ELSEVIER

Journal of Chromatography A, 764 (1997) 21–26

JOURNAL OF CHROMATOGRAPHY A

Comment on the theory of protein adsorption on a biospecific rigid matrix

Pavel G. Mingalyov, Alexander Yu. Fadeev*

Laboratory of Organic catalysis, Chemistry Department, Moscow Lomonosov State University, Vorob'yovy Gory, 119899 Moscow, Russia

Received 3 November 1995; revised 2 October 1996; accepted 2 October 1996

Abstract

A new model for biospecific sorption of proteins is proposed. The model considers adsorption on a rigid non-swelling matrix, in particular, silica. The quantity of ligand accessible to a protein is estimated using the fractal approach. Steric (not allosteric) interactions between a protein sorbed and a cluster of attached ligands are discussed. It is shown that biospecific binding of a protein decreases as the surface concentration of attached ligands is increased.

Keywords: Adsorption; Affinity chromatography; Proteins

1. Introduction

The theory of modern affinity chromatography cannot ignore the following: distribution of attached ligands over a matrix; co-operative interactions between a protein and immobilized ligands; "multivalent" binding of proteins, etc.

The early papers [1–3] consider only interactions between a protein and an isolated ligand. These theories are applicable only to highly diluted sorbents. More complex, multivalent interactions between a protein and clusters of attached ligands are considered in [4–6].

These theoretical studies provide the fundamentals for the general theory of affinity chromatography. However, the majority of the theories consider the sorbents prepared on the basis of organic matrices (agarose, cellulose, etc.). At the same time, a considerable number of bioaffinity sorbents are made on the basis of inorganic matrices (silica, alumina, etc.) [7,8]. Mineral bioaffinity sorbents have several advantages compared to organopolymeric ones. For instance, they do not swell in organic solvents, are not biodegradable and are not very expensive.

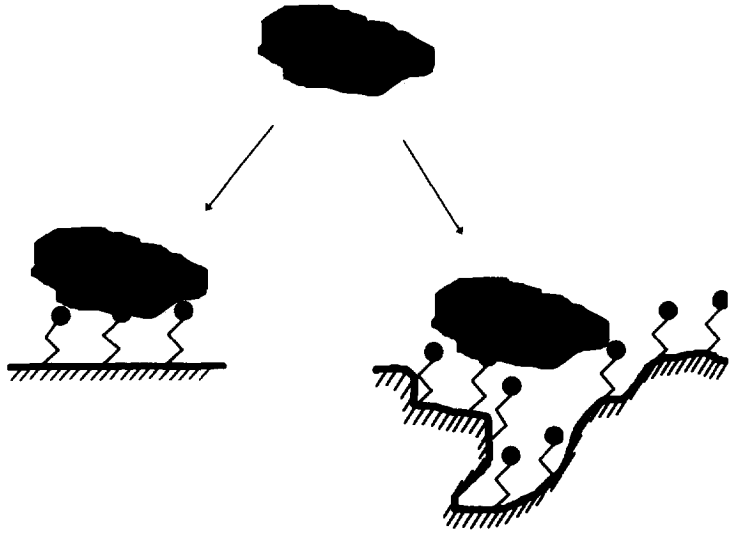
The problem is that the known theories sometimes do not give an adequate description of the biospecific sorption of a protein on an inorganic sorbent. Indeed, the sorption onto the surface of rigid, non-swelling sorbent has the following conceptual features:

– First, sorption of a protein cannot be considered as absorption into a matrix space. Therefore, the concentration of ligands cannot be expressed as a reciprocal of the volume of a sorbent. These assumptions are likely to be suitable only for swelling polymer sorbents. We believe that adsorption onto a surface of a sorbent should be considered.

– Second, it concerns the geometrical accessibility of attached ligands (Fig. 1). For organic sorbents, the assumptions are usually made that either 100% of ligands [1] or an arbitrarily chosen proportion, say

*Corresponding author.

PII S0021-9673(96)00833-3



A B

Fig. 1. Adsorption of a protein on a flat (A) and on an irregular (B) surface.

