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ORIGINAL PAPER

Ionic liquid matrices for MALDI-TOF-MS analysis of intact glycoproteins

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Abstract 2,5-Dihydroxybenzoic acid (DHB) has been demonstrated to be a more suitable matrix than 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) to obtain reliable molecular mass values of intact glycoproteins because it prevents sugar fragmentation. Lack of spot homogeneity during the crystallization step was prevented by drying the sample-matrix mixture under vacuum conditions. Nevertheless, this sample-matrix preparation procedure requires a specific experimental setup and may be time-consuming. In this work, we investigated the effectiveness of different ionic liquid matrices (ILMs) with SA and DHB on the ionization of a set of intact glycoproteins with several degrees of glycosylation. The obtained results demonstrate that some of the tested ILMs allow detection of the studied intact glycoproteins. Furthermore, the selected optimum conditions solve the reproducibility issue of using the DHB as a solid matrix without the vacuum drying method and, surprisingly, avoid sugar fragmentation when both SA and DHB were used as ILMs.

Keywords Erythropoietin · Glycoprotein · Glycosylation degree · Ionic liquid · MALDI

Introduction

Ionic liquids (IL) are salts that have a melting point at or below 100 °C and possess negligible vapor pressure. As a result of these properties, ILs have become extremely useful in many applications: organic synthesis, liquid-liquid extraction [1–3], gas chromatography [4–6], matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [7, 8], and recently in electrospray ionization mass spectrometry as ion-pairing reagent to detect anions in positive ion mode [9]. ILs used in MALDI-MS were first reported by Armstrong et al. in 2001 [7]. These novel ionic liquids, also named ionic liquid matrices (ILMs), are organic salts formed by equimolar mixtures of crystalline MALDI matrices, like α-cyano-4-hydroxycinnamic acid (CCA), 2,5-dihydroxybenzoic acid (DHB) or 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) with organic bases, e.g., tributylamine, pyridine, or 1,1,3,3-tetramethylguanidinium.

Some authors have reported that ILMs may provide extensive adduct formation and peak broadening leading to reduced sensitivity and resolution [8]. Nevertheless, the formation of Na/K adducts can be partially suppressed by the addition of acid (e.g., 0.1% TFA, trifluoroacetic acid) or when substoichiometric ILMs are used [10], and for certain analytes (e.g., isolated sugars), the increased adduct formation can even be beneficial [11]. In the last few years, ILMs have been widely used owing to some advantages compared with conventional solid matrices [12–17]. ILMs are easily prepared and require no co-crystallization with the analyte, which prevents "hot spots" and thus provides better shot-to-shot and spot-to-spot reproducibility. This higher reproducibility is crucial for quantitative analysis [13], can make the automated acquisition in MALDI-MS instruments easier, and also can improve the robustness of CE or HPLC couplings with MALDI-MS, as it is not necessary to search for "sweet spots". ILMs have been used for detection of a great number of substances and sometimes they have shown selectivity against certain analytes, e.g., glycopeptides and glycans versus peptides [12]. In this regard, ILMs have

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been reported to reduce fragmentation of some compounds with labile groups (e.g., sugars, phospholipids, glycopeptides, polymers) [12–18], although this issue needs to be studied in more depth for each type of analyte.

The analysis of glycosylated compounds such as intact glycoproteins by MALDI-MS is a difficult task as labile groups such as sialic acids or *N*-acetylglucosamine (GlcNAc) tend to fragmentation in the ion source or during acceleration [19–22]. There are solid MALDI matrices such as benzoic acid derivatives (DHB, 2-(*p*-hydroxyphenylazo) benzoic acid, and 2′,4′,6′-trihydroxyacetophenone) which may enable one to reduce this sugar fragmentation. DHB is considered a suitable matrix to obtain reliable molecular mass values of intact glycoproteins by MALDI-MS [23]. Nevertheless, it has an important tendency to form "hot spots" affecting the reproducibility of the results and increasing the time required to make the measurements because of the need to find a "sweet spot". In a previous study, we proposed a sample-matrix preparation procedure based on vacuum drying in order to prevent poor spot homogeneity using DHB as a solid matrix [23]. Nevertheless, this procedure requires a specific experimental setup and may be time-consuming.

The applicability of ILMs for the analysis of intact proteins has hardly been studied and, to our knowledge, there are no reports about their application to intact glycoproteins. Some authors advise against their use for intact proteins as they may produce peak tailing, leading to a decrease in the accuracy of molecular mass determination [7, 24]. However, recently some papers have suggested that ILMs are appropriate matrices for the analysis of glycans [12, 16, 17, 25] and glycopeptides [12, 17].

