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Research review paper

Prediction of protein retention in hydrophobic interaction chromatography

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Abstract

Hydrophobic interaction chromatography (HIC) is a powerful technique for protein separation. This review examines methodologies for predicting protein retention time in HIC involving elution with salt gradients. The methodologies discussed consider three-dimensional structure data of the protein and its surface hydrophobicity. Despite their limitations, the methods discussed are useful in designing purification processes for proteins and easing the tedious experimental work that is currently required for developing purification protocols.

Keywords: Hydrophobic interaction chromatography; Average surface hydrophobic; Hydrophobic contact area; Local hydrophobicity

Contents

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1. Introduction

Hydrophobic interaction chromatography (HIC) is a key technique that is used in purifying proteins. The HIC process consists of injecting a protein sample in a hydrophobic column under conditions of high salt concentration. Elution is typically achieved by decreasing the ionic strength or the concentration of salt in the mobile phase. During HIC, a protein coming in contact with the hydrophobic ligands of the resin or chromatography matrix experiences a spatial reorientation. The hydrophobic ligands of the resin interact with the exposed hydrophobic zones of the protein, to reversibly bind the protein to the resin. The adsorption capacity of HIC resins and the resolving power of HIC are similar to that seen in ion exchange chromatography of proteins (Fausnaugh et al., 1984).

The main system characteristics affecting protein retention in HIC are concentration and type of salt (Melander and Horvath, 1977; Sofer and Hagel, 1998) and density and type of hydrophobic ligand attached to the matrix (Jennissen, 2000). The main physicochemical property of proteins that determines chromatographic behavior in HIC is hydrophobicity. At present, no universally agreed single measure exists for hydrophobicity of proteins. There is consensus that a protein's hydrophobicity is determined by the hydrophobic contributions of its amino acid residues (Tanford, 1962; Eriksson, 1998). Hydrophobicity has been estimated in several possible ways, including measures such as "average hydrophobicity" (Tanford, 1962), "non-polar chain frequency" (Waugh, 1954), "polarity ratio" (Fisher, 1964), and "net hydrophobicity" (Eriksson, 1998). In HIC protein retention occurs because of a surface adsorption phenomenon, therefore, the use of "average surface hydrophobicity" has been suggested for characterizing retention behavior (Lienqueo et al., 2002). Average surface hydrophobicity can be estimated from a knowledge of the protein's three-dimensional structure by taking into account the hydrophobic contribution of the amino acid residues that are exposed on the surface.

Hydrophobicity is of course not uniform over the entire surface of a large protein molecule. Therefore, the distribution of surface hydrophobicity can be important in HIC. Indeed, it has been reported that protein retention in HIC is considerably affected by the distribution of hydrophobic patches on a protein's surface (Fausnaugh and Regnier, 1986; Mahn et al., 2004).

As HIC is widely used in downstream processing of proteins, we have focused on developing methods for predicting protein retention time in HIC involving gradient elution. Ability to predict retention times will greatly ease the design of protein purification processes and reduce the need for numerous tedious trial runs. Here we review three methods that have shown promise in predicting protein–resin interactions in HIC.