10% [6], is accessible to a protein. For rigid, non-swelling sorbents, accessibility of ligands to a protein can be estimated quantitatively using the idea of fractality of porous solids [9–13].The description of biospecific adsorption on a surface of rigid matrix is the goal of this study.

2. Results and discussion

2.1. Calculation of concentration of attached ligand clusters [x_i]

To estimate the extent of multivalent interactions between a protein and a biospecific sorbent one should know the number of ligands that are attached on an area equal to the cross-section of the protein (σ_p). Evidently, this value is not a constant for the surface of a real biospecific sorbent. Let us calculate this variable.

In this study, the population of attached ligands, which are attached to a randomly chosen area equal to σ_p, is referred to as a cluster of attached ligands. To calculate the concentration of clusters of attached ligands on a surface, the following model assumptions are done:

1. The sorption of a protein occurs onto a surface of a matrix and not in the matrix space, as is usually assumed [1,4,6].
2. The distribution of attached ligands over the surface follows Poisson's law. Namely, the probability of finding exactly i ligands on an area of surface, $p(i)$, is:

$$p(i) = \exp(-m) \times m^i/i! \quad (1)$$

where m is the average content of ligands in this area.

3. The surface concentration of attached ligands, which are accessible to the protein (c), can be calculated as [9–11]

$$c = \rho \times (\sigma_p/\sigma_l)^{(2-d)/2} \quad (2)$$

where ρ is the total concentration of attached ligands (mol/m^2), σ_l is cross-sectional area occupied by the attached ligand, d is the surface fractal dimension. In other words, the surface of the matrix can be treated with the fractal scaling

law [9–13]. It should be noted that the concentration of accessible ligands and the total concentration of attached ligands can differ significantly. Fig. 2 demonstrates the dependence of the share of accessible ligands (c/ρ) on the surface fractal dimension d.

4. Attached ligands are non-diffusible and their size is small in comparison with a cross-sectional area of a protein ($\sigma_p >> \sigma_l$).

Formally, related models are used for the description of "multivalent protein–DNA" interactions [14–17]. Indeed, such systems can be considered as one-dimensional analogues of protein adsorption onto the affinity sorbent. However, the models suggested in [14–17] consider the nucleic acid as a sequence of identical nucleotides arranged at an equal distance from each other. At the same time, the distribution of the affinity ligands on the surface of the sorbent is random and can be treated using Poisson's law. An additional difference lies in the fact that the present model considers the concentration of ligands as a variable, whereas the amount of nucleotides per unit length is a constant value. Thus, the model suggested in this paper and the models of [14–17] are not analogues, and they should be used in different cases.

In the framework of the model suggested, $p(i)$ can be readily calculated as follows:

$$p(i) = \exp(-c \times \sigma_p) \times (c \times \sigma_p)^i / i! \quad (3)$$

Of course $\Sigma p(i) = 1$. However, i can be equal to zero, i.e. there are areas with a size of more than σ_p, which do not contain ligands at all. Specific sorption of a protein does not occur on these areas, so allows us to exclude them from further consideration. Instead of $p(i)$, the corrected probabilities $p'(i)$ will be used:

$$p'(i) = p(i)/(1 - p(0)) \quad (4)$$

Then the concentration of i-ligand clusters $[x_i]$ is proportional to $p'(i)$:

$$i \times [x_i]/c = \frac{\text{concentration of ligands in } i - \text{clusters}}{\text{total concentration of accessible ligands}} = p'(i) \quad (5)$$

