ELSEVIER

Journal of Chromatography A, 766 (1997) 99–108

JOURNAL OF
CHROMATOGRAPHY A

Interaction between tryptophan residues and hydrophobically modified dextran
Effect on partitioning of peptides and proteins in aqueous two-phase systems

Min Lu, Folke Tjerneld*

Department of Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, S-22100 Lund Sweden

Received 20 May 1996; revised 21 November 1996; accepted 25 November 1996

Abstract

Hydrophobically modified dextrans, benzoyl dextran and valeryl dextran, have been used to study the interactions between tryptophan residues and benzoyl or valeryl groups by partitioning of tryptophan, tryptophan–tryptophan, $(tryptophan)_3$, poly(lysine, tryptophan), β-galactosidase and lysozyme in polymer aqueous two-phase systems. The two-phase systems used were polyethylene glycol (PEG)–benzoyl dextran, PEG–valeryl dextran, dextran–benzoyl dextran and dextran–valeryl dextran. Interaction between tryptophan residues and benzoyl or valeryl groups was observed by partitioning of tryptophan containing compounds to the phase containing hydrophobically modified dextran. At a certain phase composition the interactions were increased with increasing number of tryptophan per molecule. In a PEG–dextran system the partitioning of tryptophan peptides to the PEG phase was increased with increased number of tryptophan. In a PEG–benzoyl dextran system the opposite effect was obtained. At similar conditions benzoyl groups showed stronger interactions with tryptophans compared to valeryl groups. The partition coefficient of salts (sodium phosphate, NaCl, NaI and $NaClO_4$) was determined in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems. The effect of addition of these salts on partitioning of poly(lysine, tryptophan), β-galactosidase and lysozyme was studied. Salt effects on partitioning could be explained by the relative affinities of the ions for the polymers in the system. Charged molecules containing tryptophan were to an increasing degree partitioned to the phase for which the counterions had highest affinity. Strong effects on the partitioning of positively charged poly(lysine, tryptophan) and lysozyme were obtained with the ions I^- and ClO_4^-.

Keywords: Partitioning; Hydrophobic interactions; Aqueous two-phase systems; Peptides; Proteins; Dextrans; Tryptophan

1. Introduction

Aqueous two-phase systems composed of polyethylene glycol (PEG) and dextran have been widely used for separation and purification of biological materials, e.g., cells, proteins and membrane vesicles [1,2]. Different modifications of polymers were also made for special applications. PEG was modified with fatty acids and different textile dyes for affinity partitioning of proteins and enzymes [3–5]. Dextran has been modified with hydroxypropyl groups [1] and with textile dyes [6,7].

Hydrophobically modified (HM) dextrans (benzoyl and valeryl dextran) have been synthesized and

*Corresponding author.

0021-9673/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved
PII S0021-9673(96)00994-6

studied in aqueous two-phase systems [8–11]. Proteins with different surface hydrophobicity were studied in PEG–HM-dextran systems [8,9] and dextran–HM-dextran systems [10]. In both systems proteins with more hydrophobic surfaces were partitioned to the phase containing HM-dextrans.

Hydrophobic interactions are of central importance for the tertiary structure of proteins and for biological membranes. Hydrophobicity and hydrophobic interactions of biological molecules have been investigated by different methods. The average protein hydrophobicity has been calculated from the total protein amino acid composition [12]. Also, hydrophobic chromatography was used to study the hydrophobicity of proteins [13]. The surface hydrophobicity of proteins has been estimated by precipitation experiments with ammonium sulphate and the results correlated well with the partition coefficient in PEG–salt systems at high levels of NaCl [14]. The hydrophobic interactions between proteins and fatty acids have been studied by aqueous two-phase systems containing fatty acid esters of PEG [15].

Partition coefficient of protein in an aqueous two-phase system can be presented as:

$$K = K_{\mathrm{hphob}} \cdot K_{\mathrm{el}} K_{\mathrm{size}} K_{\mathrm{sol}} K_{\mathrm{aff}} K_{\mathrm{o}}$$

where K_{hphob}, K_{el}, K_{size}, K_{sol} and K_{aff} are the contributions to the total partition coefficient by hydrophobicity, electrostatic forces, size, solubility and affinity, respectively [1]. K_{o} is a parameter representing other effects on partitioning. The importance of the parameters will differ depending on the composition of the two-phase system and the properties of the protein.

