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Analysis of solute-protein interactions and solute-solute competition by zonal elution affinity chromatography

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ABSTRACT

Many biological processes involve solute-protein interactions and solute-solute competition for protein binding. One method that has been developed to examine these interactions is zonal elution affinity chromatography. This review discusses the theory and principles of zonal elution affinity chromatography, along with its general applications. Examples of applications that are examined include the use of this method to estimate the relative extent of solute-protein binding, to examine solute-solute competition and displacement from proteins, and to measure the strength of these interactions. It is also shown how zonal elution affinity chromatography can be used in solvent and temperature studies and to characterize the binding sites for solutes on proteins. In addition, several alternative applications of zonal elution affinity chromatography are discussed, which include the analysis of binding by a solute with a soluble binding agent and studies of allosteric effects. Other recent applications that are considered are the combined use of immunoextraction and zonal elution for drug-protein binding studies, and binding studies that are based on immobilized receptors or small targets.

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Contents

1. Introduction

Zonal elution is the most prevalent form of affinity chromatography that is used for the analysis of solute-protein interactions and solute-solute competition for binding sites on proteins [1–5]. In this method, a small amount of an analyte or solute (A) is injected into a chromatographic system that contains an affinity column with an immobilized biological ligand or binding agent (L). This injection is often made in the presence of a mobile phase that contains a known concentration of a competing agent or interacting agent (I). The time or mobile phase volume that is required to elute the solute from the column is then monitored and used to provide information on the interactions between the injected solute, the agent in the mobile phase, and/or the immobilized binding agent [6]. This review will examine the theory, principles and applications of zonal elution affinity chromatography method

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in the study of solute-protein binding and solute-solute competition for proteins.

A typical HPLC system for performing a zonal elution experiment is shown in Fig. 1 [6]. This particular system design includes an online detector; however, a fraction collector and an offline detector can also be employed, particularly when the experiment involves a low-performance affinity column [4,6,7]. Temperature control is ideally needed for this type of system to obtain optimum precision and accuracy [6]. Linear elution conditions are also often required, in which the amount of the injected solute is negligible compared to the amount of the binding agent in the column [2,6,8]. However, nonlinear elution conditions can be employed in some situations for zonal elution experiments [9–11].

The use of zonal elution affinity chromatography for binding studies was first described in 1974 by Dunn and Chaiken in a work based on low-performance columns [12]. Starting from this pioneering work, the same format has been used in many other studies using both high- and low-performance systems to examine solute-protein binding and solute-solute competition for sites on proteins [11,13–33]. An example of the use of an HPLC system is shown in Fig. 2 [31]. In this example, *R*-warfarin was injected as a probe into a column that contained immobilized human serum albumin (HSA) as the binding agent. The mobile phases in this example had tolbutamide as a potential competing agent for *R*-warfarin at a mutual binding site on HSA. As the concentration of tolbutamide was increased in the mobile phase, there was a decrease in the observed retention time for *R*-warfarin, indicating that either direct competition or a negative allosteric interaction was present between tolbutamide and *R*-warfarin during their binding to HSA [31]. A similar format has been used to examine the retention and competition of numerous other solutes on affinity columns [6].

There are several advantages to using zonal elution and affinity chromatography for the analysis of solute-protein binding and solute-solute competition. First, only a small amount of solute is needed per injection and the same preparation of an immobilized binding agent can often be used for many experiments [4,6]. In addition, it is possible in some situations (e.g., with chiral compounds) to examine the binding by more than one solute per run, as long as sufficient resolution is present to differentiate

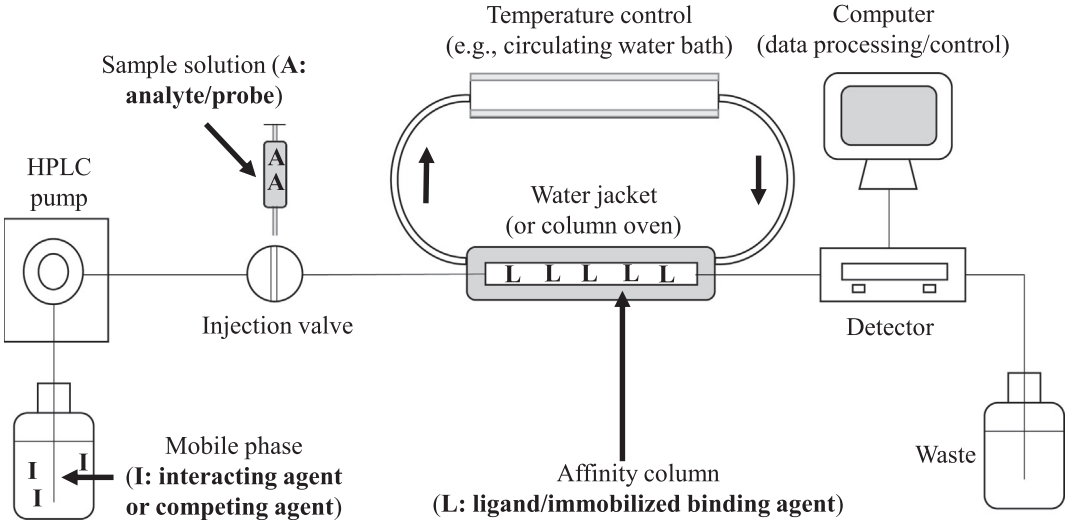

Fig. 1. A typical HPLC system for use in zonal elution affinity chromatography and studies of solute-protein binding or solute-solute competition for proteins.

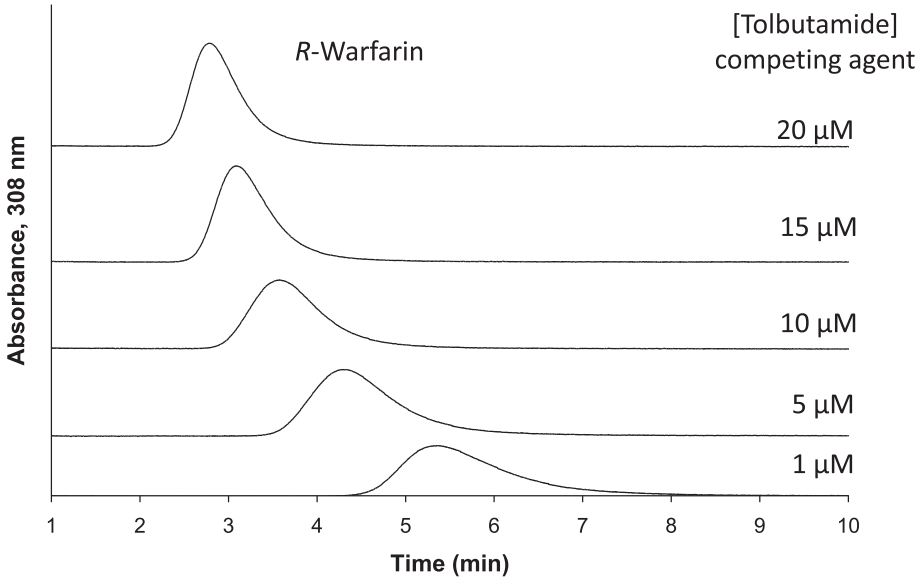

Fig. 2. Typical chromatograms obtained in zonal elution studies using injections of *R*-warfarin as a site-selective probe for HSA and mobile phases that contained various concentrations of tolbutamide as a competing agent or interacting agent. Reproduced with permission from Ref. [31].

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