

MALDI-TOF MS: Much More than only Protein Analysis!

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Carbohydrates and particularly lipids represent molecules with significant structural variabilities: this is stemming from (a) the headgroups, (b) the different fatty acyl residues and (c) the linkage types (acyl-acyl-, alkyl-acyl-, and alkenyl-acyl-). Therefore, analytical methods to clarify these aspects are of immense significance. As the amount of the available biological material is normally limited, suitable methods should also exhibit reasonable sensitivities. It is our aim to introduce MALDI (matrix-assisted laser desorption and ionization) MS (mass spectrometry) [1] (often but not necessarily with a time-of-flight (TOF) mass analyzer) as a simple and sensitive analytical method that helps to overcome many problems related to carbohydrate and particularly lipid analysis [2].

Although the history of MS dates back to the end of the nineteenth century, applications of MS to the structural analysis of biomolecules are rather new: the MS analysis of such complex molecules (often in combination with high polarity and low volatility) became only possible with the invention of “soft ionization” methods such as electrospray ionization (ESI) and “MALDI” [3]. Both techniques are nowadays widely used, whereby the focus is often on the investigation of proteins - normally subsequent to enzymatic digestion of the (unknown) protein into characteristic peptides, the MS of which enables the elucidation of the protein sequence and further structural properties [4]. It is a characteristic property of “soft ionization” MS that there is only a small extent of analyte fragmentation and, thus, the intact ions of the analyte of interest can be observed. This is a pronounced difference in comparison to the classical “electron ionization” (EI) technique.

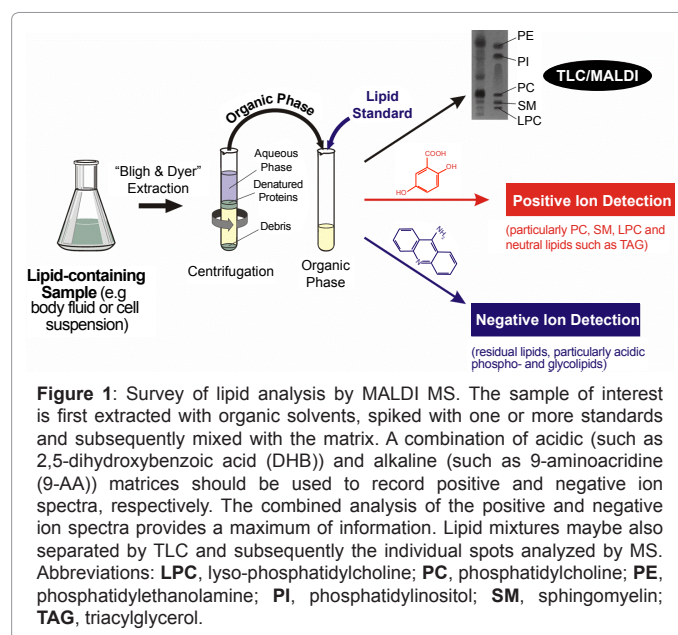
Although MALDI MS has many advantages it should be noted that the analysis of small molecules (for instance, free fatty acids) is still a problem - despite many improvements that could be recently achieved [5]. This problem with small molecules can be easily understood when the principle of MALDI MS is considered: MALDI MS is based on laser ionization, whereby normally UV lasers (emitting often at $\lambda=337$ nm) are used. Since not all potential analytes exhibit a sufficient absorption at this wavelength, a “matrix” must be used [1]. This matrix is normally a small organic molecule (such as 2,5-dihydroxybenzoic acid (DHB)) [6] that absorbs the laser energy, is evaporated and simultaneously carries the analyte into the gas phase (high vacuum). In the gas phase collisions between the analyte and cations (such as H^+ or Na^+), that are either already present in the lipid extract of interest or artificially added, occur leading to ion generation. As the mass of the observed (positive) ions is slightly higher than the mass of the analyte, these are normally termed “adducts” or “quasimolecular” ions. Of course, analytes with acidic groups are also detectable as negative ions. Since the matrix is normally in considerable excess over the analyte (at least 100:1), it is not surprising that the smaller mass range is dominated by matrix ions [7]. This is the reason why the small mass range is normally not shown when MALDI spectra are discussed. Despite this drawback, however, there are a lot of advantages:

1. The necessary sample preparation is simple and fast - and comprises often only the mixing of the analyte with the matrix and the deposition of this mixture onto the sample carrier, the “target”.
2. While ESI MS, for instance, is strongly affected by sample impurities

such as salt or buffer contaminations, MALDI MS tolerates such impurities relatively well and, thus, not even desalting of lipid extracts is normally necessary.

