

Affinity chromatography on the base of denatured proteins as a tool for purification and analysis of molecular chaperones in cell lysates

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Abstract

Molecular chaperones are mainly members of the family of heat shock proteins (HSPs), which participates in folding, transmembrane transport and degradation of numerous cellular proteins. The common property of chaperones is an interaction with polypeptides, which have no rigid spatial structure. In the present work, we demonstrate the use of affinity chromatography on the base of denatured proteins for one-step purification and the analysis of chaperones in cell lysates of different origin. Affinity sorbents on the base of cheap commercial protein products such as lysozyme, cytochrome, α -lactalbumin and pepsin, covalently attached to BrCN-Sepharose, were prepared and tested using GroEL chaperonin. The conditions for the interaction and dissociation of GroEL and the substrate proteins in different conformational states both in solution and in the affinity column were studied. The dissociation constants of some complexes and capacity of some sorbents were evaluated using titration experiments. It was shown that the affinity sorbent on the base of denatured pepsin allows simple purification of native GroEL from fluorescent impurities, tightly bound to it after standard isolation from cells. Using affinity sorbent on the base of denatured lysozyme, cell lysates of different origin (bacteria, archaea, mitochondria) were analyzed. It was shown that mainly GroEL-like chaperonins tightly interact with denatured lysozyme, although other proteins (depending on the lysate origin) were also found upon elution from this affinity sorbent.

Biography

Natalia Yu Marchenko has completed her PhD in the Kharkiv National University, Ukraine and Institute of Protein Research, RAS, Russia. Currently, she is a Researcher at the Institute of Protein Research, RAS. Her work is focused on protein-protein interactions, specifically on studying the chaperone-protein interaction using different physical and chemical methods, in particular, affinity chromatography. She has published 10 papers in peer reviewed journals.

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