In the present work, we have used two hydrophobically modified dextrans, benzoyl dextran and valeryl dextran, to study the hydrophobic interaction between tryptophans and benzoyl or valeryl groups. The aim has been to gain knowledge on the mechanisms for partitioning of proteins in aqueous phase systems containing hydrophobically modified polymers. Earlier results point to the importance of tryptophans on the protein surface for partitioning in two-phase systems [16,17]. The substances used here are compounds containing different amount of tryptophans. These materials include tryptophan, tryptophan–tryptophan, $(\text{tryptophan})_3$ and poly(lysine, tryptophan). Also β-galactosidase and lysozyme were studied, which are proteins with a relatively high content of tryptophan residues [18,19]. There is increasing interest in the partitioning of tryptophan-rich peptides in aqueous two-phase systems, due to the use of such peptides as fusion partners in order to direct partitioning of target proteins to a PEG phase [16,17] or to a thermoseparating copolymer phase [20]. The effect of salts on the partition coefficient of tryptophan compounds was investigated in both systems composed of PEG–HM-dextran and dextran–HM-dextran. The partition coefficient of salts was determined in PEG–benzoyl dextran and PEG–valeryl dextran two-phase systems, which completed earlier measurements of salt partitioning in dextran–HM-dextran systems [10].

2. Materials and methods

2.1. Chemicals

Dextran T500 with a weight-average molecular mass of 500 000, was obtained from Pharmacia (Uppsala, Sweden), and PEG with a number-average molecular mass of 8000 (PEG 8000), from BP chemicals (Hythe, UK). Benzoyl dextran and valeryl dextran were synthesized according to our previous works [8,9]. All other chemicals were of analytical grade.

2.2. Biological materials

L-Tryptophan was obtained from Merck (Darmstadt, Germany). The following materials were obtained from Sigma (St. Louis, MO, USA): tryptophan–tryptophan, poly(lysine, tryptophan) (4:1) random copolymer with a degree of polymerization of 187 and a molecular mass of 38 kDa based on viscosity (which is equivalent to 37 tryptophan and 150 lysine per molecule); β-galactosidase (EC 3.2.1.23), from *Escherichia coli* and lysozyme (EC 3.2.1.17), from chicken egg white. $(\text{Tryptophan})_3$ was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland).

2.3. Two-phase systems

The degree of substitution (DS) of benzoyl dextran and valeryl dextran is defined as the number of benzoyl (or valeryl) groups per glucose unit. PEG–benzoyl dextran, PEG–valeryl dextran, dextran–benzoyl dextran and dextran–valeryl dextran aqueous two-phase systems were prepared from stock solutions of 40% PEG 8000, 20% dextran T500, 12.26–14.56% benzoyl dextran (DS 0.054, 0.087, 0.098, 0.14, 0.16 and 0.17) and 11.51–11.83% valeryl dextran (DS 0.025, 0.12 and 0.20). All compositions are given in weight per cent. The stock solutions were weighed out together with buffer, salts, biological materials and water. The systems were mixed thoroughly and left standing for phase separation at room temperature for more than 1 h. Suitable amounts of samples were withdrawn from top and bottom phases, diluted 10–100 times and analysed. The partition coefficients (K) of substances are defined as the ratio of their respective concentration in top and bottom phases. The concentrations of tryptophan, tryptophan–tryptophan, $(\text{tryptophan})_3$ and poly(lysine, tryptophan) were determined by absorbance at 279 nm using a Shimadzu UV-240 double beam spectrophotometer. β-Galactosidase activity was measured according to Veide et al. [21]. Lysozyme activity was measured according to Shugar [22] and Worthington Manual [23]. Partition coefficients of salts were determined by measuring conductivities in both top and bottom phases using a Metrohm 644 conductometer [24]. The partition coefficients of salts were defined as the ratio of conductivity ($1/\Omega$) in top and bottom phase.

