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Analysis of lipidation of recombinant Lyme vaccine protein (rOspA) by electrospray mass spectroscopy[1]

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Abstract

Lipidation at the N-terminal of recombinant outer surface protein A (rOspA) is an important determinant of its immunogenicity. Lipidation patterns of rOspA can be sensitive to processing environments and storage conditions. In order to assure product consistency and stability, it is essential to characterize and monitor lipidation patterns of rOspA through its life-cycle. Electrospray mass spectroscopy combined with maximum entropy calculation was employed to analyze the lipidation of rOspA. The results revealed that more than 90% of protein is a tri-lipidated rOspA and the remainder is di-lipidated. It was demonstrated that the method is both sensitive and quantitative and has the potential to be used for routine quality control and stability testing. © 1997 Elsevier Science B.V.

Keywords: Electrospray mass spectroscopy; Lyme vaccine protein; rOspA; Lipidation analysis; Maximum entropy calculation

1. Introduction

Lyme disease is a tick-born infection of spirochete *Borrelia burgdorferi*, which can result in skin, joint, cardiac and neurologic manifestations [1]. A recombinant protein (Outer surface protein A, rOspA) based vaccine has been developed for preventing the disease. It has been shown that rOspA is a lipoprotein [2] and significantly, that palmitoylation at the N-terminal plays a critical role in immunogenicity of rOspA [3]. Several possible attachments of palmitic acid to the rOspA N-terminal are illustrated in Fig. 1. Lipidation patterns of rOspA can be sensitive to processing environments and storage conditions. In order to assure product consistency and stability, it is essential to characterize and monitor lipidation patterns of rOspA through its life-cycle.

Conventional lipidation analysis requires extensive sample preparation and analysis, often involving protein precipitation, acidic and/or basic hydrolysis, extraction, derivatization and chromatographic analysis [2,4]. These multi-step procedures are both time and labor consuming. The end result is the molar ratio of lipid to protein

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and no details of lipidation patterns can be obtained. Also, experimental errors, propagating in each sample preparation step, greatly reduce the accuracy of the results.

Recent developments in mass spectroscopy make it possible to characterize large molecules such as proteins. In particular, electrospray mass spectrometry (ES/MS) can be applied to molecular mass measurement with high accuracy and resolution. Accurately measured molecular mass determinations by ES/MS have been performed to characterize proteins post-translationally modified by glycosylation and acylation [5,6].

In this study, efforts were focused on the development of an ES/MS method to characterize lipidation of Lyme vaccine protein and further to investigate the feasibility of using the method for quality control and stability testing. Specifically, ES/MS was applied to rOspA of Lyme vaccine. The results revealed that more than 90% of Lyme protein is a tri-lipidated rOspA with the remainder being a di-lipidated rOspA. Mono- or non-lipidated rOspA were not detected. Based on these results, it was concluded that ES/MS is a quantitative and sensitive method for analysis of protein lipidation and has the potential to be used for routine analysis of Lyme vaccine product.

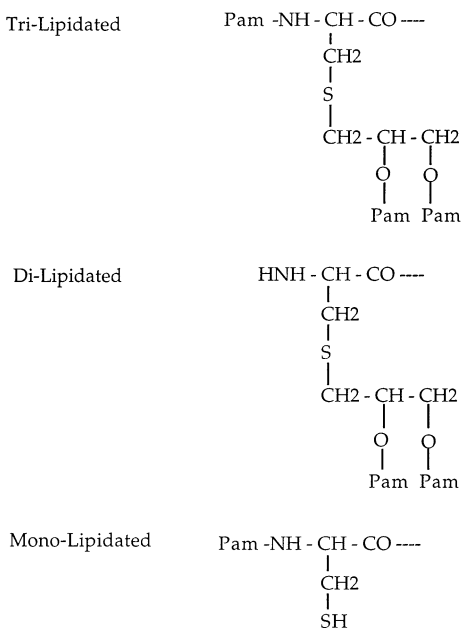

Fig. 1. Illustration of three possible lipid attachments to the N-terminal of rOspA.

2. Experimental

2.1. Samples and sample preparation

rOspA samples were from several manufacturing lots of Lyme vaccine with protein concentrations of ~ 60 μg ml^{-1}. The sample solution was dialyzed against distilled water using Pierce's 10K Dialysis Cassette for 48 h before ES/MS analysis.

2.2. ES/MS

The mass spectra were obtained using a VG Platform II (Micromass) mass spectrometer equipped with an API Electrospray source and a single quadrupole mass analyzer. The instrument was calibrated by 14 multiple-charged protein ions of horse heart myoglobin over m/z range: 650–1450. Mass scanning range was set to the calibration range. The cone voltage was optimized at 38–42 V. For each sample, 8 μl of solution was introduced into the ES ion source in a water/acetonitrile (1:1, v/v) mixture with 0.5% (v/v) of formic acid. The flow rate was 10 μl min^{-1}. The m/z spectrum was transformed to a molecular mass spectrum using MaxEnt (maximum entropy algorithm). In this molecular mass spectrum, molecular mass and peak area of each peak were measured. Based on theoretical molecular mass, possible forms of rOspA protein were identified. The relative percentage of tri-lipidated rOspA was estimated by measured peak area.