In a previous study, different solid matrices and preparation procedures were tested for the analysis of intact glycoproteins, selecting DHB with vacuum drying as the best sample-matrix preparation procedure [23]. In this work, we evaluated different ILMs with DHB and SA in simple sample-matrix preparation procedures for MALDI-TOF-MS of intact glycoproteins with the aim of improving DHB spot homogeneity and reducing the extent of fragmentation that occurs when intact glycoproteins are analyzed by MALDI-MS using solid matrices. Human transferrin, bovine fetuin, bovine α_1-acid glycoprotein, recombinant human erythropoietin, and the novel erythropoiesis stimulating protein were selected because they are biologically and therapeutically relevant and present different degrees of glycosylation. We evaluated the ability of ILMs to ionize intact glycoproteins in comparison with DHB and SA as solid matrices. Special attention was given to reproducibility of the measurements and the extent of sugar fragmentation in order to obtain reliable average molecular mass values.

Experimental

Chemicals and reagents

All chemicals and solvents used in the preparation of solutions were analytical reagent grade. Acetonitrile (MeCN), acetone, ethanol (EtOH), phosphoric acid (H_3PO_4), and trifluoroacetic acid (TFA) were supplied by Merck (Darmstadt, Germany). DHB was obtained from Sigma (St. Louis, MO, USA). SA and butylamine were supplied by Fluka (Madrid, Spain). Purified water with a conductivity value lower than 0.05 μS cm^{-1} was obtained by using a Milli-Q water purification system from Millipore (Molsheim, France).

Protein sample solutions

Bovine serum albumin (BSA, 99%), bovine α_1-acid glycoprotein (AGP, 99%), fetuin from fetal bovine serum (FET, cell culture grade), and human transferrin (Tf, ≥98%) were obtained from Sigma (St. Louis, MO, USA). Each protein was dissolved in purified water to obtain a 1,000 mg L^{-1} solution.

Recombinant human erythropoietin (rHuEPO) was provided by the European Pharmacopoeia as a Biological Reference Product (BRP-lot2). The novel erythropoiesis stimulating protein (NESP, Darbepoetin alfa, Aranesp®) was provided by Amgen (Thousand Oaks, CA, USA). Excipients of low molecular mass were removed in rHuEPO and NESP samples by passage through a Microcon-10 cartridge from Millipore (Bedford, MA, USA) and glycoprotein concentration was adjusted to 1,000 mg L^{-1} with purified water as described in [23].

Preparation of MALDI matrices

MALDI matrices were prepared daily according to the following procedures: Solid SA matrix (SA-s) was prepared by dissolving SA in MeCN/H_2O (1:1 v/v) with and without 0.1% v/v of TFA at a concentration of 10 mg mL^{-1}. A 27 mg mL^{-1} MALDI matrix solution of SA in acetone/water (99:1 v/v) was also prepared when the fast evaporation method was used as the crystallization procedure [23]. Solid DHB matrix (DHB-s) was prepared by dissolving DHB in EtOH/H_2O (1:1 v/v) with and without 0.1% v/v of TFA at a concentration of 10 mg mL^{-1}.

Three different ILM preparation procedures adapted from the literature were tested, using both SA and DHB with and without TFA [14, 26, 27]. The most important difference between the three preparation procedures was the final concentration of organic salt formed when mixing equimolar amounts of SA or DHB and the organic base. *Procedure 1*: Stock solutions of 50 mM of DHB and SA

were prepared in MeCN/H_2O (2:1 v/v) with and without 0.1% v/v of TFA and 1% v/v of H_3PO_4. ILM 1 was obtained after adding an equimolar amount of butylamine and sonicating for 5 min [26]. *Procedure 2*: ILM 2 was prepared by adding an equimolar amount of butylamine (64.1 μL) to 3,242 μL of a 200 mM solution of SA or DHB in MeOH [14]. The mixture was vortexed and sonicated for 1 min, evaporated to approximately 100 μL with air, and finally reconstituted with 100 μL of EtOH or MeCN, for DHB and SA mixtures, respectively. *Procedure 3*: Stock solutions of 600 mM of DHB, SA, and butylamine were prepared in MeCN/H_2O (1:1 v/v). ILM 3 was prepared by mixing equal volumes of DHB or SA and butylamine stock solutions [27]. Finally, for ILM 3 with TFA or H_3PO_4, the acid was added to the solution to a final concentration of 0.1% or 1% v/v, respectively.

MALDI-TOF-MS

A sample-matrix solution was prepared by mixing 1 μL of protein sample solution and 1 μL of solid matrix solution or ILM. Each mixture was vortexed and centrifuged. All sample-matrix preparation procedures were performed in a thermostatized room at 20 °C. Sample-matrix spots were obtained by using the *dried-droplet method* except for SA-s which was applied by using the *fast evaporation method* [23].

Sample-matrix spots were monitored under a BH2-UMA optical microscope from Olympus (Tokyo, Japan) coupled to a D70 digital camera from Nikon (Tokyo, Japan). Therefore, the appearance and homogeneous distribution of the spot could be easily evaluated with the aim of finding the optimal sample-matrix preparation procedure.