3. MALDI MS is characterized by the nearly exclusive presence of singly charged ions and this feature makes data analysis very simple.
4. MALDI spectra may be recorded within a minimum of time - normally within a few seconds. Of course, the required acquisition time depends on the number of laser shots that are averaged: this is, however, not a major problem because nowadays kHz lasers are available.
5. MALDI MS is characterized by an extreme sensitivity: even single cells may be analyzed and it has also been demonstrated that already 10 molecules (ca. 700 yoctomoles) of an analyte are sufficient to give a detectable signal [8]. This sheds light on the extreme low detection limits of modern MS detectors.

A typical workflow of lipid analysis by MALDI MS is shown in Figure 1. In contrast to other MS methods where solutions of the analytes are normally investigated, a solid sample, i.e. a co-crystal



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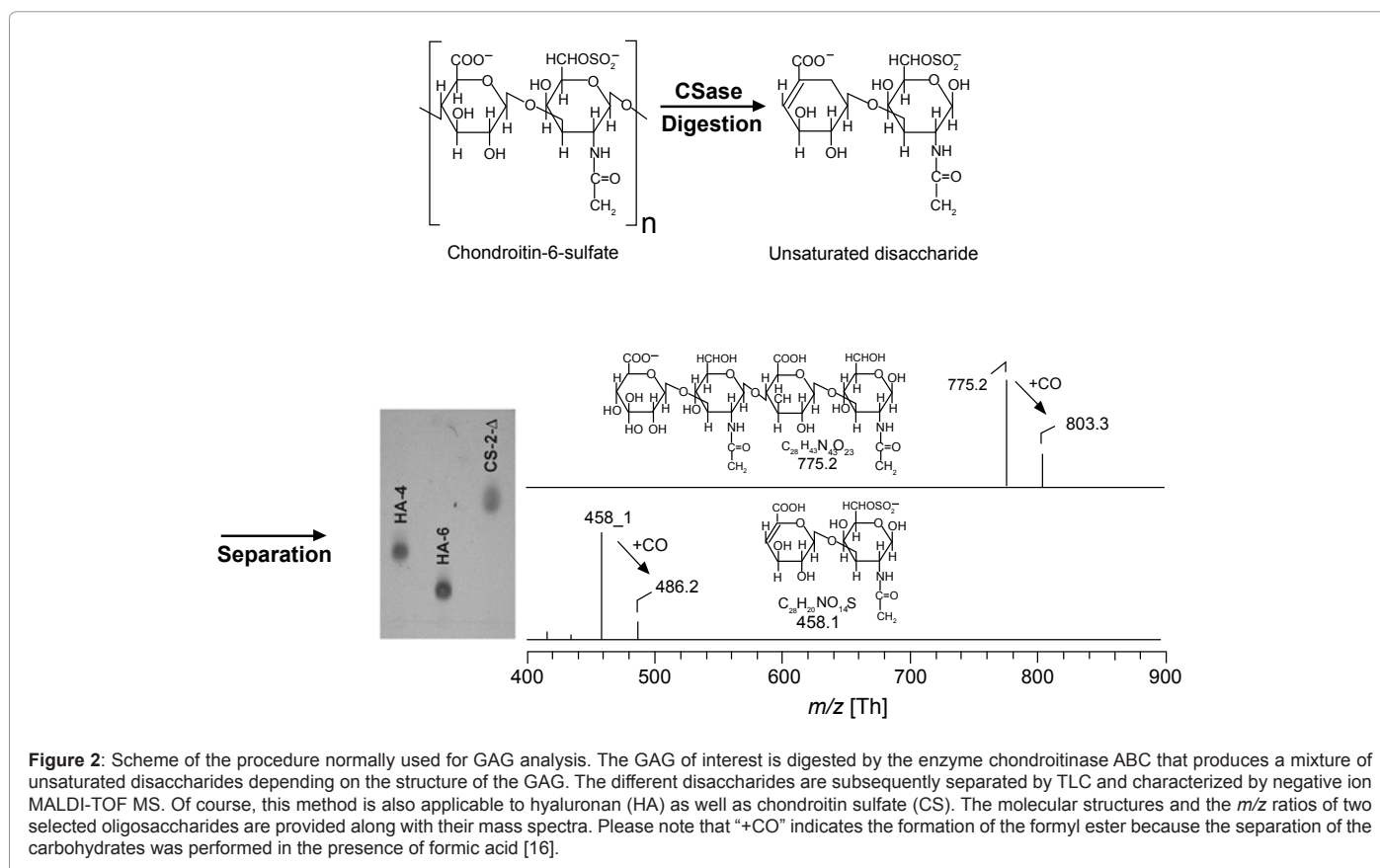
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between analyte and matrix is analyzed by MALDI MS. Since a co-crystal is never completely homogeneous, this limits the shot-to-shot reproducibility; however, this problem can be reduced (if quantitative data have to be obtained) by averaging a high number of laser shots at different positions at the sample. In contrast, the fact that “solid” samples are analyzed provides also significant advantages: first, this enables the recording of MALDI MS “images”. This is a rapidly growing field [9] and of particular interest for clinicians since thin tissue slices (e.g. from brain or other organs) may be investigated. In order to perform MS imaging (MSI), the tissue slice is evenly covered with the matrix (normally by spraying with the matrix solution) and subsequently spatially resolved MS spectra are recorded, whereby the achievable resolution is determined by the laser spot size (typically 20 μm) and the size of the matrix crystals. The spectral intensities of a given peak are subsequently converted into a grey or colour scale and this allows the evaluation of the distribution of certain metabolites [10]. There are significant expectations regarding this new method and it is expected that MSI will support medical diagnosis, for instance, the discrimination between healthy and tumour tissue.

