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Evaluation of the microheterogeneity of transthyretin reference standard materials by MALDI-TOF-MS

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Introduction

Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) is able to detect proteins in complex mixtures in the presence of an excess of salts and buffer components. This study describes the measurement of the microheterogeneity of transthyretin (TTR) by MALDI-TOF-MS.

TTR (formerly known as prealbumin) is involved in the transport of thyroxine and retinol-binding protein-vitamin A complex and is a tetramer of non-covalently bound identical subunits, each with an average mass of 13761 Da. Because the majority of TTR (> 85% of total TTR) in the circulation is post-translationally modified and various mutations are known, a large number of possible TTR variants exists (1). Native TTR is normally only present in blood as a minor variant, while cysteinyl-TTR is the dominant one.

The relevance of this study is the need for methods that can distinguish TTR variants in the diagnosis of ovarian cancer (2) and amyloidosis (3) and evaluate TTR reference standard materials for quality assessment (4). This part deals specifically with the evaluation of TTR reference standard materials.

Materials and Methods

The purchased reference TTR standard preparations were either of a commercial source [Sigma-Aldrich, BioMac and Dade Behring] or an international reference institute [European Reference Material ERM®-DA470] (table 1). According to the product specifications and as far as described, all standard materials were isolated from pooled human plasma samples and as a consequence the standards had a heterogeneous composition. The standard materials were measured by low resolution MALDI-TOF-MS analysis (PBS IIc analyzer, Ciphergen) on a gold chip ProteinChip® array (Ciphergen) using sinapinic acid as the energy absorbing matrix and ammonium phosphate as a matrix additive. Pure TTR standards were analyzed directly without removing salts and buffer components, while TTR standards, which were part of a mixture of proteins, were treated by centrifugal ultrafiltration (Centricon® YM-50, Millipore) to remove high mass proteins (table 1).

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Additionally, all standards were also measured after reduction with dithiothreitol (DTT) in order to convert modified TTR variants with a disulfide bridge into the native TTR variant. Because pure TTR variants are not available, assignment of the TTR variants to the peaks in the mass spectra was based on the measured *m/z* values *versus* reported *m/z* values of the respective $[M + H]^+$ ions in literature assuming that the measured TTR variants were the wildtype TTR variants (5). Two point internal calibration using bovine cytochrome C (12230.92 Da) and equine apomyoglobin (16951.51 Da) was applied. The overall mass accuracy was determined as the mean mass accuracy of 4 different spot measurements.

Results

Figure 1 shows typical examples of the MALDI-TOF-MS mass spectra of the reference TTR standard materials analyzed. The intra- and interassay overall mass accuracy of the major variant in a TTR profile were < 0.03%. Assigned TTR variants were besides native TTR (*c*), three post-translationally modified variants, namely sulfonated TTR (*e*), cysteinyl-TTR (*f*) and glycinecysteinyl-TTR (*g*). Other peaks in the mass spectra (*a, b* and *d*) probably were TTR artefacts in the respective preparations, although it cannot be excluded that TTR variants of mutations were present also. Based on these overall TTR profiles the Sigma-Aldrich preparation was dominated

Table 1. Product specifications of purchased reference TTR standard preparations

Preparation	Supplier	Lot number of standard
Sigma-Aldrich	Sigma-Aldrich Chemie BV (Zwijndrecht)	014K0582
*Purity > 95% (based on SDS gel electrophoresis) from plasma		
Dade Behring	Dade Behring GmbH (Marburg)	08365316
*Mixture of proteins as part of serum control sample		
BioMac	BioMac GmbH (Leipzig)	#04-07
*Purity > 98% (based on SDS gel electrophoresis) from plasma		
ERM®-DA470	IRMM (Geel)	15049
*Mixture of proteins as part of serum control sample		

by post-translationally modified variants, the BioMac and ERM®-DA470 preparation by artefacts, while the Dade Behring preparation was a mixture of both (figure 1).
The data of the DTT reduced TTR preparations were complementary. Only the Sigma-Aldrich preparation showed the appearance of native TTR (figure 1) and the others not (data not shown). The rationalisation is that by the disappearance and appearance of certain TTR variants the dominant presence of either post-translationally modified variants or those of artefacts is confirmed. After all, the appearance of native TTR proves the presence of post-translationally modified variants, while any other result confirms the dominant presence of artefacts.

Discussion

The intra- and interassay variations proved that the profiling of TTR variants by MALDI-TOF-MS was reproducible. Although the mass resolution was not sufficient to distinguish all TTR variants, significant differences were observed between the profiles of TTR variants of some reference standard materials (Figure 1). Of particular interest is the presence of the so-called TTR artefacts, which were dominant in the BioMac and ERM®-DA470 preparations. Based on these data the nature of these artefacts and therefore as well the theoretical *m/z* values of their $[M + H]^+$ ions remains unknown.
Especially for quality assessment of immunological methods to measure TTR, one might wonder to which extent the different TTR variants and artefacts have equimolar responses to the antibodies used and to which extent reference standards resembles the TTR profile in physiological and pathological samples. The role of the internationally accepted standard ERM®-DA470 preparation (equivalent to the CRM 470) in such a sense might be very critical. Moreover, the exact composition of the TTR reference material might explain a certain contribution to the poor inter-method commutability of serum TTR values (4).

Conclusions

MALDI-TOF-MS can provide in a simple way detailed analytical information about proteins. TTR reference standard materials have a heterogeneous composition, which may differ from material to material.

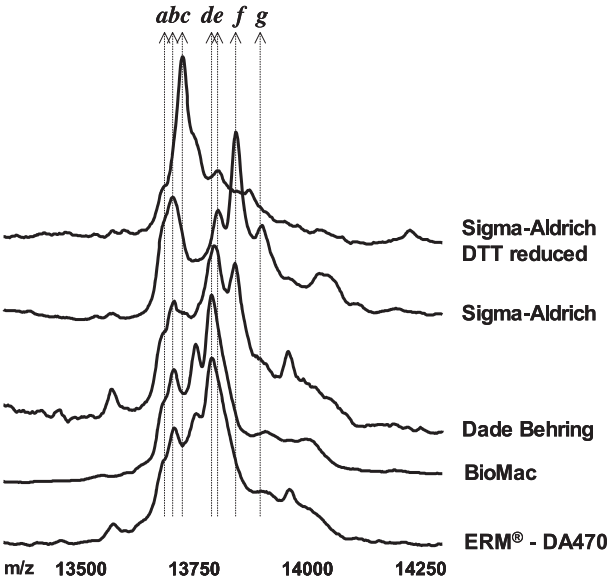

Figure 1. MALDI-TOF-MS mass spectra of reference TTR standard preparations; TTR artefacts (*a, b* and *d*) at unknown theoretical *m/z* values; native TTR (*c*) at theoretical *m/z* 13762.4; sulfonated TTR (*e*) at theoretical *m/z* 13842.3; cysteinyl-TTR (*f*) at theoretical *m/z* 13881.4; glycinecysteinyl-TTR (*g*) at theoretical *m/z* 13938.4. Of the Sigma-Aldrich preparation also the DTT reduced mass spectrum is shown, whereas those of the remaining preparations are not.

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