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Bovine serum albumin, α-lactoalbumin and β-lactoglobulin partitioning in polyethylene glycol/maltodextrin aqueous-two-phase systems

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Abstract

The polysaccharide maltodextrin (MD) can provide a low cost alternative to substitute the fractioned dextran for the use with polyethylene glycol (PEG) in aqueous two-phase, two-polymers systems. In this work we have studied the partitioning of Bovine Serum Albumin (BSA), α-Lactoalbumin (α-La) and β-Lactoglobulin (β-Lg) in PEG/MD aqueous two-phase systems. The experiments were performed at local atmospheric pressure (727 mmHg) and 25°C. The influence of the polymers molecular weights and tie-line length were investigated.

Keywords: Partitioning; Aqueous two-phase systems; Bovine serum albumin; α-Lactoalbumin; β-Lactoglobulin

1. Introduction

The industrial production of biological compounds is strongly dependent upon the separation technique applied. Liquid–liquid extraction is increasingly used for the isolation and purification of proteins and enzymes (Coimbra, Thömes, Meirelles & Kula, 1995; Coimbra, Mojola & Meirelles & Kula, 1998; Johansson, Karlstörm, Tjerneld & Haynes, 1998; Peng, Li & Li, 1995; Rito-Palomares & Hernandez, 1998). Over the last three decades many research groups have been using the aqueous two-phase system technique for the separation of proteins, cells and other biological materials in laboratory scale (Atkinson & Johns, 1994; Chistian, Manley-Harris & Kula, 1998; Fisher, 1981; Johansson, 1985; Zaslavsky, 1995).

The most common aqueous two-phase systems used for biomolecule separation are polyethylene glycol (PEG)/dextran or PEG/salt systems. A major set back to an ample adoption of PEG/salt systems is that they may damage fragile proteins and also that they present waste disposal problems (Vernau & Kula, 1990). In the case of the PEG/dextran system the high cost of dextran prevents its use in large scale.

Maltodextrin (MD) can provide a low cost alternative to the fractionated dextran. MD is a polysaccharide obtained by the hydrolysis of starch, it is water-soluble and commercially available with polydispersity near that of dextran.

This paper presents the behavior of the partition coefficients of Bovine Serum Albumin (BSA), α-Lactoalbumin (α-La) and β-Lactoglobulin (β-Lg) in PEG/MD systems at 25°C, with several PEG/MD polymer concentrations and different polymer molecular weights.

2. Materials and methods

MDs 2000 and 4000 were kindly supplied by Companhia Lorenz (Blumenau/SC, Brazil). PEGs 1450, 8000 and 10 000 were purchased from Sigma. The average molecular weight and the polydispersity index of the various polymers used in the present work can be found in Silva and Meirelles (1999). The proteins used in this work were BSA 96–99%, α-La and β-Lg from bovine milk, electrophoresis grade, all purchased from Sigma.

2.1. Proteins partitioning and analysis

The tie-lines were prepared from polymer stock solutions (55% MD and 50% PEG). Proteins were dissolved in the PEG stock solution. Mixtures of known weights of the stock solutions were made up to a final mass of 12 g. All systems contained nearly 50 mg (accurately weighed) of the selected protein. This mixture was gently stirred for 10 min at ambient temperature. Complete phase separation was

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Table 1
Partition coefficients of BSA, α-La and β-Lg in PEG/MD systems

System PEG/MD	Total Composition % PEG/MD	TLL (%w/w)	V_r	K_{BSA}	$K_{\alpha\text{-La}}$	$K_{\beta\text{-Lg}}$
8000/2000	11.23/34.80	26.78	3.64	0.06	1.50	0.13
	12.97/36.81	33.43	2.71	0.13	0.87	0.27
	15.06/38.78	43.14	2.20	0.20	0.53	0.39
	16.69/40.50	49.94	1.71	0.28	0.41	0.43
10 000/2000	10.72/34.63	27.34	3.64	0.13	1.09	0.19
	12.38/35.77	34.47	2.42	0.19	0.62	0.30
	15.15/38.79	44.26	1.67	0.32	0.42	0.37
	16.82/40.26	48.52	1.67	–	0.38	0.46
1450/4000	10.96/33.82	17.82	1.50	0.90	4.75	0.95
	12.55/36.18	29.28	1.68	0.89	4.93	0.89
	13.75/43.18	43.19	1.77	0.89	4.87	0.90
8000/4000	11.02/34.98	39.75	1.45	0.09	1.05	1.14
	13.24/36.71	46.58	1.36	0.16	0.58	1.11
	15.06/38.93	53.96	1.41	0.16	0.68	1.22
	16.81/40.12	61.07	1.33	0.20	–	1.17
10 000/4000	10.61/34.03	39.69	1.36	0.08	0.97	1.04
	12.52/35.78	46.41	1.33	0.11	0.65	1.07
	14.55/37.84	52.47	1.27	0.16	0.61	1.28
	16.43/39.86	58.78	1.34	0.13	–	1.15

