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Bovine serum albumin as a universal suppressor of non-specific peptide binding in vials prior to nano-chromatography coupled mass-spectrometry analysis

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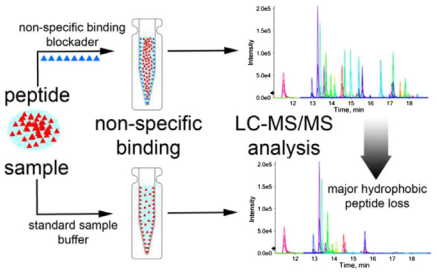
HIGHLIGHTS

- NSB to polypropylene vials for complex peptide mixtures was analyzed.
- Different agents were analyzed for NSB suppression and nanoLC-MS/MS compatibility.
- Peptide and protein matrices block NSB.
- In the peptide matrix ionization suppression of target peptides was observed.
- 0.1% BSA NSB blockader is fully compatible with nanoLC-MS/MS analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Non-specific binding (NSB) is a well-known problem for any application that deals with ultralow analyte quantities. The modern nano-flow chromatography coupled tandem mass-spectrometry (nanoLC-MS/MS) works with the lowest conceivable analyte concentrations. However, while the NSB problem is widely accepted and investigated for metabolomics and single-peptide medicine-related assays, its impact is not studied for complex peptide mixtures in proteomic applications. In this work peptide NSB to a common plastic autosampler vial was studied for a model mixture of 46 synthetic peptides. A significant NSB level was demonstrated for total peptide concentrations of up to 1 mg mL^{-1}. Different agents were tried for NSB suppression and compatibility with nanoLC-MS/MS analysis: a chaotropic agent, an amino acid mixture, a peptide mixture and a protein solution. The first two were inefficacious. The peptide matrix blocked NSB, however, it also led to analyte ionization suppression in nanoLC-MS/MS. The protein solution (0.1% BSA) efficiently eliminated NSB, while a trap-elute nanoHPLC configuration together with a small-pore reverse-phased sorbent effectively and quantitatively extracted the model peptides and depleted protein material from the sample. Higher protein concentration partially impeded peptide extraction. Thus, the 0.1% BSA solution might be

Abbreviations: NSB, non-specific binding; BB, basic buffer; AbsRate, absorption rate; InitInt, initial intensity; RT, retention time; GuHCl, guanidine-hydrochloride.

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regarded as an effective non-interfering blockader of NSB for sample resuspension and storage in an autosampler prior to LC-MS/MS analysis.

1. Introduction

Non-specific binding (NSB) to container material is a universal problem for all applications which deal with ultra-low analyte concentrations (e.g. radio-ligand analysis or immunochemistry [1–3]). Modern chromatography-coupled tandem mass-spectrometry analysis has an absolute sensitivity in zeptomole (10^{-21} mol) range [4,5] and it is one of the most sensitive techniques known. Such concentrations are low enough not only to introduce very high error rate due to NSB but it is possible for analytes to disappear from the solution completely.

It is obvious, that NSB would become more and more troublesome with the decrease in the concentration of an analyte and in the complexity of the matrice. The NSB problem is widely recognized in LC-MS assays in clinical studies, e.g. in pharmacokinetics or biomarker analysis, especially for small molecules and metabolomic studies [6–8], while most of the modern proteomic experiments deal with highly complex mixtures (especially in bottom-up proteomics after tryptic digestion) with rather high material concentrations (e.g. 1–10 mg mL^{-1}) and usually the impact of biological or technical variations would greatly surpass that of any possible NSB to storage container material. In the proteomics field NSB is mostly known to affect samples during an ultrafiltration sample preparation step [9,10], however the problem is generally recognized.

Lately, a new branch of proteomics aimed at high-throughput analysis of peptides of endogenous origin (peptidomics) has been actively developing. And peptidomics deals with just low enough peptide concentrations (e.g. analysis of peptide antigens presented on MHC proteins [11] or of endogenous plasma peptides [12]) where NSB might lead to grave consequences especially when going quantitative. Another case with a very high NSB probability due to very low material concentration is a LC-MS/MS identification of in-gel digested proteins from 1D or 2D electrophoresis gels, especially, silver-stained gels.

