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用多维**NMR**谱和圆二色**CD**光谱研究蛋白质亲环素**A**的结构稳定性

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摘要 本文结合异核多维NMR谱和圆二色CD光谱研究蛋白质人亲环素A的结构稳定性。由CD测量亲环素A的去折叠自由能变化，用H/D交换NMR实验测量慢交换的酰胺质子的交换速率，用2D SEA-HSQC实验测量快交换酰胺质子的交换速率，用二维变温HSQC实验测量酰胺质子化学位移的温度系数。结果表明蛋白质亲环素A的结构相当稳定，酰胺质子的交换速率和温度系数与蛋白质空间结构显示了很好的相关性。经过86天的H/D交换，仍有44个残基有残留的谱峰，其中26个残基位于二级结构α_2，β_1，β，β_5，β_6和β_7上（73%是疏水性残基），可能构成了亲环素A稳定的疏水核。

关键词 亲环素A,结构稳定性,质子交换,NMR,圆二色光谱

分类号

Study of Structural Stability of Cyclophilin A by NMR and Circular Dichroism Spectra

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Abstract The structural stability of cyclophilin A (CypA) was investigated using H/D exchange and temperature coefficients of chemical shifts of amide protons, monitored by 2D heteronuclear NMR spectroscopy. Amide proton exchange rates were measured by H/D exchange experiments for slow-exchange protons and measured by SEA (Solvent Exposed Amides)-HSQC experiments for fast-exchange protons. Temperature coefficients of chemical shifts and hydrogen exchange rates of amide protons show reasonably good correlation with the protein structure. Totally, 44 out of 153 non-proline assigned residues still exist in 86 d of hydrogen-deuterium exchange at 4 ℃, suggesting that CypA structure should be highly stable. Residues in secondary structures of $\alpha 2$, $\beta 1$, $\beta 2$, $\beta 5$, $\beta 6$ and $\beta 7$ might constitute the hydrophobic core of the protein. The change in free energy of unfolding ( ) of CypA was estimated to be (21.99±1.53) kJ•mol^{-1} by circular dichroism (CD). The large free energy change is also an indicator of the high structural stability.

Key words cyclophilin A structural stability hydrogen exchange NMR circular dichroism

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