3. Results and discussion

Benzoyl dextran and valeryl dextran can form aqueous two-phase systems with PEG. However, these two modified polymers can only form aqueous two-phase system with dextran when their degree of substitution is higher than 0.1 [8,9]. In PEG–benzoyl dextran and PEG–valeryl dextran two-phase systems PEG is enriched in the top phase and benzoyl dextran or valeryl dextran is enriched in the bottom phase. In dextran–benzoyl dextran and dextran–valeryl dextran systems dextran is enriched in the top and benzoyl dextran or valeryl dextran in the bottom phase. β-Galactosidase shows strong interaction with benzoyl dextran and valeryl dextran in aqueous two-phase systems composed of PEG–benzoyl dextran, PEG–valeryl dextran [9], dextran–benzoyl dextran and dextran–valeryl dextran [10]. The content of tryptophan is an average of 1.1% in *E. coli* proteins [25]. β-Galactosidase (from *E. coli*) has a higher tryptophan content (3%) compared with other *E. coli* proteins [18,26,27]. Also the partitioning of lysozyme was strongly affected by HM-dextran [10]. Is the strong interaction between β-galactosidase (or lysozyme) and benzoyl (or valeryl) groups due to the relatively high content of tryptophan in these proteins? And if so, is there a difference between the interaction of aromatic (benzoyl) and aliphatic (valeryl) groups with tryptophans? To answer these questions, a series of molecules containing different amount of tryptophan were partitioned in aqueous two-phase systems containing benzoyl dextran and valeryl dextran.

3.1. Partitioning of tryptophan, tryptophan–tryptophan and $(\text{tryptophan})_3$ in PEG–HM-dextran and dextran–HM-dextran systems

The effect of DS of benzoyl and valeryl dextran on the partition coefficients of tryptophan in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems is shown in Table 1. The systems were composed of 6.0% PEG 8000 and 6.0% benzoyl dextran or valeryl dextran, 10 mM sodium

Table 1
Partition coefficients of L-tryptophan in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems

	K	K (100 mM NaCl)
DS of BzDx		
0	0.93	0.94
0.054	0.94	0.88
0.087	1.01	1.04
0.14	0.94	1.14
DS of VaDx		
0.025	0.93	0.93
0.12	0.87	0.93
0.20	0.86	1.02

The system composition was: 6.0% PEG 8000, 6.0% BzDx (or VaDx), 10 mM sodium phosphate buffer (pH 7.0), with or without 100 mM NaCl; room temperature; measured at 279 nm.

phosphate buffer (pH 7.0), with or without 100 m*M* NaCl. The partition coefficients were around 0.9 and were not affected by the increased dextran hydrophobicity. The results indicate that a single tryptophan does not give significant interaction with benzoyl or valeryl groups and the interaction was hardly affected by NaCl. In general, small molecules are partitioned evenly in polymer aqueous two-phase systems [1,28]

Tryptophan–tryptophan molecules were partitioned in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems with the same phase composition as used in Table 1. The results are shown in Table 2. The *K* values were decreased with increasing of DS for both benzoyl dextran and valeryl dextran systems. In PEG–valeryl dextran system the partition coefficient of tryptophan–tryptophan decreased from 1.50 to 0.81 when the DS of valeryl dextran increased from 0 to 0.20. In the system of PEG–benzoyl dextran the *K* value was changed from 1.50 to 0.49 when DS of benzoyl dextran increased from 0 to 0.17. This indicates that tryptophan–tryptophan has stronger interaction with benzoyl groups compared with valeryl groups. This is in agreement with our previous studies on protein partitioning. Six proteins were partitioned in dextran–benzoyl dextran and dextran–valeryl dextran systems. The results showed that at same polymer concentration the partition coefficients in the dextran–valeryl dextran system for all the studied proteins were closer to 1 compared with the dextran–benzoyl dextran system [10]. Salt (NaCl) had no significant effect on the partitioning of tryptophan–tryptophan (Table 2).

Table 3
Partition coefficients of tryptophan and tryptophan–tryptophan in dextran–benzoyl dextran and dextran–valeryl dextran aqueous two-phase systems

	K	*K* (100 m*M* NaCl)
Trp (BzDx)	0.88	0.95
Trp–Trp (BzDx)	0.48	0.64
Trp (VaDx)	0.98	1.11
Trp–Trp (VaDx)	0.76	0.76

The system composition was: 3.6% Dx T500, 3.3% BzDx (DS=0.17) or 3.6% Dx, 3.3% VaDx (DS=0.20), 10 m*M* sodium phosphate buffer, (pH 7.0), with or without 100 m*M* NaCl; room temperature (22 °C).