The MALDI-TOF mass spectrometer used to acquire the spectra was a Voyager-RP-DE system from Perseptive Biosystems (Framingham, MA, USA) equipped with a nitrogen laser (337 nm). Laser intensities ranging from 66.2% to 85,0% of the maximum (4,000 V) were used. The acceleration voltage was set at 25 kV. The voltage on the first grid was 81% of the total acceleration voltage, and the delay time between ion production and extraction was 400 ns. No significant effects were observed on the spectra when the delay time was increased or decreased. The guide wire voltage was set at 0.08%.

The spectra shown were not smoothed and they represent summation of 100 consecutive laser shots acquired by using the linear positive mode. The spectra were externally calibrated in terms of mass-to-charge (m/z) ratio, using the singly and doubly charged ions from BSA using SA-s and the fast evaporation method and spotting the calibrant solution next to the samples being analyzed. Data calibration and data processing were performed by using Data Explorer version 4.4 software (Perseptive Biosystems).

Results and discussion

ILM selection

In this paper, different ILMs were studied with the aim of evaluating their effectiveness in the ionization of intact glycoproteins and improving the homogeneity and reproducibility of the spots obtained previously with DHB-s and a vacuum drying-based sample-matrix preparation procedure [23]. The methods for the preparation of ILMs were adapted from some recommended for biomolecules, low molecular weight compounds, and phosphopeptides [14, 26, 27]. Three different preparation procedures were tested by using both DHB and SA, containing in all cases butylamine as organic base, and with and without acid (0.1% of TFA or 1% of H_3PO_4). To allow comparisons to made, these ILMs were tested with rHuEPO and laser energy was maintained near the threshold value required for generation of glycoprotein molecular ions. The spot homogeneity, the quality of the MALDI mass spectra, the signal-to-noise (S/N) ratio of the singly charged molecular ion of rHuEPO, and the standard deviations of these S/N ratios were selected as evaluation criteria.

Similar results were obtained with the ILMs containing DHB or SA. Figure 1 shows the images of the spots obtained for rHuEPO, using the three different ILM preparation procedures with SA without acid (SA-ILM). As can be observed in Fig. 1a and c, SA-ILM 1 and SA-ILM 3 formed “hot spots”. In contrast, SA-ILM 2 spots appeared as dense and homogeneous glycerin-like drops without any presence of crystals (Fig. 1b). The appearance of the spot between SA-ILMs seems to be more related to the final concentration of the organic salt formed by reaction of SA and butylamine (i.e., 50 mM, approx. 3 M, and 300 mM before preparing sample-matrix mixtures for ILM 1, 2, and 3, respectively) than to the ILM preparation method. The higher the concentration of organic salt, the more homogeneous the spot that was obtained. Therefore, as can be observed in Fig. 2, SA-ILM 1 and SA-ILM 3 provided glycoprotein signal only at the rim of the spot (Fig. 2a(ii) and c(ii), respectively) whereas SA-ILM 2 allowed reproducible mass spectra of rHuEPO to be obtained throughout the spot (Fig. 2b(i)). ILM 1 was discarded because it provided broad peaks and signal intensity was too low (Fig. 2a(ii)). These poor results obtained with ILM 1 could be explained as this preparation/concentration was originally optimized for low molecular weight compounds [27], which require lower amounts of matrix to efficiently ionize the analytes of interest. If we compare SA-ILM 2 with SA-ILM 3 mass spectra, the former only showed the singly charged molecular ion of rHuEPO, whereas with SA-ILM 3 mass spectra showed the

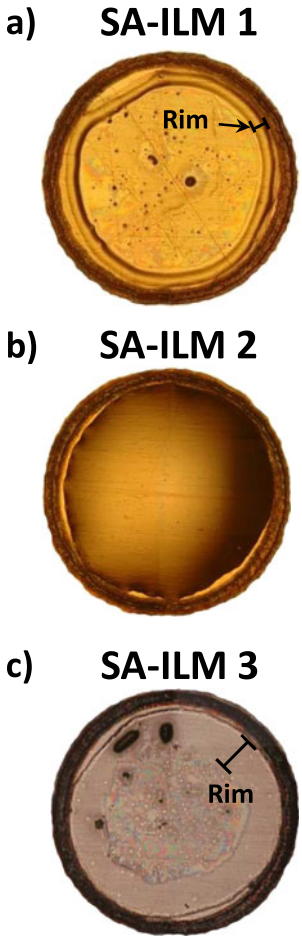

Fig. 1 Optical microscope images of the spots obtained for rHuEPO with SA using **a** ILM 1, **b** ILM 2, and **c** ILM 3 without the addition of acid