achieved by centrifugation at 2900 g for 40 min at 25°C. After centrifugation the tubes were placed into a thermostatic bath at 25 ± 0.1°C for 5 h to equilibrate. Visual estimates of the volumes of top and bottom phases were made in graduated centrifuge tubes. The volumes of phases were then used to estimate the volume ratio (V_r = volume of top phase/volume of bottom phase). Aliquots of 5 ml were withdrawn using syringes. The top phase was sampled first, with care being taken to leave a layer of material at least 0.5 cm thick above the interface. The lower phase was withdrawn using a syringe with a long needle. A tiny bubble of air was retained in the needle tip and expelled once in the bottom phase to prevent contamination from top phase material. Protein concentration in the samples was estimated by the method of Bradford (1976) with BSA as standard. In general, four different tie-line compositions were utilized to determinate the protein partition. The effects of polymers molecular weight and tie-line length (TLL) were studied.

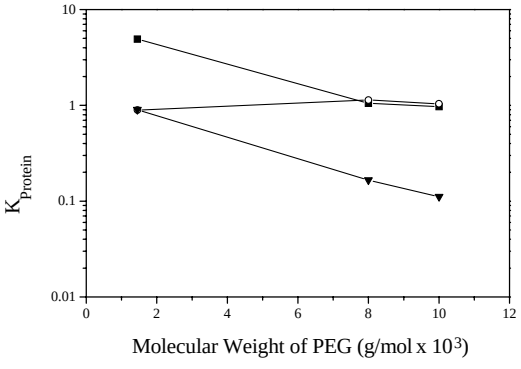

Fig. 1. Effect of PEG molecular weight on the partition coefficients of α-La (■), β-Lg (○), BSA (▼). The system composition was 11% (w/w) PEG/ 35% (w/w) MD 4000.

3. Results and discussion

Protein partitioning in the PEG/MD systems was studied at 25°C using BSA, α-La and β-Lg. The partition coefficient (K) was calculated as $K = C_T/C_B$ where C_T and C_B are the protein concentrations in mg/g of the top and bottom phases, respectively. The quoted K value is the average of triplicate determinations. The mean standard deviation for the protein concentration was ±0.0005 mg/g. For the partition coefficients the standard deviation was ±0.001. The obtained results are given in Table 1. Johansson (1985) reported that the K values for proteins partitioned in systems containing PEG 3350/dextran 500 or PEG 8000/dextran 500 are usually in the range 0.01–1.0, similar to most of the results obtained in the present work. But for some of the systems containing MD 4000, α-La and β-Lg concentrated in the top PEG-rich phase.

3.1. Influence of PEG molecular weight

The partition coefficients of α-La, β-Lg and BSA as a function of PEG molecular weight are shown in Fig. 1. For α-La and BSA the partition coefficient decreases as the molecular weight of PEG increases. The trend observed here for partitioning in PEG/MD systems agreed well with the literature data for PEG/dextran systems (Albertsson, 1986; Johansson, 1985). The partition coefficient for α-La was markedly higher than that for β-Lg in the PEG 1450 system, but they show similar values for the systems containing PEG 8000 or 10 000. The partition coefficients for BSA were the lowest for the entire PEG molecular weights studied in the present work. A dramatic decrease in α-La partition coefficient was observed in the PEG molecular weight range of 1450–8000. A similar behavior

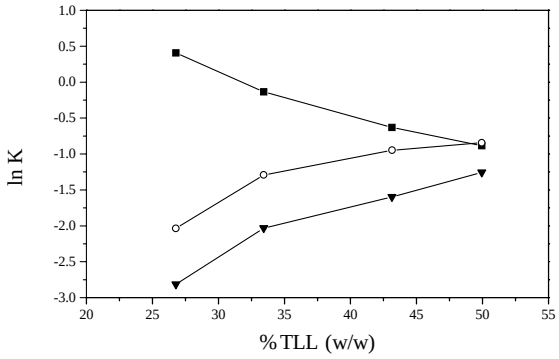

Fig. 2. Effect of TLL on partition coefficients in the PEG 8000/MD 2000 system. α-La (■), β-Lg (○), BSA (▼).

