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DNA 和 *L*-精氨酸胺非共价结合常数测定中的电喷雾质谱研究

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Determination of Dissociation Constant of Noncovalent Complex of DNA with L-argininamide Using ESI-FTICR/MS

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Abstract: Electrospray ionization mass spectrometry (ESI-MS) has been developed fast in more than one decade and widely used for studies of noncovalent interactions between protein and protein, protein and nucleic acid, as well as bio-molecule and drugs. As a result, determinations of binding stoichiometry and binding sites are available. In addition, it has been used for determinations of binding constants. However, precise determinations of binding constants calculated from ion abundances depend on the precise determinations and calculations of gas-phase ion abundance. This study selected a well studied 24mer hairpin DNA strand that specifically interacts with L-argininamde as a model studied the relationship of gas-phase ion abundances and ion concentrations in varieties of solution (water, fixed concentrations of ammonium acetate, various concentrations of ammonium acetate) using elelctrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR MS) in negative ion mode. The purpose is to determinate dynamic range of multiply charged DNA ions. The binding constants were detected in different ratios of DNA and L-argininamide (1∶1, 1∶2, 1∶3 and 1∶4), the constant obtained in 1∶4 ratio shows good agreement with CD determination, in which both abundances of DNA and DNA-Largininamide complex ions fall in a linear dynamic range. The determination of ion dynamic range and the calculation of multiply charged ion abundance presented in this work will benefit others who intend to detect precise concentrations of multiply charged ions and binding constants of noncovalent complexes.

Key words: electrospray ionization fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR/MS); dissociation constant; noncovalent complex and ion abundance

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近十几年来电喷雾质谱(ESI-MS)技术迅速发展并广泛地用于研究蛋白与蛋白,蛋白与核酸以及生物分子与药物的相互作用。这一技术不仅可以直接测定非共价复合物的化学量、结合位点,而且已经用于测量复合物的结合常数[1-3]。由测定的相对离子强度计算的复合物的结合常数,其准确性不仅取决于离子从溶液到气相的转化效率,而且取决于气相离子强度的准确测定和计算。这一研究工作以发卡构型的 24 个碱基 DNA 与 *L*-精氨酸胺形成特殊的非共价相互作用为模型 A[4-5],使用傅立叶变换离子回旋共振电喷雾质谱仪(ESI-FTICR/MS)在负离子模式下考察了带有多电荷的 DNA 离子在变化的溶液条件下(水、固定浓度乙酸胺、相对浓度乙酸胺)其相对离子强度与浓度的关系,确定了多电荷 DNA 离子线性浓度测定范围。此外测定了 50 μM DNA 分别与 50,100,150 及 200 μM *L*-精氨酸胺混合下的解离常数。结果显示不同的 DNA-*L*-精氨酸胺配比下测得的解离常数不同,在 50 μM DNA 与 200 μM *L*-精氨酸胺下测得解离常数与文献报道 CD 测定结果呈现好的吻合,而在此配比下 DNA 及复合物离子强度落入线性浓度测定范围。由于 ESI-FTICR/MS 生成多电荷和多同位素的复杂离子,增加了离子强度计算的复杂性,这项研究工作结果对定量测定多电荷离子浓度,以及用 ESI-MS 法准确测定复合物的解离常数具有重要的参考价值。

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