singly and doubly charged molecular ions and the singly charged dimeric form of rHuEPO as usually happens with conventional solid matrices like SA and DHB [23]. Signal-to-noise (S/N) ratios obtained with SA-ILM 3 were better than those obtained with SA-ILM 2 but only at the rim of the spot (compare the insets in Fig. 2b, c(ii)). The higher sensitivity obtained with ILM 3 might be caused by the formation of hot spots because glycoprotein molecules were concentrated at the rim, whereas with ILM 2 they were distributed homogenously throughout the spot. But also we cannot discard that the high concentration of organic salt (~3 M) in ILM 2 could have a negative influence on the ionization due to the very dense plume. For that reason, ILM 2 with 1.5 M of organic salt was also tested. Figure 2b(ii) shows the spectrum of rHuEPO under these conditions. S/N ratios did not improve and the quality of the MALDI mass spectra was the same, but we started to have a thin rim at this lower concentration of organic salt. On the other hand, no differences were found on spot appearances and mass spectra in any case with the addition of TFA. Moreover, 1% of H_3PO_4 formed a microcrystalline surface which improved ILM 3 spot homogeneity but the analytical signal was lost (data not shown). Consequently, we continued our studies with ILM 2 with 3 M of organic salt without the addition of any acid.

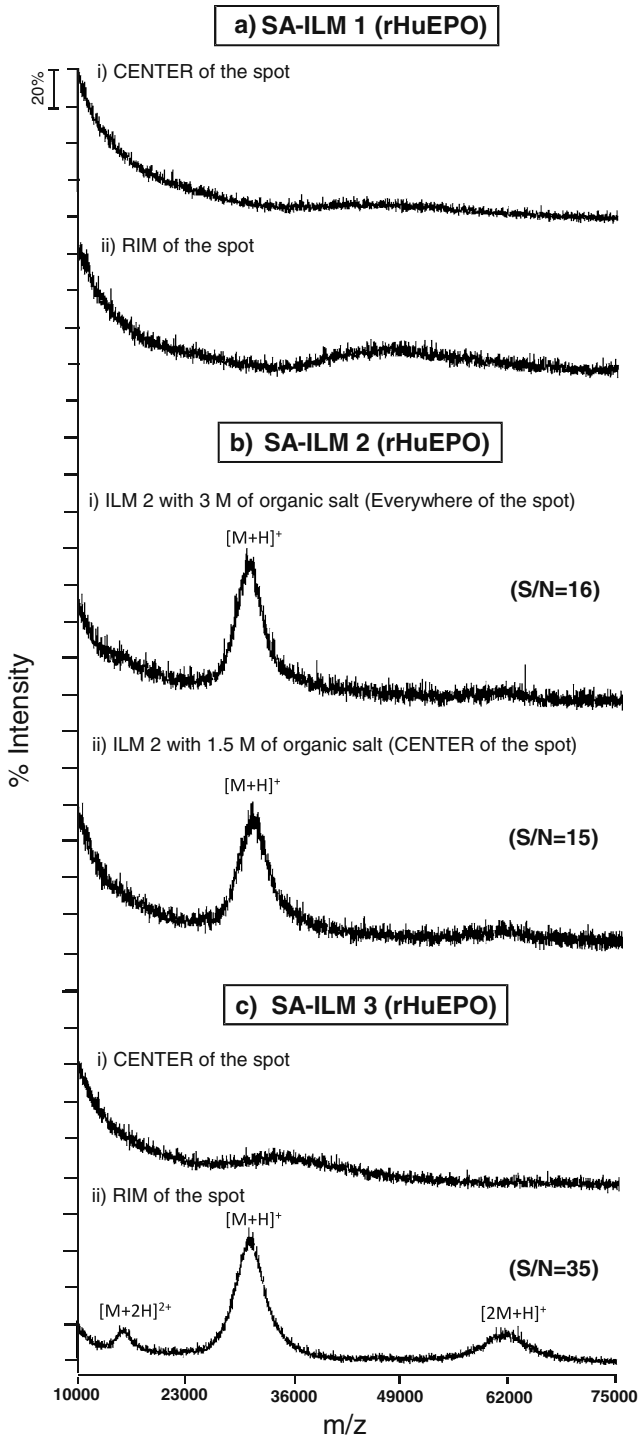

Fig. 2 MALDI-TOF mass spectra of rHuEPO with **a** SA-ILM 1, **b** SA-ILM 2, and **c** SA-ILM 3 at a laser intensity of 72.5% of the maximum (4,000 V). The signal-to-noise (S/N) ratio was calculated by using the software provided by the manufacturer of the Voyager-RP-DE mass spectrometer