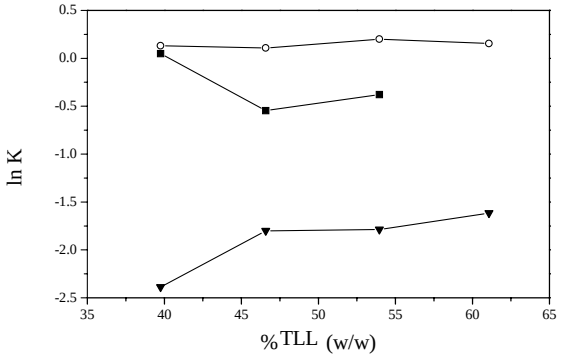

Fig. 4. Effect of TLL on partition coefficients in the PEG 8000/MD 4000 system. α-La (■), (○) β-Lg, BSA (▼).

was reported by Chen (1992) for the partitioning of α-La in PEG/potassium phosphate systems.

For PEG/dextran systems Johansson (1985) observed that in general the protein partition coefficient can be increased by reducing the molecular weight of the polymer in the top phase or by increasing the molecular weight of the polymer in the bottom phase. Our results concerning PEG molecular weight confirm such behavior, except for β-Lg. In this last case only small changes are observed and no clear trend is evident (Fig. 1). Chistian et al. (1998) also reported that in the PEG/arabinogalactan system the PEG molecular has no significant effect upon the partition coefficient of BSA. In contrast, Forciniti, Hall and Kula (1991) observed that in PEG/dextran systems the BSA partition coefficient decreases as PEG molecular weight increases. With regard to the MD molecular weight, only for β-Lg we have observed the same behavior as reported by Johansson (1985) for PEG/dextran.

The PEG 1450/MD 4000 system shows higher partition coefficient values for α-La and β-Lg than those reported for other aqueous two-phase systems: 4.92 for α-La and 0.89 for β-Lg. Chen (1992) quoted partition coefficients of 3.0 for α-La and 0.05 for β-Lg in 14% PEG 1500/14% potassium phosphate. Coimbra et al. (1995) quoted a partition coefficient of 3.4 for α-La and 0.04 for β-Lg in 14% PEG 1550/18% potassium phosphate. Despite the higher values obtained for α-La in PEG/MD systems, the selectivity is better in PEG/salt systems because the partition coefficients for β-Lg are expressively lower for the PEG/salt system.

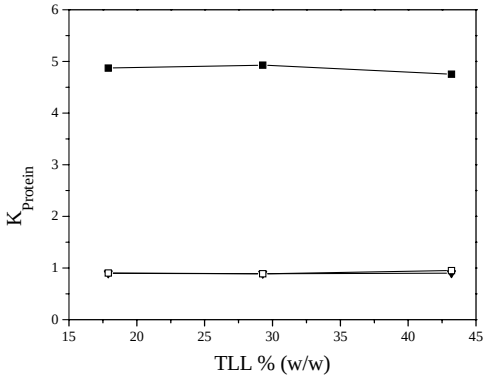

Fig. 3. Effect of TLL on partition coefficients in the PEG 1450/MD 4000 system. α-La (■), β-Lg (○), BSA (▼).

3.2. Effect of tie-line length on partitioning

Phase composition may be usefully characterized by TLL, since systems sharing a common tie-line have the same composition of upper and lower phases, and proteins exhibit similar partition coefficients in such systems. The effect of TLL on partition coefficient was evaluated in all PEG/MD systems (Table 1). Fig. 2 illustrates the impact of TLL on the protein partition coefficient in PEG 8000/MD 2000 system. Results show that partition coefficients for BSA and β-Lg increase as TLL increases. In contrast, the partition coefficient of α-La decreases as TLL increases. The same behavior was obtained for all proteins in the PEG 10 000/MD 2000 system. Albertsson (1986) observed the same trends for serum albumin and phycoerythrin partitioned in PEG/dextran systems: the partition coefficient of the first protein (serum albumin) increases as TLL increases, while for the last (phycoerythrin) it decreases.

TLL has no significant effect upon the protein partition coefficients in the PEG 1450/MD 4000 system (Fig. 3). For the other PEG/MD 4000 systems the partition coefficients of BSA and α-La exhibit a behavior similar to that obtained for PEG/MD 2000, but in this case the effect of the TLL is less significant. In such systems β-Lg partitions preferentially to the top phase ($K_p > 1$) and its partition coefficient remains nearly constant as TLL increases (Fig. 4).

In all systems studied in the present work, an increase in the partition coefficients of BSA and β-Lg was accompanied by a decrease of the volume ratio, V_r (see Table 1). A similar trend was observed by Johansson et al. (1998) for partitioning of amino acids and proteins in several aqueous two-phase systems. For α-La we have observed an opposite behavior, which is similar to that reported by Marcos, Fonseca, Ramalho and Cabral (1998) for *penicillin acylase* in PEG/sodium citrate systems.

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