

论文

电喷雾离子阱质谱法研究蛋白与小分子配体间非共价结合的方法学探讨

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摘要:

以小檗碱与 α_1 -酸性糖蛋白间的非共价结合作为例,提出了一种新的质谱滴定法。其主要假定条件是:当蛋白与小分子配体间有一定亲合力、且作用配体的摩尔量大于作用蛋白的摩尔量时,蛋白复合物总浓度与作用蛋白总浓度近似相等。通过不同摩尔量比的小檗碱对 α_1 -酸性糖蛋白的电喷雾离子阱质谱(ESI-IT/MS)滴定实验,分析小檗碱与 α_1 -酸性糖蛋白复合物的结合特点,并应用荧光法进行验证。结果表明应用质谱滴定法与荧光法得到的小檗碱与 α_1 -酸性糖蛋白复合物的结合特性常数(如稳定常数、结合位点数、作用力类型)基本相同。与传统质谱滴定法相比,本文提出的ESI-IT/MS滴定法用于蛋白与小分子配体间非共价结合特性研究,所用仪器相对便宜,方法简单且适应性强,在一些研究体系中可得到更准确的实验结果。

关键词: 电喷雾离子阱质谱 荧光 小檗碱 α_1 -酸性糖蛋白

Methodology of electrospray ion trap mass spectrometry for analyzing the non-covalent binding of protein and low-molecular-weight ligand

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Abstract:

A new MS-titration method for the non-covalent binding of protein-ligand based on the research of berberine and α_1 -acid glycoprotein was established. The major presumption of new method is that the total concentration of protein-ligand complex is approximately the same as the total concentration of acting protein if a certain extent of affinity is existed between protein and ligand, in addition, the mole amount of acting ligand is more than that of acting protein. The non-covalent binding behaviours between berberine and α_1 -acid glycoprotein was studied by using electrospray ionization ion trap mass spectrometry (ESI-ITMS), and the results were verified by fluorescence quenching method. The results showed that the binding behaviours between berberine and α_1 -acid glycoprotein, for example, stability constant, number of binding site, type of the main binding force, were almost the same by using the new MS-titration method and fluorescence quenching method. Comparing with the reported MS-titration method, the presented MS-titration method in this paper is more simple and applicable, does not demand much for the devices, and can lead to reliable results in same cases.

Keywords: fluorescence berberine α_1 -acid glycoprotein electrospray ion trap mass spectrometry

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