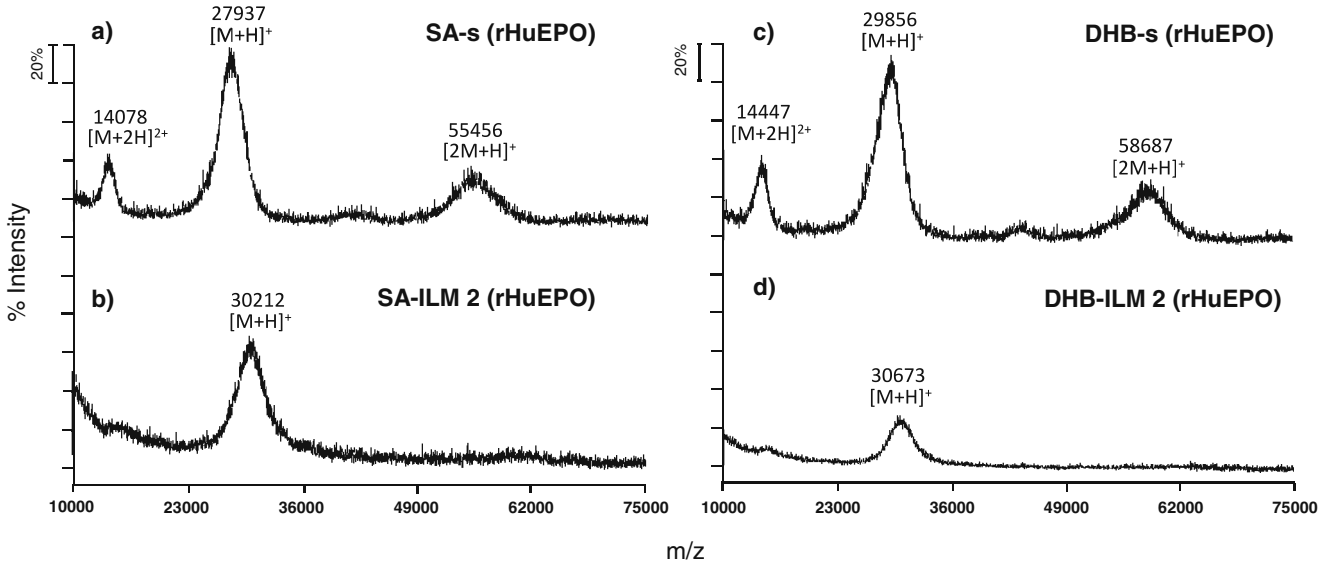

Fig. 3 MALDI-TOF mass spectra of rHuEPO with **a** SA-s, **b** SA-ILM 2, **c** DHB-s, and **d** DHB-ILM 2 at a laser intensity of 72.5% of the maximum (4,000 V)

Comparison of ILM 2 with solid matrices

Mass spectra and S/N ratios

We compared the performance of SA-ILM 2 and DHB-ILM 2 for intact glycoprotein analysis with that obtained with the corresponding solid matrices (SA-s and DHB-s, respectively). Figure 3 shows rHuEPO mass spectra using ILMs 2 and conventional solid matrices with both SA and DHB at the same laser intensity. As can be observed in Fig. 3b and d, following the ILM 2 preparation procedure, we did not observe any peak broadening caused by neutral losses and/or adduct formation, as other authors have suggested for intact proteins using other ILMs [8]. The mass spectra only showed the singly charged molecular ion of rHuEPO, in contrast to those obtained with the solid matrices that perfectly showed the doubly charged molecular ion and the singly charged dimeric form of the glycoprotein (Fig. 3a, c). These differences on mass spectra suggested that the amount of energy transferred to the rHuEPO molecules during the desorption/ionization process with SA-ILM 2 or DHB-ILM 2 was not enough to form the multiply charged ions.

Sensitivity was also affected by the sample-matrix preparation used. As can be observed in the mass spectra in Fig. 3d, DHB-ILM 2 provided lower glycoprotein signal than DHB-s (Fig. 3c). SA-ILM 2 (Fig. 3b) also elicited lower signal intensity than SA-s (Fig. 3a) but the difference was not as striking as that obtained for DHB. Figure 4 shows the average S/N ratio with the corresponding standard deviations (*s*) for the singly charged molecular ion of rHuEPO, obtained from the mass spectra of twelve different spots of glycoprotein with the solid matrices (SA-s and DHB-s) and ILM 2 (SA-ILM 2 and DHB-ILM 2) at the same laser intensity. As can be observed, solid matrices gave better S/N ratio values than ILM 2 preparations, probably because a co-crystallized solid matrix allowed higher ion production because the transfer of energy was higher. This was also the reason why the threshold laser intensity required for signal generation for ILMs 2 was higher than for the solid matrices. However, signal reproducibility significantly improved with ILMs 2 as shown by the standard deviation of the S/N ratio values (*s*) in Fig. 4. The shot-