The partition coefficients of tryptophan and tryptophan–tryptophan in dextran–benzoyl dextran and dextran–valeryl dextran systems were determined (Table 3). In these systems tryptophan is partitioned evenly and tryptophan–tryptophan is partitioned to the more hydrophobic benzoyl dextran or valeryl dextran phase. Again the benzoyl group showed stronger effect.

The partition coefficients of $(\text{tryptophan})_3$ in the systems of 6.0% PEG 8000 and 6.0% benzoyl dextran or valeryl dextran, 10 m*M* sodium phosphate buffer, pH 7.0, with or without 100 m*M* NaCl is shown in Table 4. In the PEG–dextran aqueous two-phase system $(\text{tryptophan})_3$ was partitioned to the more hydrophobic PEG top phase ($K=2.6$). This is because $(\text{tryptophan})_3$ is more hydrophobic than

Table 2
Partition coefficients of tryptophan–tryptophan in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems

	K	*K* (100 m*M* NaCl)
DS of BzDx		
0	1.50	1.52
0.054	1.26	1.24
0.17	0.49	0.69
DS of VaDx		
0.025	1.62	1.52
0.12	1.27	1.21
0.20	0.81	0.94

The system composition was: 6.0% PEG 8000, 6.0% BzDx (or VaDx), 10 m*M* sodium phosphate buffer, pH 7.0, with or without 100 m*M* NaCl; room temperature; measured at 279 nm.

Table 4
Partition coefficients of $(\text{tryptophan})_3$ in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems

	K	*K* (100 m*M* NaCl)
DS of BzDx		
0	2.60	2.28
0.054	2.65	1.49
0.16	0.22	0.37
DS of VaDx		
0.025	2.25	2.21
0.12	1.28	1.27
0.20	0.39	0.44

The system composition was: 6.0% PEG 8000, 6.0% BzDx (or VaDx), 10 m*M* sodium phosphate buffer, pH 7.0, without or with 100 m*M* NaCl; room temperature; measured at 279 nm.

single tryptophan and tryptophan–tryptophan. The partition coefficients of $(\text{tryptophan})_3$ were significantly decreased with increasing DS for both benzoyl and valeryl dextran. In a PEG–valeryl dextran system, the K values were decreased from 2.6 to 0.39 when the DS of valeryl dextran was increased from 0 to 0.20. The K value was lowered to 0.22 at a DS of benzoyl dextran of 0.16. The effect of increasing length of tryptophan–peptides on partitioning in PEG–dextran and PEG–benzoyl dextran systems is shown in Fig. 1. In the PEG–dextran system PEG is the more hydrophobic polymer and in the PEG–benzoyl dextran (DS=0.14 to 0.17) system the hydrophobically modified dextran is more hydrophobic. In the former case $(\text{Trp})_2$ and $(\text{Trp})_3$ were partitioned to the PEG phase and in the latter case both molecules were partitioned to the HM-dextran phase. The difference in partitioning behaviour increased with increasing number of tryptophans in the peptide.

3.2. Partitioning of poly(lysine, tryptophan)

Poly(lysine, tryptophan) with a molar ratio of 4:1 was partitioned in PEG–valeryl dextran aqueous two-phase systems with 6% PEG and 6% valeryl dextran (Table 5). In a system with 10 mM sodium phosphate buffer (pH 7.0), poly(lysine, tryptophan) was mostly partitioned to the dextran bottom phase (K=0.031) in PEG–dextran system. With increasing DS of valeryl dextran the partition coefficient of poly(lysine, tryptophan) was increased slightly to 0.055 at DS 0.20. The partitioning of poly(lysine, tryptophan) is dominated by electrostatic forces due to the high content of lysine in the polypeptide. The pK_a of lysine is 10.8 and in a pH 7.0 system poly(lysine, tryptophan) has about 150 positive

Table 5
Partition coefficients of poly(lysine, tryptophan) in PEG–valeryl dextran aqueous two-phase systems

DS of VaDx	K	K (100 mM NaCl)
0	0.031	0.321
0.025	0.029	0.300
0.12	0.040	0.220
0.20	0.055	0.155

The system composition was: 6.0% PEG 8000, 6.0% VaDx, 10 mM sodium phosphate buffer (pH 7.0), with or without 100 mM NaCl; room temperature.