The most NSB dangerous step of modern LC-MS/MS analysis is when the samples are stored in autosampler vials or plates. Before that, it is possible to dry them, to freeze them, to control storage conditions in any possible way, but when the samples are set in a queue for a round-the-clock LC-MS analysis it is impossible to prepare each sample just before the injection. And even this might not solve the problem if a peptide NSB rate is fast enough – there would always be a several minute time lag between the sample transfer into the vial and the injection.

Usually, NSB blocking conditions are selected for a single analyte under analysis and it might end up with some very special agents [13,14]. It is obviously not applicable for research peptidomic analysis where a set of different peptides is supposed to be analyzed simultaneously, thus a kind of a universal storage solution is needed.

Besides, the LC-MS/MS technology itself restrains the range of possible NSB blocking agents. The most important thing for any NSB suppressing additive is the lack of interference with the analytical method [15–17], thus any component which might affect chromatography, electrospray ionization process or mass-spectrometry analysis should be best avoided or at least thoroughly checked. It is obvious that such complex mixtures of uncontrollable composition as serum or milk, which are among the most popular unspecific interaction suppressors, would be very risky due to potential effects on LC and MS stages. Among the suggested LC-MS compatible solutions are special container material use, such as silicon-coated glass or low-sorption plastic (these are however not a universal remedy [18]), preliminary plastic coating with unrelated peptides [12] and a mixture of BSA and detergents as a peptide resuspension sample buffer [18]. These were however tested for micro-flow LC-MS/MS analysis, while most of the modern research studies use nano-chromatography, which on one hand is much more sensitive, while on the other hand is much more fragile and sensitive to physical contamination both in LC and MS parts (e.g. any detergent must be avoided).

In this work we studied NSB in a common plastic autosampler vial for a set of 46 synthetic peptides of very different amino acid composition, charge and hydrophobicity. In contrast to the usual way to test NSB action which is to analyze signal response factor to the analyte concentration (titration curves), we analyzed NSB dynamics measuring peptide intensities immediately after putting them in a vial and after different times of incubation. Overall we checked 5 different storage compositions. All of them were based on (1) the basic buffer for LC-MS/MS analysis (2% ACN, 0.1% TFA): (2) a chaotropic agent – 6 M guanidine-hydrochloride (GuHCl) (known to block NSB in ultrafiltration spin-cartridges [9]), (3) a 0.1% amino acid mixture (since peptides consist of amino acids, an excess of free amino acids in a solution should theoretically block all unspecific binding sites), (4) a 0.1% peptide mixture – several other unrelated peptides in an excessive concentration (somewhat similar to unrelated peptide surface coating [12]), (5) a 0.1% BSA solution – this solution is commonly used for blocking non-specific interactions in many applications [18,19].

2. Materials and methods

2.1. Chemicals and consumables

Amino acid derivatives and all the other chemicals for peptide synthesis were from Novabiochem (Merck). Dimethylformamide and dichloromethane were from ChimMed (Moscow, Russia). Formic acid (FA), trifluoroacetic acid (TFA), free amino acids, guanidine-hydrochloride and BSA were from Sigma–Aldrich. HPLC grade solvents for peptide isolation and LC-MS grade solvents for LC-MS/MS analysis were from Merck. C18 SPE 50 mg Discovery cartridges were from Supelco (Sigma–Aldrich). Autosampler vials were from Thermo Scientific National (Thermo Fisher Scientific) (product number C4011-13).

2.2. Peptide and NSB blocking solution preparation

Peptides were prepared by standard solid-phase Nα-Fmoc chemistry and isolated by HPLC up to >95% purity (UV 210 nm). Target product fidelity was confirmed by MALDI TOF MS analysis. Individual peptide stock solutions were prepared gravimetrically at 1.5 mM concentration. 46 target peptides were mixed in a stock solution with individual peptide concentration 32 pmol mL^{-1}. The solution was aliquoted and frozen. All the experimental dilutions were made immediately before the analysis by no more than 50