THE INFLUENCE OF SALTS ON THE CHROMATOGRAPHIC BEHAVIOR OF NATURAL AND THERMAL DENATURED ALBUMIN

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The presence of KCl, KBr or KI ions in the eluent can modify the reverse phase thin layer chromatography separation of BSA conformers obtained in solution by controlled –temperature refolding.

Introduction

Preliminary studies shown that natural albumin has different physical chemical behavior comparatively with the denatured one. The denaturation process is reflected in structural changes of albumin, including an increased lipophilicity of the molecule. Albumin contains in its structure some charged amino acid radicals able to give interactions with different chemical species. This kind of interactions are implicated in the formation of pairs between the amino acids moieties (Lys, Glu, Asp, Arg) [1], the presence of a salt in the solvent being able to modify the ionic bridges established between these moieties. Because in this way the ion-ion interaction is disturbed, it is expected a change in the chromatographic behavior of albumin, the most used chromatographic technique being the reversed-phase thin layer chromatography [2,3].

The aim of this paper is to study four samples of albumin solutions in water: 1 – natural albumin 2, 3 and 4 – thermal denatured (15 minutes at 65 ^{0}C) with gently refolding (2), with refolding at 4^{0}C (3) and with fast refolding at –25^{0}C (4). Salts used as eluent modifiers were KCl, KBr, and KI. From chromatographic data it was calculated the molar lipophilicity (R_M), which is a sensitive parameter to changes in eluent composition.

Experimental

The albumin probes (Sigma, fraction V, used without further purification) were dissolved in water (0.22 mg/ml) and 2 μl of each solution were spotted on silica gel plates (Merck) impregnated by overnight predevelopment with chloroform / paraffin oil (95: 5 v/v). The plates were developed in parallelepiped chamber at room temperature using as eluent water and water solutions of KCl, KBr, and KI (Chimopar, used without further purification) with molal concentration 0.05, 0.10, 0.375, 0.50, 0.75, 1.00 and 1.50 each salt. After

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development, the plates were dried and the albumin spots were detected using nynhidrin reagent (Merck). Independent parallel determinations were carried out in each instance and the data were omitted from calculation when the relative standard deviation of parallel determination was above 8% [2]. Because after development the spot is not circular, we considered for measurements the value recorded at the top of each spot. The R_M values of albumin's probe were determined according to the eqn. 1 [2]:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \quad (1)$$

Results and discussion

The retention of albumin probes decreases from sample (1) to (4), meaning that after thermal denaturation and different temperature –controlled refolding the albumin molecule became more lipophile. We can explain these observation if we consider that after thermal denaturation it follows a reordering of amino acid rests in albumin molecule , as a result of temperature-controlled refolding processes, a sequence of conformational modifications being supposed to be obtained, from the largely featureless ensemble of denaturated conformations to structures more or less modified in respect with the unique structure of native albumin molecule. In the natural albumin the polar amino acid rests are mostly oriented to the surface of macromolecule and in the case of denatured one the hydrophobic rests of some amino acid could be oriented to the surface of this macromolecule. Therefore, the mixture of obtained conformers with different composition are supposed to present various interaction with alkali halide ions.

In Tables 1-3 are presented the R_M values of albumin probes obtained in each chromatographic system.

Table 1: The R_M values of albumin probes obtained at different concentration of KCl dissolved in eluent

Albumin probe	0.00	0.05	0.10	0.37	0.50	0.75	1.00	1.50
1	-1.12	-0.82	-0.75	-0.74	-0.86	-0.55	-0.55	-1.06
2	-0.43	-0.57	-0.75	0.09	-0.29	0.05	0.43	-0.104
3	-0.50	-0.86	-0.43	0.07	-0.52	0.07	0.07	0.09
4	-0.14	-0.16	-0.39	0.05	-0.50	-0.16	-0.14	-0.07

Table 2: The R_M values of albumin probes obtained at different concentration of KBr dissolved in eluent

Albumin probe	0.00	0.05	0.10	0.37	0.50	0.75	1.00	1.50
1	-1.12	-0.90	-0.63	-1.004	-0.14	-0.69	-0.21	-1.20
2	-0.43	-0.33	-0.43	-0.20	0.07	-0.21	0.23	-0.14
3	-0.50	-0.68	-0.75	-0.21	-0.18	-0.14	-0.03	-0.09
4	-0.14	-0.16	-0.79	-0.23	-0.18	-0.19	-0.23	-0.21

Table 3: The R_M values of albumin probes obtained at different concentration of KI dissolved in eluent

Albumin probe	0.00	0.05	0.10	0.37	0.50	0.75	1.00	1.50
1	-1.12	-1.005	-1.12	-0.95	-1.00	-0.95	-0.63	-1.004
2	-0.43	-0.57	-0.41	-0.39		-0.29	0.16	-0.19
3	-0.50	-0.75	-0.39	-0.25	-0.21	-0.29	-0.02	-0.14
4	-0.14	-0.82	-0.45	-0.50	-0.25	-0.43	-0.19	-0.07

The salt presence in the eluent modifies the R_M of albumin, but generally preserves the relationship between the R_M value corresponding to the natural albumin comparatively with the R_M values of denaturated albumins. In order to evidence the different chromatographic behavior of albumin samples interaction with alkali halide ions we considered a new parameter ΔR_M:

$$\Delta R_M = R_{M\,(\text{inpresenceofsalt})} - R_{M\,(\text{inabsenceofsalt})} \tag{2}$$

R_M represents an equilibrium constant, for the repartition process of the albumin between the eluent (the mobile phase) and the impregnated silica gel (stationary phase) then $RT\Delta R_M$ is the supplementary free enthalpy which accompanies the partition process due to the modifier presence (salt) in eluent. We note this quantity ΔG_{ionic}. From the mean value of ΔG_{ionic} for each used concentration of KCl, KBr or KI in all albumin probes, respectively, the results are presented in Table 4.

Table 4- Values of mean ΔG_{ionic} (KJ/mol)

Albumin probe	KCl	KBr	KI
1	0.89	1.08	0.36
2	0.66	0.83	0.37
3	0.44	0.50	0.51
4	-0.14	-0.36	-0.61

These data reveal that the interaction between charged rest of amino acids plays an important role in stabilization of protein structure [4,5]. With some exceptions we note that, for each halide, the intensity of interaction appears to be gradually modified from the natural albumin to more severe perturbation of its conformation, from probe (2) to (4). The obtained negative values in the last row is probably an indication of the modification from polar to hydrophobic character of macromolecular aminoacid rests in interaction with ions, the values being gradually more significant according to the polarizability of ions.

The obtained numerical values are in agreement with some results from the literature concerning such interaction [6], the free energy of the formation of a single Glu-Lys salt bridge being found 2.1 kJ/mol at -4^0C and neutral pH in 10 mM salt. In our case, the presence of KCl, KBr or KI ions in eluent can influence the salt-bridges in the albumin macromolecule, modifying the repartition process.

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