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Biophysical Chemistry 119 (2006) 137 – 145

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Binding of chemicals to melanins re-examined: Adsorption of some drugs to the surface of melanin particles

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Received 4 March 2005; received in revised form 10 June 2005; accepted 10 June 2005
Available online 1 September 2005

Abstract

This work presents a first attempt to study the interaction of some drugs with melanins, realistically considered as solid aggregates of primary particles. This situation appears similar to the adsorption of organic molecules onto the surface of colloidal absorbers, as active carbon, zeolites or titanium dioxide. We have applied some of the most popular theoretical models used in technological applications with the aim to give a more realistic picture of the melanin–drug interaction responsible for some observed side effects in vivo. Moreover, this approach can simplify the problem of the search of the physical parameters dominating the binding processes, by reducing the phenomenon to a simple physisorption/chemisorption, at least in a first approximation.

We have studied the binding to melanin of gentamicin, methotrexate and chlorpromazine, molecules with different physico-chemical and structural characteristics. Our study demonstrates the possibility to fit experimental adsorption data with Langmuir, Freundlich, Tempkin and Dubinin–Radushkevich equations. In such a way we obtain binding parameters useful to characterize the drug–surface interaction in terms of energy and of mean affinity.

Keywords: Melanin; Drugs; Adsorption isotherms; Sorption kinetics; Surface interaction; Chemisorption

1. Introduction

The problem of the interaction of drugs with melanins, the natural brown/black pigments present in many different regions of the human and animal body, has attracted a considerable interest since 1962, the year of the publication of the first paper on the binding of phenothiazines to eye melanin [1]. Successively, a noticeable number of works devoted to this topic have appeared and many drugs were studied with the aim to determine the possible toxic effects that their accumulation could cause at the level of pigmented tissues and organs, with particular attention to oculotoxicity and ototoxicity.

Ings, in 1984 [2], reviewed first the biological experiments and the techniques adopted to reveal the localization of drugs in the various pigmented compartments of living organisms. More recently, another important review pointed out that "the binding of drugs to eye melanin is not predictive of ocular toxicity" [3]. This last paper contains a very large number of references, covering drugs of disparate and uncorrelated structure, demonstrating that the affinity of neutral and charged molecules for melanins does not follow a general chemical rule, but melanins behaves as efficient absorber thanks to their physical characteristics.

The data generated by these studies attracted the attention of some scientists interested in physical chemistry and structure of melanins. At those times (from the seventies to the nineties) the pigment was generally considered as a huge insoluble macromolecule whose reactivity could give valuable information about chemical structure. The Scatchard analysis was consequently the method of choice, even if rather poor information could be obtained in terms of number of binding sites and equilibrium constants.

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doi:10.1016/j.bpc.2005.06.004

In more recent times, more sophisticated hypotheses on melanin structure have been proposed and widely accepted, in which the insolubility and the (photo)reactivity found a satisfactory explanation. The presently accepted model, valid for any type of melanin, is based on a hierarchical organization of basic molecules (mainly, but not only, 5,6-dihydroxyindole, indole-5,6-quinone, 5,6-dihydroxyindole-2-carboxylic acid) covalently bound to form the building blocks of protoparticles, further interacting in the form of irregular observable particles, displaying highly complicated surfaces with a large area [4–6].

The adsorption of neutral molecules on such solid aggregates can therefore be analysed by assuming that the binding is analogous to the adsorption on a solid surface, i.e. making use of the classical Langmuir isotherm [7]. This way was followed in the past by some authors [8] with remarkable results.

In the recent years, many new models and theories have been developed to analyse the processes of adsorption of gas and solutes on materials of technological interest and low cost as methods of intervention in environmental contamination or to recover wastes in industrial plants. The interest has been focused mainly on activated carbon, zeolites, colloidal metal oxides and chitosan.

The aim of the present work is to employ such models in a completely different framework in order to achieve results useful for understanding the biophysical behaviour of melanins. The main reason to perform this attempt lies in the surface structure of melanin particles suggesting some parallelisms with activated carbons. In fact, both the BET surface area and the micropore volumes of melanins and amorphous carbon are comparable [9] even if the physical and chemical processes leading to the adsorption can be in principle very different. Moreover, there are experimental indications that the melanin structure can be better described in terms of fractal geometry as in the case of some activated carbons.

The main criticism that can be done to this procedure (but this can be an objection to all the works employing adsorption technologies) is the lack of a strong theoretical justification to apply theories, valid for gas adsorption, to adsorption from solutions on solid surfaces. This is a debate still in course, but, in any case, a number of valid results obtained in the last years encourage such an approach.

2. Binding equations

Though structural features of different melanins differ depending on sources and methods of preparations, some indicative values of their surface characteristics are: the specific surface area varying from 18 m^2/g for synthetic pigments to 25 m^2/g for Sepia pigment and the micropore volume of about 0.01 cm^3/g [9].

Following the Brunauer classification [7], in the case of melanins the adsorption isotherms of drugs from solution are all of type I. Therefore, following the trace of a great number of technical papers, we have performed the analysis of the binding taking advantage of the isotherm equations type Langmuir, Freundlich, Tempkin and Dubinin–Radushkevich. In recent times some more sophisticated models have been added to the list of the classical theories such as those discussed in [10]. Our choice of these few models is due to the poor knowledge of the structure of the adsorber, the melanins, that doesn't encourage at this stage deeper and more quantitative studies and also to the widespread diffusion of these more popular equations.

It should be noticed that the point $(0,0)$, though not experimentally determined, has been included in our graphs. This apparently not rigorous procedure was adopted in order to add a further constraint in the fittings of the data.

Moreover, in the case of the drug gentamicin, we have tested some kinetic models of adsorption.

We will present here a short list of such equations with the information that can be obtained from the analysis of the data. All these equations can be linearized and some authors have used the linear form to better evaluate the parameters. Rigorous theoretical derivations can be found elsewhere [7,10].

2.1. The Langmuir isotherm

It has been widely used to describe many real sorption processes. A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites. Theoretically, a saturation value is reached beyond which no further sorption can take place. The curve is represented by the expression:

$$q = \frac{q_0 K C}{1 + K C}$$

where q is the amount adsorbed ($mmol \cdot g^{-1}$), q_0 and K are related to monolayer adsorption capacity and energy of adsorption, respectively, and C is the equilibrium solution concentration of solute. In the analysis of the results, q_0 is assumed as the total number of binding sites, N_t.

2.2. The Freundlich isotherm

It is often used for heterogeneous surface energy systems. In this equation the concentration of the solute at equilibrium is raised to the power $1/n$ and the semi-empirical expression can then be written:

$$q = q_0 (KC)^{1/n}$$

When the heterogeneity index $1/n < 1$, the adsorption rate decreases with solution concentration as the low-energy sites are occupied. Being all the concentrations expressed with the same units, the Freundlich constant K_F ($=q_0 K^{1/n}$) gives an estimate of the adsorption capacity.