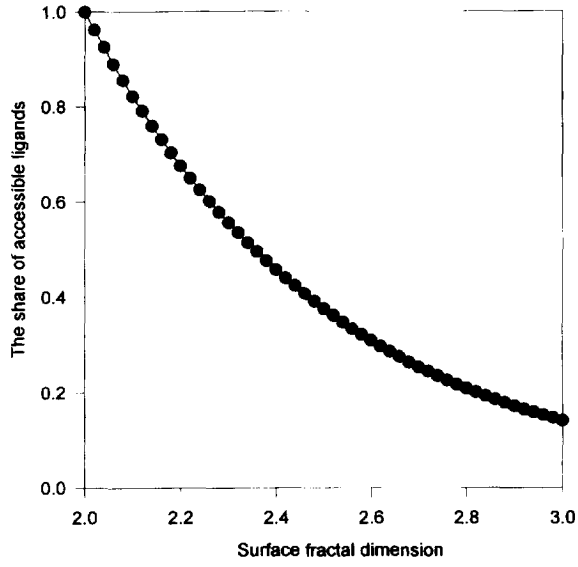


Fig. 2. Share of ligands with σ_l equal to 1 nm^2, which are accessible to a protein with σ_p equal to 50 nm^2, as a function of the surface fractal dimension.

Combining Eqs. (3–5) and taking into account that $p(0) = \exp(-c \times \sigma_p)$ one can obtain:

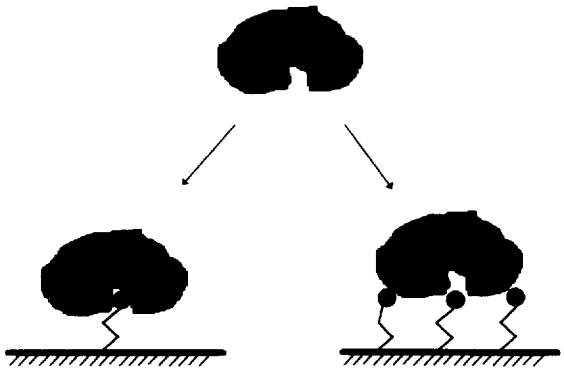
$$[x_i] = c \times \exp(-c \times \sigma_p) \times (c \times \sigma_p)^i / i \times i! \times (1 - \exp(-c \times \sigma_p)) \quad (6)$$

This equation is the goal of this section. Using Eq. (6), one can describe adsorption of a protein on a surface and interactions between a protein and a ligand cluster (co-operative, allosteric, steric, etc.).

2.2. Model of steric interactions between protein and attached ligands

This section considers the reduction of biospecific binding of proteins due to possible steric interactions between the protein sorbed and attached neighbouring ligands (Fig. 3). Indeed, affinity ligands are usually bulky molecules. Therefore, the ligand can be sterically hindered by proximate neighbours.

It should be noted that the probability of interaction between sorbing protein and "neighbouring" ligands can be considerable. Let us calculate the proportion of isolated and clustered attached ligands for the following typical example: Total concentration of attached ligands (ρ) is equal to 0.1 groups



A B

Fig. 3. Interactions between a protein and an isolated attached ligand (A) and a cluster of attached ligands (B).

per nm^2 (about 0.06 mmol/g for sorbents with a specific surface of 100 m^2/g); cross-sectional area of a protein is equal to 50 nm^2 and the surface fractal dimension is equal to two. Then, using Eq. (6) we find that the proportion of ligands in clusters with the number of ligands ranging from two to eleven is about 68%, whereas the proportion of isolated ligands is 32%.

It is difficult to give a quantitative description of such non-specific interactions. In this paper we make the following simplifying assumptions: the specific binding constant for a protein and an i-ligand cluster is expressed as follows:

$$K_i = \text{Constant} \times K_1 / i^b \quad (7)$$

where K_1 is a binding constant for an isolated attached ligand and b is a constant that characterizes the strength of the interactions. It should be noted that the essence of parameter b is now out of our consideration.

The case with $b=0$, evidently, means that steric interactions between the enzyme sorbed and the ligands are non existent.

Furthermore, we would like to consider two cases: $b=\infty$ and $0<b<\infty$.

