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Note

Behaviour of biomolecules in water–organic solvent–inorganic salt two-phase ternary systems

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Among methods for the fractionation of biomolecules, methods based on non-uniform partition of substances in two-phase liquid–liquid systems are of great interest. An example is systems formed by polymers incompatible in aqueous solutions, which are widely employed for the fractionation of biomolecules[1]. Two-phase systems can also be obtained by salting-out organic solvents that are completely miscible with water with the use of various salts[2]. It is known that the addition of salts to aqueous solutions of polar organic solvents, such as alcohols and ketones, leads to the separation of the system into two phases: a top phase rich in the organic solvent and a bottom phase rich in water and salt. Taking into account the differences in the compositions of equilibrium phases, and a sufficient solubility of different biopolymers and biogenic low-molecular-weight compounds in aqueous–organic systems[3,4], it can be assumed that the given systems tend to have a definite ability to induce fractionation and can thus be used for the separation and purification of various classes of biomolecules. An important advantage of such systems is the easy removal of phase-forming substances, working at sub-zero temperatures, and also the wide variation of characteristics of the medium such as dielectric constant, pH, concentration and nature of the salt introduced.

In this work we investigated the behaviour of various types of biomolecules (proteins, peptides, amino acids, lipids) in a typical two-phase ternary system formed by salting-out of *n*-propanol from an aqueous solution by sodium chloride.

EXPERIMENTAL

Materials

The following materials were used: deionized water (doubly distilled); *n*-propanol, purified according to the procedure described in ref. 5; sodium chloride of extra-purity grade (Reachim, Moscow,U.S.S.R.); amino acids and peptides (Reanal, Budapest, Hungary); bovine serum albumin (Koch-Light, Colnbrook, Great Britain); β-lactoglobulin, isolated according to ref. 6; isovaleric and capric acids (Reakhim), purified by methyl ester distillation; and egg lecithin, isolated according to ref. 7.

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Methods

A batch of the substance was partitioned by dissolving it in a known amount of water with stirring at 25 ± 0.1°C, adding known amounts of sodium chloride solution and *n*-propanol, allowing the contents to stand for 1–3 h. After the separation of the phases, aliquots were taken for analysis.

Concentrations of the various substances in the phases were determined in the following manner: amino acids and peptides by reaction with *o*-phthalic aldehyde[8]; proteins by absorption of the biuret complex[9]; lecithin by determination of phosphorus[10]; and fatty acids by the bichromate method[11]. The amount of the partitioned substance adsorbed at the interface was estimated from the material balance equation.

The composition of the equilibrium phases (Table I) was determined by analysing the content of sodium chloride gravimetrically and the content of *n*-propanol by gas–liquid chromatography on Porapak-Q (80–100 mesh) (Waters Assoc., Milford, MA, U.S.A.) using a Varian 1860 chromatograph with a stainless-steel column (1.5 m × 2 mm I.D.) and helium as the carrier gas.

TABLE I

COMPOSITIONS OF EQUILIBRIUM PHASES

The error in the determination of the composition of equilibrium phases was ≤2.5%.

Phase	*System 1 (%, w/w)*			*System 2 (%, w/w)*		
	NaCl	H_2O	C_3H_7OH	*NaCl*	H_2O	C_3H_7OH
Top	2.50	37.50	60.00	1.00	17.00	82.00
Bottom	9.75	74.00	16.25	21.10	74.00	4.90

RESULTS AND DISCUSSION

Various biomolecules (proteins, peptides, amino acids, lipids) are partitioned non-uniformly in the two-phase *n*-propanol–water–sodium chloride system. The partition can be characterized by the partition coefficient $K_p = C_1/C_2$, where C_1 and C_2 are concentrations of the partitioned substance in the top and bottom phases, respectively. The value of K_p does not depend on the concentration of the partitioned substances, at least up to a concentration of 3 mg/ml.

Analysis of the K_p values (Table II) shows that the K_p value in a given system is determined by the nature of the partitioned substance. For example, in system 2, glycine is fully concentrated in the bottom phase, fatty acids quantitatively transfer to the top phase and lecithin is partitioned between the two phases with $K_p = 6.9$.

Definite regularities are observed in amino acid partition. Thus, in system 1 the K_p values of amino acids decrease in the order $K_p^{Phe} > K_p^{Leu} > K_p^{Glu} > K_p^{Gly} > K_p^{Lys} \approx K_p^{Ser}$. This series correlates with the hydrophobicity scale of amino acids[12,13], which indicates a definite dependence of the partition on the hydrophobicity of the compounds being partitioned. Probably for this reason replacement in diglycine of glycine

TABLE II

K_p VALUES OF BIOMOLECULES

The error in the determination of K_p values was $\leqslant 5\%$.

Class	*Substance*	*System 1**	*System 2**
Amino acids	Gly	0.18	Not detected in TP
	Leu	0.70	0.35
	Phe	0.80	0.50
	Glu	0.26	0.07
	Ser	0.11	0.02
	Lys	0.12	0.01
Peptides	Gly–Gly	0.08	Not detected in TP
	L-Leu–Gly	0.51	0.15
Proteins	Bovine serum albumin	Not detected in TP (4% at the interface)	Partition was not performed
	β-Lactoglobulin	1.17 (20% at the interface)	Partition was not performed
Lipids	Isovaleric acid	Not detected in BP	Not detected in BP
	Capric acid	Not detected in BP	Not detected in BP
	Lecithin	6.90	Partition was not performed

* TP = top phase; BP = bottom phase.

with leucine, corresponding to an increase in the hydrophobicity, leads to a considerable increase in K_p.

However, hydrophobicity is not the sole factor that influences the partition of substances in the given system. Thus, the order of the K_p values in system 2 differs from that in system 1: $K_p^{Phe} > K_p^{Leu} > K_p^{Glu} > K_p^{Lys} > K_p^{Ser} > K_p^{Gly}$, which indicates of a more complicated nature of the interaction of the phase-forming and partitioned substances.

Investigation of the behaviour of proteins using albumin and β-lactoglobulin as an example showed that the K_p values for these proteins differ. Albumin has a greater affinity for the bottom phase, whereas the β-lactoglobulin distribution is practically uniform. It is important to note that the percentage of the proteins precipitating at the interface is comparatively small (4% with albumin and 20% with β-lactoglobulin).

The considerable differences in the partition of biomolecules lead to the conclusion that two-phase systems formed by salting-out of the organic solvent may be used for the fractionation and purification of various classes of biomolecules. Taking into account the great diversity of two-phase systems of this type, and the possibility of varying the composition of the coexisting phases over a wide range, the use of such systems opens up new prospects for the separation and purification of biomolecules.

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