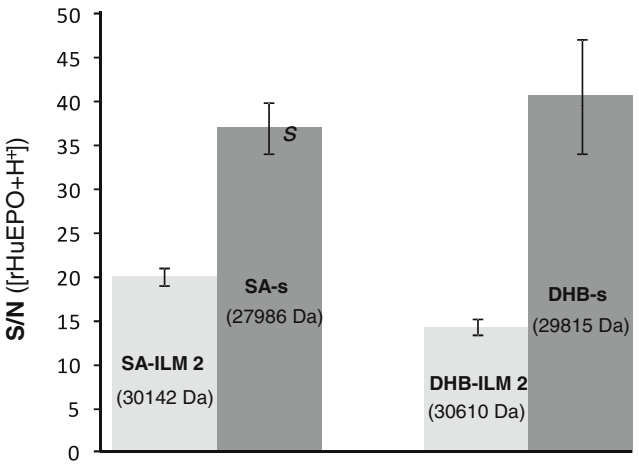

Fig. 4 Signal-to-noise (S/N) ratios (n=12) obtained for the rHuEPO singly charged molecular ion with the studied matrices at a laser intensity of 72.5% of the maximum (4,000 V). *Error bars* show the standard deviations (*s*)

to-shot and spot-to-spot reproducibility with ILM 2 preparations exceeded the reproducibility achieved with s-SA and the fast evaporation method, which is considered one of the best sample-matrix preparation procedures to ensure spot homogeneity and thus highly reproducible results with solid matrices [28]. On the other hand, the average molecular mass values of rHuEPO obtained with DHB-s and SA-s were lower than those obtained with DHB-ILM 2 and SA-ILM 2 (see Figs. 3 and 4). Hence, we decided to study in depth the effect of laser intensity in these novel matrices and its influence on glycoprotein sugar fragmentation.

