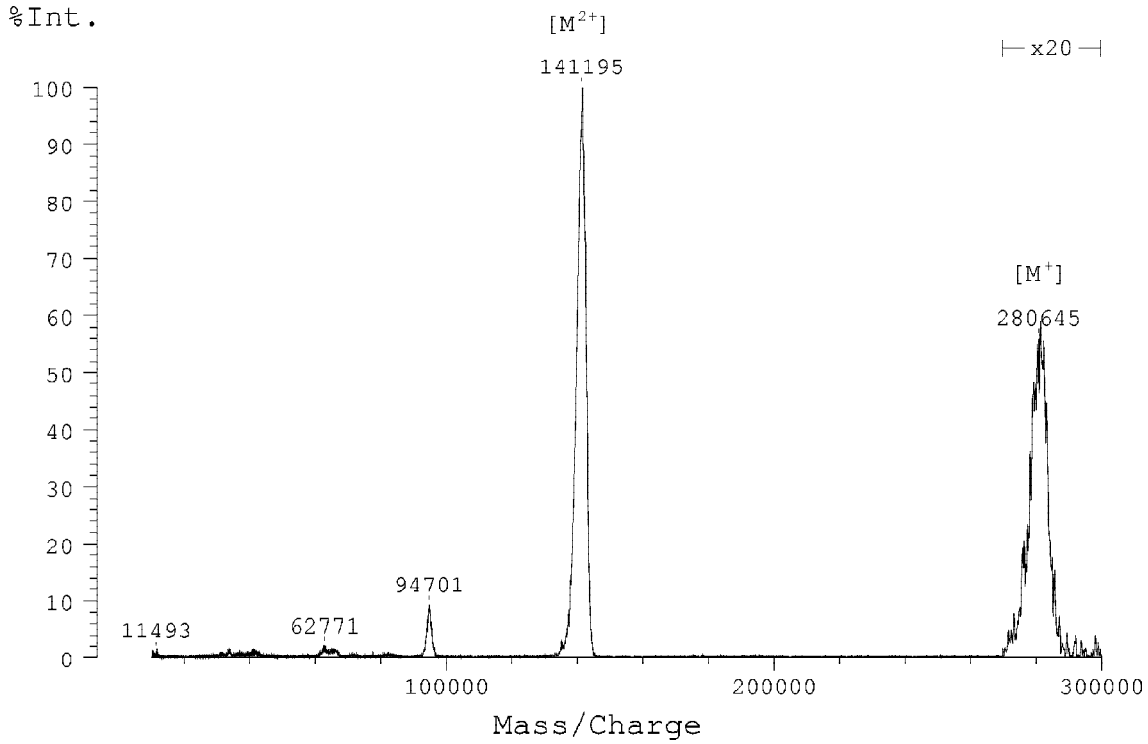

Figure 3: Spectrum after dialysis of a protein with a molecular mass of 280 kDa

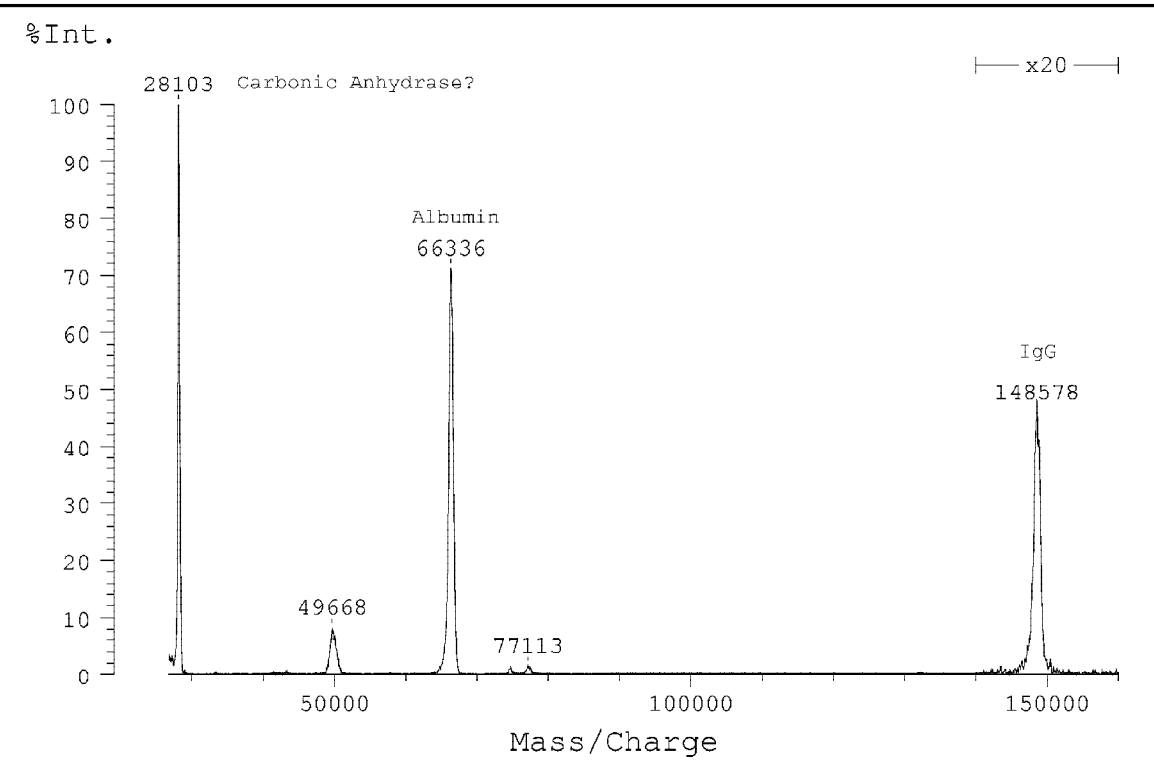

Figure 4: spectrum of a serum sample (murine) after dialysis

0.2/E/10/97/mr/bmu

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