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蛋白质水力学半径的测定及用于蛋白质变性过程的实时监控

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摘要

采用纳米粒度分析仪Zetasizer Nano测定了牛血清白蛋白（BSA）的水力学半径，考察了pH、离子强度、表面活性剂等的影响，并用于BSA热变性和脲变性过程的实时跟踪。随pH增大，BSA水力学半径呈“U”形变化趋势;酸性条件下，分子膨胀，随着盐浓度升高，BSA分子先小幅减小后显著增大;中性pH范围，离子强度对蛋白质分子尺寸影响很小，蛋白质性质相对稳定。离子型表面活性剂在蛋白质表面吸附，从而影响BSA水力学半径。通过水力学半径的测量实时跟踪蛋白质变性过程的分子变化，发现增加热变性中离子强度可加快变性速率，SDS加入增大了BSA变性温度Tm。脲在促使BSA分子扩张的同时，对链伸展有抑制作用;在DTT存在下，BSA的水力学半径随着时间变化逐渐增大。结果表明，简便的水力学半径测量可以用于蛋白质大小的表征，并可实时跟踪蛋白变性过程的分子尺度变化。

关键词

水力学半径 牛血清白蛋白 表面活性剂 热变性 脲

分类号

Measurement of protein hydrodynamic radius and its applications to monitor protein denaturation

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Abstract

The hydrodynamic radius（Rh）of bovine serum albumin（BSA）was measured with the Zetasizer Nano instrument.The influences of pH，ionic strength and surfactants were investigated，and the real-time measurement of BSA size was used to monitor the thermal denaturation and urea denaturation of BSA.The results indicated that R_h showed a typical “U” form with the increase of pH.In acid solution，with increasing ionic strength the Rh decreased a little first，and then increased remarkably.At neutral pH the influence of ionic strength could be neglected.The adsorption of ionic surfactants on BSA molecular surface resulted in the change of R_h.For the thermal denaturation of BSA，the rate of denaturation increased with the increase of ionic strength.When sodium dodecyl sulfate（SDS）was added，the melting point T_m of BSA could increase.For urea denaturation，urea could cause BSA molecule to expand but also restrain the extension of peptide chains.By adding dithiothreitol（DTT），the Rh of BSA increased gradually with denaturation time.The results indicated that this convenient measurement of Rh could be used for characterizing the molecular size of protein，and monitoring the change of protein molecule during the denaturationprocess.

Key words

hydrodynamic radius bovine serum albumin surfactant thermal denaturation urea

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