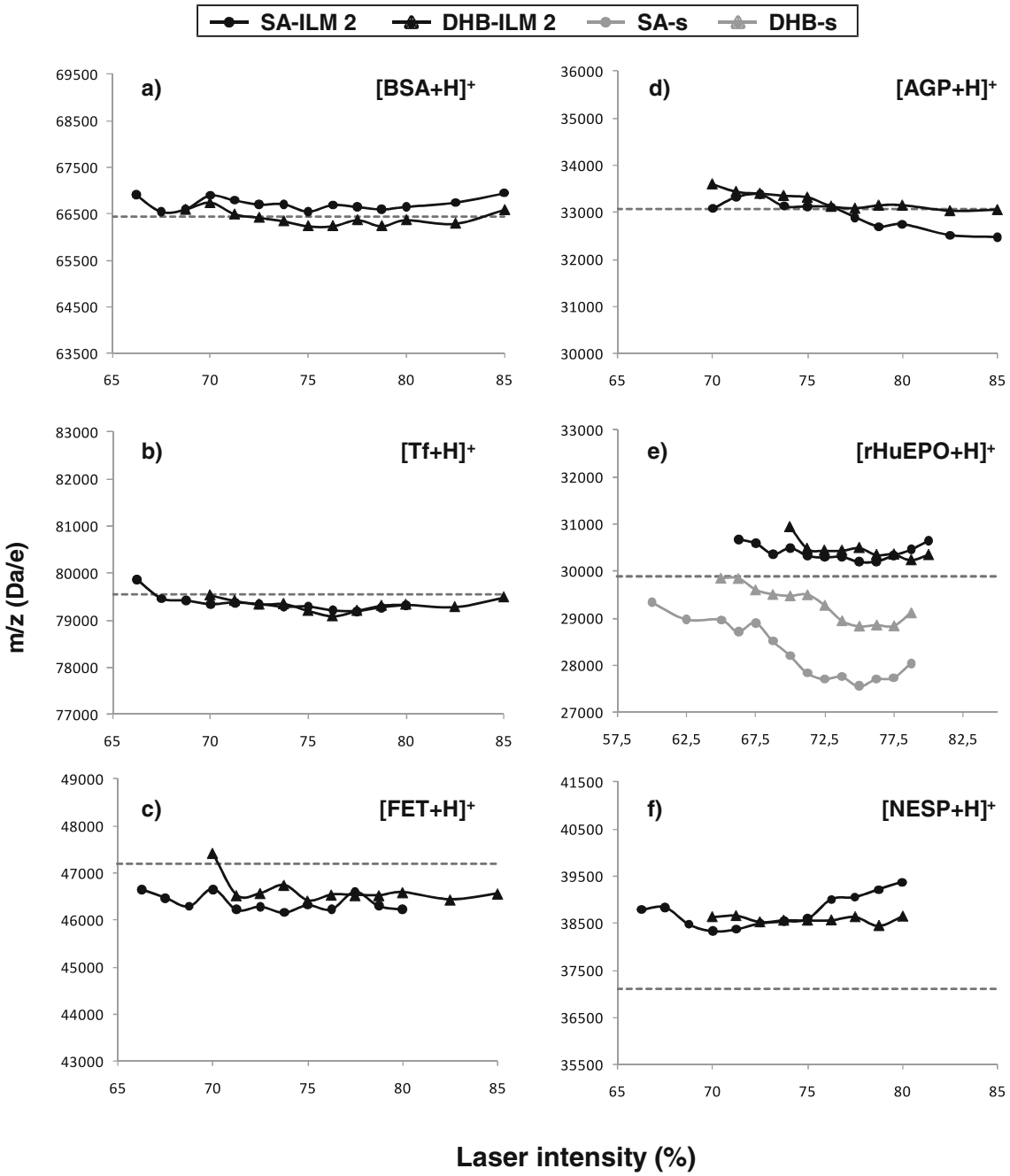

Fig. 5 Influence of the laser intensity on MALDI-TOF mass spectra. Variation of the *m/z* ratio corresponding to the singly charged molecular ion of all the studied proteins: **a** $[BSA+H]^+$, **b** $[Tf+H]^+$, **c** $[FET+H]^+$, **d** $[AGP+H]^+$, **e** $[rHuEPO+H]^+$, and **f** $[NESP+H]^+$ with laser intensity using SA-ILM 2 (—●—), DHB-ILM 2 (—▲—), and SA-s (—●—) and DHB-s (—▲—), the last two of these only in the case of rHuEPO. Each *m/z* ratio value represents the average of three replicates. The *dashed line* represents the molecular mass value reported in the literature for each protein (see Table 1)

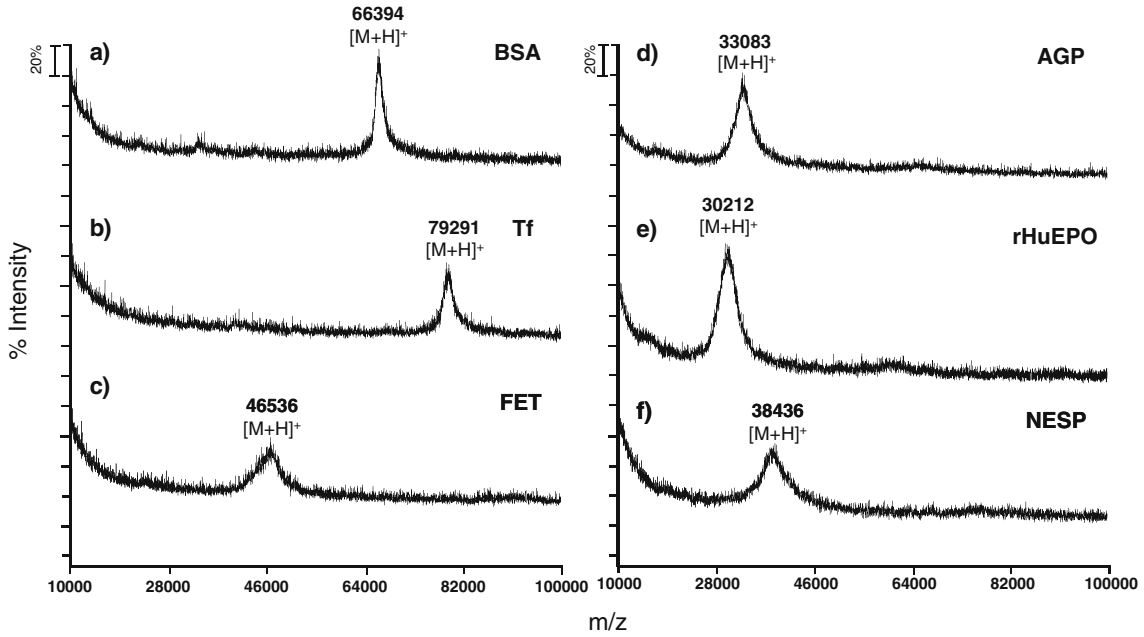

Fig. 6 MALDI-TOF mass spectra of the studied proteins at a laser intensity of 72.5% (maximum 4,000 V) using SA-ILM 2: **a** BSA, **b** Tf, **c** FET, **d** AGP, **e** rHuEPO, and **f** NESP

Laser intensity influence

In a previous study, we demonstrated that intact glycoproteins which presented different degrees of glycosylation could be fragmented to some extent at certain laser intensity values when they were analyzed by MALDI-TOF-MS with SA and DHB solid matrices [23]. In the present work, we evaluated the effect of laser intensity for the same intact glycoproteins but, in this case, using SA and DHB with the ILM 2 preparation. Figure 5 shows the *m*/*z* ratio of the singly charged molecular ion of all the studied glycoproteins and the unglycosylated BSA against the laser intensity, using SA-ILM 2 and DHB-ILM 2. A dashed line shows the *m*/*z* ratio of the molecular mass value reported in the literature for each studied protein (see Table 1) [29–33]. rHuEPO was also analyzed with DHB-s and SA-s at different laser intensity values to allow a direct comparison between ILM 2 preparations and the solid matrices (see Fig. 5e). As can be observed in Fig. 5e, using the solid matrices, increased laser intensities resulted in an *m*/*z* ratio shifting to lower values due to sugar fragmentation in the ion source and/or during acceleration [23]. Sugar loss was greater with SA-s as it is considered to be a "hotter" matrix. Nevertheless, DHB-s also promoted a slight decrease of molecular mass values when laser intensity increased (see Fig. 5e), which suggested that some fragmentation also occurred although it was less dramatic than with SA-s. On the contrary, SA-ILM 2 and DHB-ILM 2 provided better results (Fig. 5). The measured *m*/*z* ratio values remained practically constant over the studied laser intensity range, even with SA-ILM 2, suggesting that sugar fragmentation was strongly reduced. In addition, these values were always higher for all the intact glycoproteins [23] than those obtained with the solid matrices at any laser intensity and hence the molecular mass values obtained with ILM 2 may be a better estimation of the average molecular mass values of each glycoprotein. It is important to mention that the molecular mass value of NESP reported in the literature and shown in Table 1 is theoretically calculated [33], and Tf, AGP, FET, and rHuEPO mass values correspond to the mass of the most abundant glycoform determined by ESI-