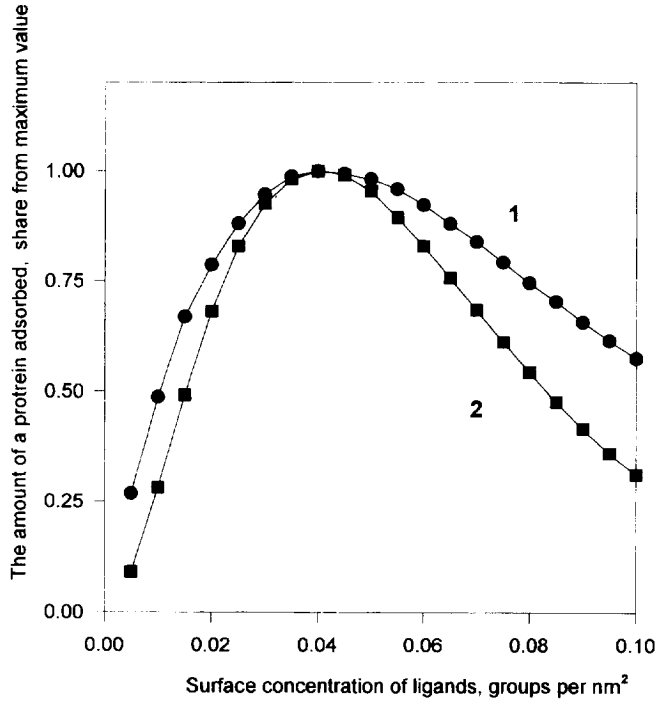


Fig. 4. Dependence of a relative amount of a protein adsorbed specifically on the surface concentration of attached ligands (c). Surface fractal dimension was taken to be equal to two, σ_p was taken to be equal to 50 nm^2.

(1) $b=\infty$. This means that clusters with $i>1$ do not bind enzyme at all ($K_i=0$). Taking into account that the mass of a sorbed protein (M) is expressed as follows:

$$M=\sum K_i \times [x_i] \tag{8}$$

and combining Eqs. (6,8), we have

$$M=\text{Constant} \times c^2 \times \sigma_p \times \exp(-c \times \sigma_p) \tag{9}$$

Fig. 4 shows the dependence between the relative amount of protein adsorbed and the total concentration of attached ligands. Note that concentration of accessible ligands (c) and total concentration of attached ligands (ρ) are unambiguously connected for rigid matrices (see Eq. (1)).

As one can see, the curve have well-pronounced extremuma. The increase in the surface fractal dimension d from two (corresponding to a flat surface) to three (geometrically non-uniform, microporous support) leads to the displacement of the maximum to the region where the concentration of ligands (ρ) is higher. The decrease in the cross-sectional area of the protein (σ_p) gives similar results.

(2) $0<b<\infty$. We believe that the above-mentioned case ($b=\infty$) is unlikely to occur in practice. Indeed, steric interactions are hardly so strong, and the sorption of a protein on the clusters with $i>1$ does occur. Thus, it is worth considering the case where the value of parameter b is small. Without loss of generality, let $b=1$. Then, from Eqs. (6–8), we have

$$M=\text{Constant} \times \sum c \times \exp(-c \times \sigma_p) \times (c \times \sigma - p)^i / i^2 \times i! \times (1-\exp(-c \times \sigma_p)) \tag{10}$$

Fig. 4 shows the dependence of the relative amount of protein adsorbed on the total concentration of attached ligands.

The general behaviour of the dependence is similar to that discussed above. Unlike the case with $b=\infty$, this curve has a less pronounced maximum.

It should be noted that the mass of adsorbed protein depends on the cross-sectional area of a

