

A synthesis of new, bi-labeled peptides for quantitative proteomics.

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Artykuł

Isotopically labeled peptides are often used in proteomics as internal reference allowing quantification of peptides by isotopic dilution method. Although the synthesis of peptides labeled with stable isotopes is relatively simple, there are several factors limiting application of these standards in proteomic research: cost of labeled derivatives of amino acids, time needed to obtain labeled peptide and problems with quantification of the standard. To solve these problems we developed a method of synthesis of peptides labeled with heavy oxygen and with a dabsyl moiety. The chromophoric group facilitates the determination of peptide concentration while sequence of peptide allows enzymatic cleavage of fragment containing dabsyl from peptide leaving "natural" sequence with incorporated (18)O atoms. The approach proposed herein is based on the "analytical construct" concept. The experiments performed on model peptides demonstrated that response factors in HPLC analysis of labeled peptides does not depend on the sequence and tryptic hydrolysis of obtained conjugates is completed in minutes producing labeled standards useful in quantitative proteomics.

BIOLOGICAL SIGNIFICANCE:

The reported method allows for a cheap and efficient synthesis of peptides labeled with heavy isotopes, and for their precise quantification. Peptides of our design are stable, and the isotopic label, which is a part of the peptide backbone, is stable as well. Moreover, they can be quickly quantified in solution at any time, so the possible decomposition of standard or a non-uniform distribution of the peptide in lyophilisate does not pose a problem. Therefore, we deem our synthesis to be useful for a broad range of quantitative proteomics methods. In addition, the procedure described herein allows direct application of crude peptides as the analytical standards. The elimination of expensive and time-consuming chromatographic purification reduces the cost of AQUA peptides and gives the possibility of a rapid preparation of large libraries of proteolytic fragments.

Słowa kluczowe

Isotopic dilution, Isotopic labeling, MRM, quantitative proteomics

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