Table 1 Values reported in the literature for the molecular mass ($M_{reported}$) and degree of glycosylation for each studied protein

Glycoprotein	Glycosylation degree (%)	$M_{reported}$ (Da)	Reference	$\overline{M} \pm s^c$ (Da)
BSA	0	66,430	[29]	66,512±165
Tf	~6	79,561[a]	[30]	79,338±431
FET	~20	47,188[a]	[31]	46,425±256
AGP	~35	33,059[a]	[31]	33,361±92
rHuEPO	~40	29,888[a]	[32]	30,289±153
NESP	~51	37,100[b]	[33]	38,312±224

Average molecular mass values $(\overline{M})$ obtained by MALDI-TOF-MS using SA-ILM 2 at a laser intensity of 72.5% of the maximum

[a] Molecular mass corresponding to the most abundant glycoform

[b] The average molecular mass of the glycoprotein is an average value of the molecular mass of the component glycoforms

[c] $M(\pm s)$ was calculated as an average of three replicates and s is the standard deviation

MS [30–32]. Hence, the molecular masses in Table 1 are appropriate reference values but in some cases they did not fit with the values obtained with SA/DHB-ILM 2 as the masses in MALDI-TOF-MS of the studied glycoproteins are an average of the molecular masses of all the glycoforms, taking into account their abundance. In the literature there are inconsistent results concerning the fragmentation of the analytes when they are analyzed with ILMs. Most of the conclusions are drawn for low molecular weight compounds and some authors have reported a total suppression of the fragmentation [14], whereas others have still observed loss of labile groups, although to a lower extent [12, 15–17]. In our case, as results with SA-ILM 2 and DHB-ILM 2 were similar, ILM preparation seemed to transfer enough energy to ionize the glycoprotein, but at the same time, it absorbed part of the unnecessary matrix energy, which prevented analyte fragmentation.

We should point out that, as other authors have indicated with other ILMs [12, 14, 24, 25], the ionization efficiency with ILM 2 was slightly worse for unglycosylated BSA or glycoproteins with a lower sugar content (i.e., Tf or FET) than those that presented higher degrees of glycosylation such as AGP, NESP, or rHuEPO (see Table 1). The mass spectra obtained for the unglycosylated BSA at certain laser intensities showed broader peaks than those obtained with SA-s or DHB-s, resulting in a slightly lower mass accuracy than before for the same protein with solid matrices [23].

In general, at the same laser intensity, no significant differences were achieved between the molecular mass values obtained with SA-ILM 2 and with DHB-ILM 2 (see Fig. 5). However, DHB-ILM 2 occasionally provided slightly broader peaks, and it allowed one to work in a more limited laser intensity range in comparison with SA-ILM 2 as the threshold to obtain mass spectra was higher. Consequently, as the most appropriate matrix for the analysis of intact glycoproteins, we propose SA-ILM 2 with a laser intensity slightly higher than the threshold. Figure 6 shows the MALDI-TOF mass spectra of all the studied proteins using SA-ILM 2 at a laser intensity of 72.5%, and Table 1 shows the molecular mass values with their corresponding standard deviations (*s*) obtained under the optimal conditions. As can be observed, the obtained values agree with those reported in the literature, indicating that SA-ILM 2 may be regarded as a simple and reproducible alternative to obtain reliable average molecular mass values for intact glycoproteins.

Conclusions

We have demonstrated that the chemical composition of an ILM very strongly influences the analysis of intact glycoproteins by MALDI-TOF-MS. Ionization efficiencies and spot homogeneity were better using ILMs with higher amounts of organic salt. ILM 2, containing 3 M of butylamine and an equimolar amount of DHB or SA, was selected as the optimal ILM preparation procedure. SA-ILM 2 and DHB-ILM 2 provided less sensitivity than SA-s and DHB-s, but higher shot-to-shot and spot-to-spot reproducibility. Moreover, the obtained average molecular mass values were close to the values reported in the literature at any laser intensity, suggesting that sugar fragmentation was prevented in both cases. SA-ILM 2 was proposed as the best alternative for a reliable analysis of intact glycoproteins by MALDI-TOF-MS as it provided a slightly better sensitivity, sharper ion peaks, and it allowed one to work over a wider range of laser intensities.

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