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Short Communication

Elimination of the temperature-induced loss of the enantioselectivity of chemically bonded albumin

ZDENĚK ŠIMEK[a] and RADIM VESPALEC*
Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Kounicova 42, 611 42 Brno (Czechoslovakia)
and
JAN ŠUBERT
Drug Control Laboratory, Institute of National Health. Jilemnického 2–4, 614 00 Brno (Czechoslovakia)
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ABSTRACT

The enantioselectivity of bonded bovine serum albumin (BSA) which has been destroyed by high temperatures can be restored by exposure to methanol, common water-based mobile phases are ineffacious. The effectiveness of methanol demonstrates that BSA enantioselectivity, though conformationally dependent, is not conditioned by native conformation of BSA. Therefore, a systematic study is advised of the influence of denaturing factors on BSA enantioselectivity.

INTRODUCTION

The idea of using bovine serum albumin (BSA) as a chiral selector in liquid chromatography arose from the fact that albumin is capable of selectively binding compounds that differ only in their steric structures. Biological functions of albumin in living organisms are ascribed to, among other factors, the existence of a particular steric conformation of the albumin molecule, called the native form. In view of the sensitivity of BSA conformation to medium effects such as pH, ionic strength and chemical composition [1,2], it is improbable that the conformation of albumin in the separation system is identical with its conformation in living organisms. This applies in particular to albumin bonded to a solid matrix. In spite of this, bonded albumin retains the ability to distinguish steric conformations of solutes [3,4].

[a] Present address: Department of Chemistry, Technical University, Gorkého 7,662 38 Bmo (Czechoslovakia).

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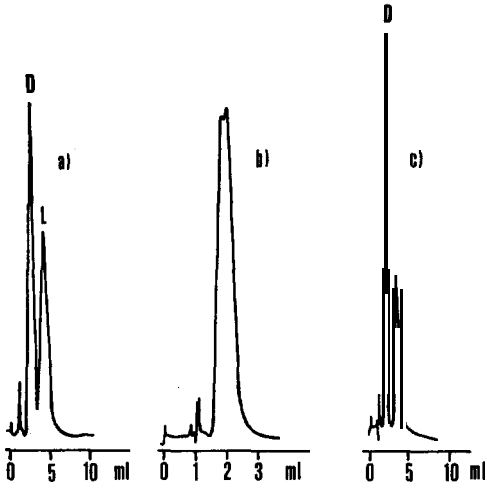

Fig. 1. Separation of D,L-tryptophan on Separon HEMA1000–BSA sorbent (a) prior to heating, (b) after heating to 96°C and (c) after heating and washing with methanol. Separation conditions: CGC column (150 × 3.3 mm I.D.) packed with Separon HEMA 1000–BSA; temperature, ambient; mobile phase, 50 m*M* phosphate buffer in water (pH 7.5); flow-rate, 0.5 ml/min; injection, 2 μl of 0.46 m*M* D,L-tryptophan; detection, UV (254 nm). Quantitative characteristics of separations: (a) k_1 = 1.42, α = 2.21, R_s = 1.60; (b) k_1 = 0.87, α = 2.61, R_s = 3.42.

The chromatographic properties of the separation system can be controlled effectively by temperature. However, heating results in changes in albumin conformation and leads to its denaturation [1,2].

EXPERIMENTAL AND RESULTS

To test the effect of heating BSA on the enantioselectivity of the separation, we used BSA chemically bonded to a hydroxyethylmethacrylate matrix (Separon HEMA 1000–BSA sorbent; Tessek, Prague, Czechoslovakia). D,L-Tryptophan, whose L-form is transported by albumin in living organisms, was selected as a model solute. Fig. la shows the selectivity of the separation of D,L-tryptophan on a column filled with Separon HEMA1000–BSA stored for cu. 1 year at 5°C in phosphate buffer (pH 7.5) containing 0.1% sodium azide. After being equilibrated with 50 m*M* phosphate buffer (pH 5.93), the column was heated in a stream of this mobile phase in a thermostat up to 96 ± 1°C within 40 min and allowed to stand at 96 ± 1°C for the following 30 min. The heated column almost lost its ability to separate tryptophan (Fig. lb). Conditioning of the heated column with 50 m*M* phosphate buffer (pH 7.45) at ambient temperature for 40 h did not change the selectivity of the separation and conditioning with 50 m*M* buffer (pH 2.53) (at ambient temperature for 24 h) improved it only negligibly. The enantioselectivity of the separation of D,L-tryptophan was reestablished by washing the column with methanol (Fig. lc). The separation selectivity in Fig. lc agrees with that on the same sorbent immediately after BSA bonding (k_1 = 1.80, o! = 2.64) [5].

DISCUSSION

The finding that methanol, which also has a denaturing effect [1,2], re-established the enantioselectivity of bonded BSA is remarkable. First it reveals the reversibility of the loss of BSA enantioselectivity caused by thermal treatment. It confirms that the ability of BSA to bind solutes enantioselectively is conditioned neither by the native conformation nor by any other conformation close to it. Chemically bonded albumin, having been heated and subsequently washed with methanol, could not acquire any structure analogous to the native one in the mobile phase of pH 7.45. The native form exists only in aqueous medium in the pH range 4.8-7.0 [6,7]. The results of the experiment do not cast doubt on the likely link between the selectivity of the separation of D,L-tryptophan and the steric conformation of bonded BSA. The experiment also shows that neither an identical nor an unambiguously negative effect of denaturating factors on the enantioselectivity of BSA as a chiral selector can be expected in advance. The reason can be found in different mechanisms of the actions of the various denaturating factors.

The possibility of restoring the selectivity of tryptophan separation reduced by heating by exposure to methanol has immediate practical importance. Provided that BSA in free solution and BSA bonded to a solid carrier are influenced similarly by the environment and temperature, speculations leading to important conclusions for practical applications can be based on the influence of temperature and methanol on the enantioselectivity of tryptophan separation.

Conformational changes of albumin are caused not only by temperature and methanol but also by other factors, e.g., the pH of the environment [1,2]. Therefore, it cannot be discounted that a decrease in the selectivity of tryptophan separation on Separon HEMA 1000–BSA stored for almost 1 year at pH 7.5 (with respect to the selectivity on the freshly prepared sorbent) may be connected with changes in albumin conformation at $pH > 7$.

Each enantiomer is sorbed at particular points of the BSA chain. Its retention depends on the accesibility of appropriate sorption centres and, as a consequence, also on the BSA conformation [6,7]. Therefore, changes in BSA conformation manifested by a decrease in tryptophan retention need not necessarily lead to a decrease in retention of any enantiomeric solute. If this expectation is proved, the management of BSA conformation can serve as a new method for the regulation of the enantioselectivity of bonded BSA.

Systematic studies on the enantioselectivity of bonded BSA are a prerequisite for a decision on the correctness of these two and other possible speculations. The information that BSA enantioselectivity is conditioned neither by the native form nor a conformation close to it should be one of principal starting points.

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