Second, MALDI MS can be easily combined with thin-layer chromatography (TLC). TLC is an established method of lipid analysis [11] and somewhat similar to the investigation of a tissue slice since the lipid molecule is here also entrapped in a solid (not biological) matrix namely the material of the stationary phase [12]. Although the individual lipid classes can be easily separated by means of normal phase (NP) TLC, the information of the fatty acyl compositions of the individual fractions can be hardly obtained by NP TLC and requires additional separation by reversed phase (RP) TLC. However, this information is readily available when the obtained lipid spots are

directly (on the TLC plate) investigated by MALDI MS [13]. It has to be emphasized that this approach can be performed on each available MALDI mass spectrometer and there is no need of purchasing a dedicated device.

Lipids are (at least from the MS viewpoint) very nice molecules and exhibit two significant advantages: first, their molecular weight (ca. 500-1500 Da) is high enough to avoid interferences with the matrix, but sufficiently low to result in high ion yields, i.e. reasonable sensitivities. Second, lipids are polar molecules that are normally extracted by organic solvents from the sample of interest (Figure 1). This confers the considerable advantage that salts or other water-soluble molecules are nearly completely removed. Unfortunately, carbohydrates do not have these nice properties and, thus, their MS characterization is much more challenging. We are particularly interested in the analysis of glycosaminoglycans (GAG) such as hyaluronan (HA) or chondroitin sulfate (CS). Natural GAG possess high molecular weights and represent polyelectrolytes (carboxylate and/or sulfate residues). It is impossible to detect these polysaccharides by MS: proteins with molecular weights of the 500 kDa range have been already detected by MALDI MS; dextran (a polysaccharide without charged groups!) is detectable up to about 70 kDa [14], while heparin (a strongly acidic GAG) was only detectable up to a mass of 2 kDa. Therefore, degradation of the polysaccharide of interest is necessary to obtain defined oligosaccharides that are indicative of the homogeneity of the polysaccharide (Figure 2). Degradation is best performed enzymatically because chemical degradation is normally accompanied by unwanted side reactions [15]. Of course, the obtained digestion products may be also separated by TLC and the individual fractions characterized by combined TLC/MALDI [16] as it is illustrated in Figure 2. Although



only some selected examples could be given, we hopefully succeeded in showing that MALDI MS is a useful, sensitive, fast and convenient method to characterize lipids and carbohydrates. Thus, MALDI MS is much more than protein analysis! We would like to encourage all readers to use eventually available MS mass spectrometers: although MALDI MS is not necessarily the optimum method to solve a dedicated problem, it is a very simple, easy to learn method. Every lipid and carbohydrate (bio)chemist should explore the capabilities of MALDI MS.

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