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A theory of protein–resin interaction in hydrophobic interaction chromatography

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Abstract

Docking simulations were performed in order to investigate surface area of interaction between several ribonucleases and a reduced model for the hydrophobic moiety used in Phenyl Sepharose™ using the program AutoDock 3.0. For each ribonucelase, 80 independent simulations with populations consisting of 100 random structures were performed and from these the most probable docked protein–ligand conformations were obtained. A new methodology was used to select the most probable conformations, based on qualitative and quantitative considerations. The interacting amino acids in each protein were identified. The average surface hydrophobicity of the interfacial zone (local hydrophobicity, LH) was determined. The LH showed a high correlation level ($r^2 = 0.99$) with the "hydrophobic contact area" (HCA) experimentally determined for the different ribonucleases as well as with the dimensionless retention time ($r^2 = 0.90$). This study allowed us to identify the zones on the protein surface most probably involved in protein retention in HIC, without tedious experimental work. Given the good correlation level obtained, this new methodology may constitute a novel approach that could be used to predict protein behavior in HIC.

Keywords: Molecular docking; Local hydrophobicity; Dimensionless retention time; Hydrophobic accessible area

1. Introduction

Hydrophobic interaction chromatography (HIC) is a powerful technique for protein separation, based on the reversible interaction between the hydrophobic zones of a protein's surface and the hydrophobic ligands of a chromatographic resin [1]. HIC is widely used in the downstream processing of proteins, as it provides separation properties complementary to other protein purification techniques such as ion-exchange chromatography, affinity chromatography or gel filtration chromatography [2]. The main protein property determining retention in HIC is hydrophobicity, which can be estimated as "average surface hydrophobicity" starting from the protein 3D-structure data and considering the hydrophobic contribution of the exposed amino acids [3,4]. A novel methodology to predict protein retention time in HIC starting from protein's average surface hydrophobicity has been proposed, with a reasonable degree of success [4,5]. However, some proteins did not follow the expected behavior. This was attributed to a heterogeneous distribution of the hydrophobic zones on the protein surface [5]. The surface hydrophobicity distribution of proteins related to retention in HIC has been investigated by Mahn et al. [6]. Based on a classical thermodynamic model [7], the contact area between the hydrophobic ligands of the HIC matrix and the protein when adsorbed (hydrophobic contact area, HCA) was experimentally determined. This parameter was found to give an idea of the surface hydrophobicity distribution of proteins. HCA correlated extremely well with the dimensionless retention time showed by different ribonucleases in HIC with salt gradient elution. However, a high number of experiments are necessary to determine this parameter.

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The aim of this work was to identify the zone of a protein's surface which most probably interacts with the hydrophobic ligands of resins used in HIC. We carried out molecular docking simulations to identify the interaction zone. This identification would allow us to find any parameter that could be used to predict chromatographic behavior of proteins in HIC, reducing in this way the experimental work.

1.1. Molecular docking

Computational methods are increasingly being used in the identification and characterization of protein–ligand interactions. The ligand docking and the selection algorithms are commonly used in drug design as well as in biochemical process elucidation [8]. In the protein–ligand docking process, a huge number of degrees of freedom has to be taken into account for both molecules, as well as the combination of energetic forces that acts on them. Molecular docking consists of a conformational sampling procedure, in which different protein–ligand conformations are examined to find the correct one. The sampling procedure is normally based on methods such as genetic algorithms and Monte Carlo simulation, among others. Besides, the conformational sampling involves an energy function ("score function") used to evaluate the fitness between the protein and the ligand [9]. The molecular docking has three steps: identification of the binding sites, a search algorithm to efficiently perform the conformational sampling in the search space, and a score function [8].

In this work, we simulated the interaction between different ribonucleases of known three-dimensional structure and the hydrophobic ligand used in the resin Phenyl Sepharose^{TM}, using the program AutoDock 3.0.5^{TM} [10]. For each protein eight simulations were carried out, each of them consisting of ten grids, obtaining eighty possible conformations of the protein–ligand complex for each ribonuclease. Based on qualitative (location of the interaction zone) and quantitative (free energy of the complex) considerations, the most probable protein–ligand conformations were chosen. Once the interaction zone was identified, the local hydrophobicity (LH) was determined, considering the amino acid residues that belong to that zone and their exposure level, using a methodology similar to that proposed before [4].

1.2. The main factors that affect protein retention in hydrophobic interaction chromatography (HIC)

In previous work [5], we have demonstrated that the main factor affecting protein retention in HIC is a protein's hydrophobicity. On reference to the chromatographic conditions, we have investigated the effect of different chromatographic conditions on protein retention in HIC. A linear correlation has been found between a protein's retention time using different hydrophobic matrixes (butyl and phenyl sepharose) or different initial salt concentration in the elution buffer. It was concluded that the types of matrix do not affect the elution order of proteins, despite the hydrophobic moieties in these matrixes interact with protein in a different way [11]. A correction factor was obtained (Eq. (5)), which can be used to estimate a protein's retention time using phenyl sepharose, starting from that obtained using butyl sepharose [5].

In addition, it has been possible to compare protein retention time using ammonium sulphate and sodium chloride at similar ionic strength [5]. Proteins showed a very different behaviour. Selectivity was reduced when using sodium chloride and the elution order of proteins was indeed affected. It was concluded that ammonium sulphate allows a much more predictable behaviour of proteins in HIC, because this salt stabilises a protein's structure in solution [12].

Then, if ammonium sulphate is used to build the elution gradient, the dimensionless retention time of a protein in HIC using the matrix phenyl sepharose, can be estimated from that obtained with butyl sepharose.

In this work we propose to investigate if it is possible to identify the zone of a protein's surface that interacts with a hydrophobic resin used in HIC. The results obtained in the docking simulations will be analysed based on the chromatographic behaviour of proteins, using the experimental conditions that favour a protein's structural stability.

2. Experimental

2.1. Proteins

In the simulations we used the crystal structures of four different ribonucleases, which have been used in our previous paper [6]: RNAse A (PDB code 1AFU), RNAse S (PDB code 1RBC), RNAse T1 (PDB code 1RGC) and a variant of RNAse T1 (PDB code 1TRP). The spatial coordinates were retrieved from The Protein Data Bank [13].

In the chromatographic runs we used the ribonucleases mentioned before. Ribonuclease T1 wild type (1RGC) and the variant Y45W/W59Y (1TRP) were obtained by expressing both enzymes in *E. coli* strain DH5α. Competent cells were transformed with the corresponding plasmids. RNase variants were produced and purified after the protocol published by Grunert and coworkers [14]. Both plasmids were kindly donated by Prof. Dr. Ulrich Hahn (University of Hamburg, Germany). Ribonuclease A (1AFU), Ribonuclease S (1RBC) and Tris buffer were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Water prepared from a Milli-Q water cleaning system (Millipore, Bedford, MA, USA) and analytical-reagent grade ammonium sulfate (Merk) was used in the preparation of the buffers.

2.2. Hydrophobic ligand

The interaction between the crystal structure of the different RNAses and the hydrophobic ligand used in Phenyl Sepharose^{TM} was studied. This ligand consists of a phenyl group linked to the hydrophobic resin through a three-carbon