3. Results and discussion

3.1. Sample preparation

The vaccine formulation contains salts and detergent. The presence of salts and detergent in the sample solution not only suppresses protein ionization but also interferes with the data processing since their m/z ratios are similar to those of

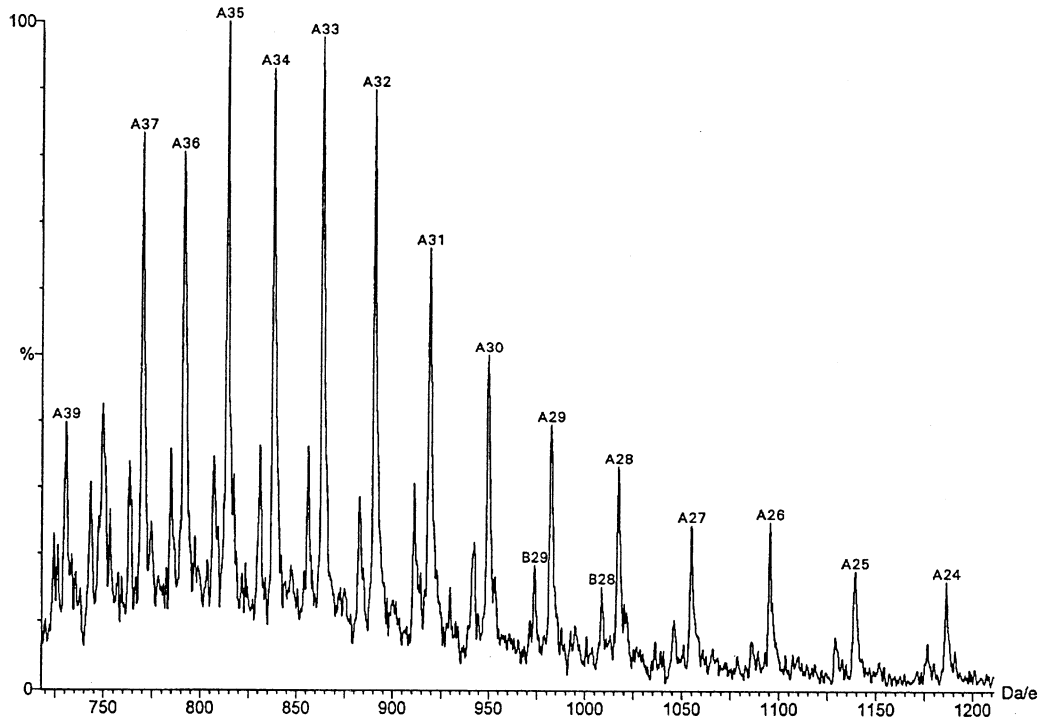

Fig. 2. Typical m/z spectrum of Lyme vaccine protein (rOspA).

protein ions. Dialysis against H_2O, using Pierce's Dialysis Cassette, effectively removed the salts and detergent. Unlike the acetone precipitation method [6], this method avoids using formic acid to redissolve the protein. This will eliminate the potential for hydrolysis of lipid-protein linkage during sample preparation, which is important if the method will be used quantitatively.

3.2. Molecular mass

Fig. 2 is a typical m/z spectrum of rOspA, showing multi-charged protein ion peaks. The charge state profiles for two visible components are very similar, suggesting that these are very closed related proteins. Fig. 3 is a corresponding deconvoluted molecular mass spectrum derived from the m/z spectrum by MaxEtn. The molecular masses measured are listed in Table 1. The theoretical molecular mass of four possible forms of rOspA were calculated based on the DNA sequence and also listed in Table 1 for reference. Comparison between theoretical and experimental molecular mass clearly indicates that the major component (Peak A) is a tri-lipidated rOspA and the minor component (Peak B) is a di-lipidated rOspA. Neither mono-lipidated nor non-lipidated rOspA is present. The peak at 28 490 Da was not identified. One possible explanation is that it is a di-sodium adduct peak since it is 44 Da higher than 28 446 of tri-lipidated rOspA. It should be noted that the molecular mass spectrum produced by MaxEnt yields very narrow peaks: < 10 Da for rOspA. This high resolution molecular mass spectrum provides a sensitive means to detect modification of the protein.

