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荧光研究表明去辅基天青蛋白突变体M121L至少存在两种不同的构象

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以往对绿脓杆菌去辅基天青蛋白变性机制的研究认为它经历了一个复杂的反应过程，相比之下，锌离子替代的天青蛋白的变性符合简单的二态模型（Engeseth and McMillin (1986) Biochemistry 25, 2448-2455; Leckner et al. (1997) Biochim. Biophys. Acta 1342, 19-27）。本文以脲为变性剂对去辅基天青蛋白突变体M121L的变性过程进行了研究。结果表明，虽然稳态条件下突变体的变性/复性符合二态模型，但其动力学过程复杂，并可用溶液中存在着两种可以相互转化的构象的变性/复性来解释。天然态N1去折叠的速度快，其重折叠的速度也快，N1的折叠机制可用存在着折叠途径上的快速折叠中间体模型来描述；天然态N2的去折叠速度慢，其重折叠主要是首先生成天然态N1，然后再缓慢的转化成N2。添加Zn2+能够把两种构象整和成一种构象，相应地，Zn2+替代的天青蛋白突变体的变性过程简化为单指数过程。

Heterogeneity of apoazurin mutant M121L from Pseudomonas aeruginosa : A fluorescence study

Unfolding of Pseudomonas aeruginosa apoazurin has been suggested to be more complex than the Zn2+ substituted form (Engeseth and McMillin (1986) Biochemistry 25, 2448-2455; Leckner et al. (1997) Biochim. Biophys. Acta 1342, 19-27). This complexity was investigated using a mutant M121L with urea as denaturant. Although the equilibrium unfolding/refolding showed a two-state transition, its kinetic behaviour was complex and could be best understood as two interconvertible conformations coexisting in solution. One conformation (N1), which was unfolded fast, was found to be refolded independently through a three-state mechanism with a fast-populated intermediate on its pathway, while refolding of the other (N2), which was unfolded slow, was dominantly through N1 folding pathway, then to be isomerised into N2. Adding of the extraneous ligand Zn2+ could integrate these two native conformations into a unique complex and the corresponding unfolding kinetics was reduced to a monophasic process. This provides new insight on the unfolding behaviour of this protein.

关键词

天青蛋白(urea denaturation); 变性(unfolding); 折叠机制(azurin); 荧光(fluorescence); 多种构象(stopped-flow)