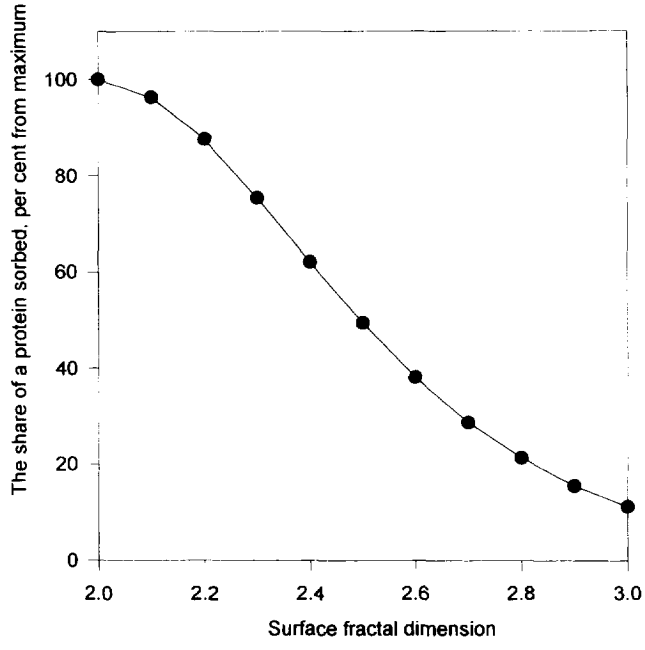


Fig. 5. Dependence of a relative amount of a protein adsorbed specifically on the surface fractal dimension of a matrix. The surface concentration of attached ligands is 0.04 groups per nm^2; parameter $b=1$ (curve 1), $b=\infty$ (curve 2). σ_p was taken to be equal to 50 nm^2; σ_p/σ_1 was taken to be equal to 50.

protein (σ_p) (see Eqs. (9,10)) and on the surface fractal dimension (d) (see Eqs. (1,9,10)). The increase in the surface fractal dimension d (passing from wide to narrow pore supports) leads to a decrease in the mass of protein adsorbed (Fig. 5). A decrease in the cross-sectional area of a protein also results in a decrease in the mass of adsorbed protein.

3. Conclusions

The following conclusions can be drawn.

The model discussed above considers sorption of a protein onto a surface of a rigid non-swelling biospecific sorbent. The proportion of attached ligands that are geometrically accessible to a protein is estimated using the idea of surface fractality. It was demonstrated that biospecific sorption can decrease as the ligand loading is increased, due to neighbouring ligands sterically hindering the formation of an affinity complex.

The model predicts the maximum on the dependencies between biospecific binding on the sorbent and the ligand loading. In other words, the maximum in affinity binding does not correspond to the maximum of ligand loaded.

Acknowledgments

We are grateful to Professors A.N. Pryakhin and A.S. Yerokhin (Moscow State University, Department of Chemistry) for helpful discussions. This work was supported by International Science Foundation (ISF) grant number MEL 300.

References

[1] D.J. Graves and Y.T. Wu, Methods Enzymol., 34 (1974) 140.
[2] P. Andrews, B.J. Kitchen and D.J. Winzor, Biochem. J., 135 (1973) 897.
[3] B.M. Dunn and I.M. Chaiken, Proc. Natl. Acad. Sci. U.S.A., 71 (1974) 2382.
[4] P.D.G. Dean, W.S. Johnson and F.A. Middle (Editors), Affinity Chromatography, IRL Press, Oxford–Washington, DC, 1985, Ch. 6.
[5] J. Hubble, Biotech. Bioeng., 30 (1987) 208.
[6] R. Yon, J. Chromatogr., 457 (1988) 13.
[7] J. Turkova, Bioaffinity Chromatography, (Journal of Chromatography Library, Vol. 55), Elsevier, Amsterdam, 1993.
[8] L.A. Osterman, Khromatografiya belkov i nukleinovykh kislot. (Chromatography of Proteins and Nucleic Acids), Nauka Press, Moscow, 1985.
[9] P. Pfeifer and D. Avnir, J. Chem. Phys., 79 (1983) 3558.
[10] D. Avnir, D. Farin and P. Pfeifer, J. Chem. Phys., 79 (1983) 3566.
[11] D. Farin and D. Avnir, J. Chromatogr., 406 (1987) 317.
[12] D. Farin and D. Avnir, Colloids and Surfaces, 37 (1989) 155.
[13] D.Avnir (Editor), The Fractal Approach to Heterogeneous Chemistry, Wiley, New York, 1992.
[14] J.D. McGhee and P.H. von Hippel, J. Mol. Biol., 86 (1974) 469.
[15] R.C. Kelly, D.E. Jensen and P.H. von Hippel, J. Biol. Chem., 251 (1976) 7240.
[16] I.R. Epstein, Biopolymers, 18 (1979) 765.
[17] I.R. Epstein, Biopolymers, 18 (1979) 2037.