3.3. Relative percentage of tri-lipidated rOspA

Since the molecular mass spectrum was produced from m/z spectrum using MaxEnt, a maximum entropy calculation algorithm, it is possible

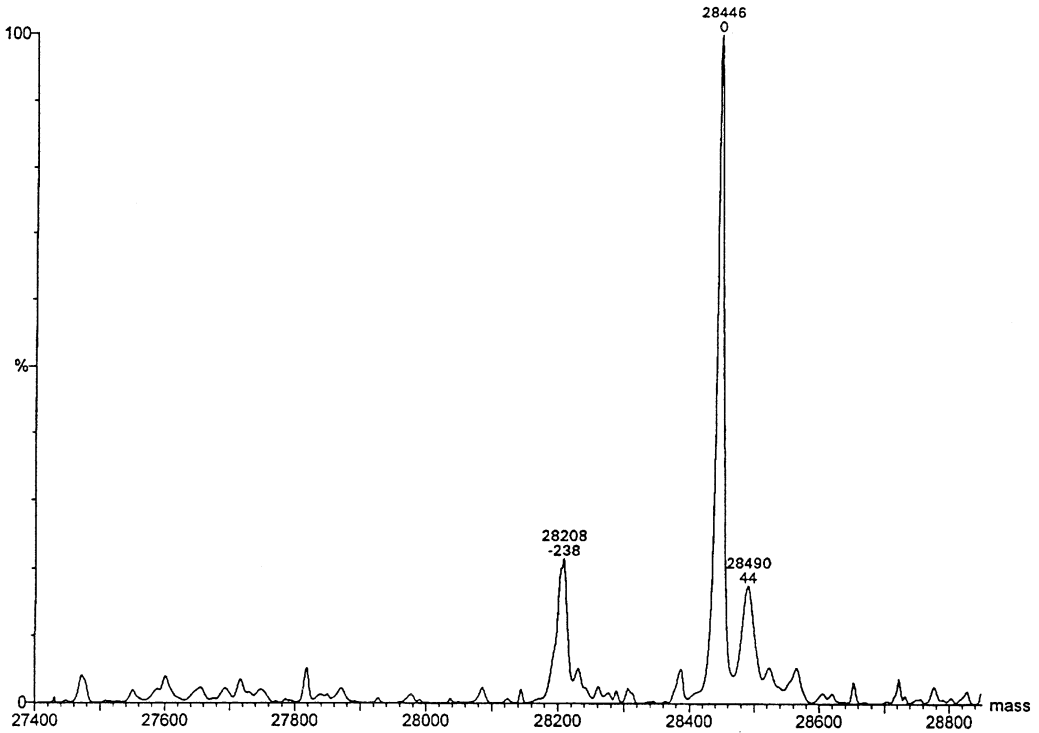

Fig. 3. Molecular mass spectrum of Lyme vaccine protein (rOspA), produced by maximum entropy calculation (MaxEnt).

to evaluate the data quantitatively [7,8]. The area of the peaks in the molecular mass spectrum were integrated and are also listed in Table 1. Due to lack of a standard for any single form of lipidated rOspA, no attempt was made to correlate peak area to protein concentration. Instead, the relative percentage of tri-lipidated rOspA was estimated. By measuring the relative percentage instead of absolute values, the accuracy of the results is much less dependent on instrumental conditions and other variables. A LC/UV/MS experiment was carried out to determine if the ionization (MS response) of different lipidation patterns of rOspA might vary due to lipid attachment, since such variation affects the relative percentages determined. It was observed that peak area ratio of tri- to di-lipidated rOspA in the UV profile was similar to that in TIC profile. This suggests that different lipid attachment to rOspA does not affect the MS response and that relative peak area ratios can be correlated to relative molar ratios of different forms of rOspA.

3.4. Feasibility of ES/MS method for QC and stability testing

As demonstrated above, this method, with accurate and precise mass measurements and the quantitative MaxEnt calculations, provides a powerful tool to detect lipidation variations of rOspA. The method is also fast and relatively simple to be carried out. Thus, it has the potential to be used for routine quality control and stability testing.

4. Conclusion

ES/MS was applied to several lots of recombinant Lyme vaccine to characterize lipidation of rOspA. The measured molecular mass of rOspA matched well with the theoretical value calculated from its DNA sequence and degrees of lipidation. The resolution of the spectrum was sufficient to

Table 1
ES/MS data of Lyme vaccine protein (rOspA)

Sample	Peak A		Peak B		A (%)
	MW (Da.)	Area	MW (Da.)	Area	
1	28 449	280	28 212	26	91.5
2	28 447	266	28 208	18	93.7
3	28 445	250	28 208	20	92.6
Average	28 447		28 209		92.6
S.D.	1.6		1.9		0.9
Lipidation form:		Tri-	Di-	Mono-	Non-
Theoretical MW (Da):		28 444	28 205	27 893	27 655

resolve different degrees of lipidation. Based on the integration of the peaks in the molecular mass spectrum produced by maximum entropy calculation, it was estimated that more than 90% of Lyme vaccine protein is a tri-lipidated rOspA and the remainder is di-lipidated. Mono- and non-lipidated rOspA were not found. Preliminary validation data suggested this ES/MS method has the potential to be used for routine quality control and stability testing.

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