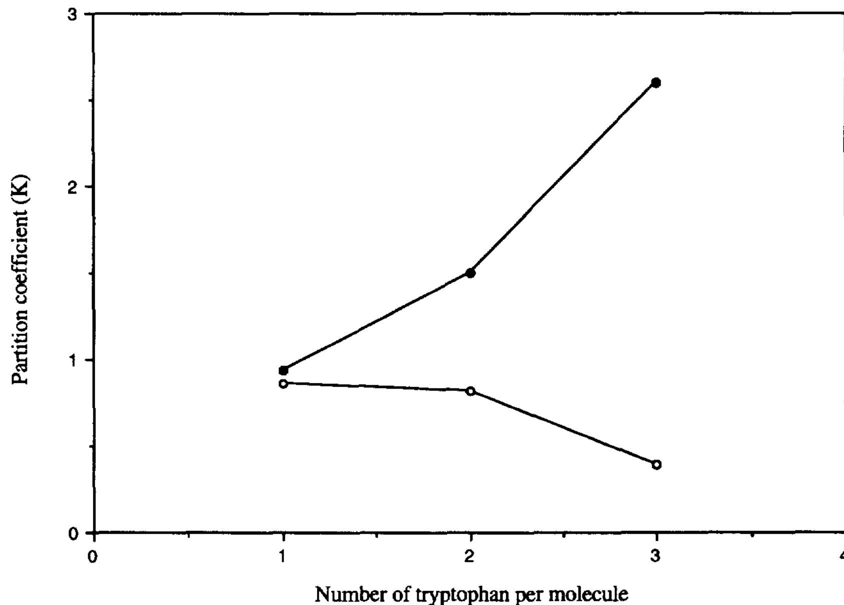

Fig. 1. The effect of increasing length of tryptophan–peptides on their K values in PEG–dextran (●) and PEG–benzoyl dextran (○) systems. The system composition was: 6.0% PEG 8000 and 6.0% dextran T500 or 6% PEG and 6% benzoyl dextran (DS=0.14 to 0.17), 10 mM sodium phosphate buffer (pH 7.0). Room temperature (22 °C).

Table 6
Partition coefficient of salts in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems

	K					
	Sodium phosphate buffer (pH 7.0)	Na_2HPO_4	NaH_2PO_4	NaCl	NaI	$NaClO_4$
BzDx						
DS=0	0.77	0.76	0.90	0.99	1.10	1.19
DS=0.098	0.91	0.87	0.99	1.10	1.07	1.18
VaDx						
DS=0.025	0.78	0.78	0.90	1.02	1.11	1.23
DS=0.12	0.88	0.92	1.00	1.03	1.01	1.09

The system composition was: 6% PEG 8000, 6% BzDx (or 6% VaDx), 100 m*M* salts, room temperature (22 °C).

lysine residues. In PEG–dextran aqueous two-phase systems phosphate is partitioned to the bottom dextran phase [24] (see also Table 6). The interfacial electrical potential difference which is created leads to partitioning of the positively charged polypeptide to the bottom phase [1]. The hydrophobicity of the bottom phase is increased with increasing DS of valeryl dextran. The partition coefficient of phosphate is also increased by valeryl groups on dextran (Table 6), which can explain the increase of the partition coefficient of poly(lysine, tryptophan) with the increased DS of valeryl dextran. When 100 m*M* NaCl was added to the system, the 10 times excess of this salt determines the partitioning of poly(lysine, tryptophan). The partition coefficient of poly(lysine, tryptophan) was increased by a factor of 10 by the addition of NaCl in the PEG–dextran system. The partition coefficient of NaCl in the PEG–dextran system is about 1 (Table 6), which is higher than the *K* value of sodium phosphate buffer ($K=0.77$). The higher affinity of Cl^- ions for the top phase, when compared with the phosphate ions, leads to higher *K* values for poly(lysine, tryptophan) (Table 5). The partition coefficient of poly(lysine, tryptophan) in 100 m*M* NaCl was decreased with increasing DS of valeryl dextran. This could reflect stronger hydrophobic interaction between valeryl dextran and poly(lysine, tryptophan) by the addition of NaCl, and that this effect increased with increasing DS of valeryl dextran. Hydrophobic interactions are strengthened by increasing salt concentration.

Poly(lysine, tryptophan) was also partitioned in aqueous two-phase systems composed of 3.6% dextran T500 and 3.3% valeryl dextran. The two-phase forming polymers (dextran and valeryl dextran) have the same molecular backbones. Therefore the properties of the two phases are quite similar. Different salts at 100 m*M*, including NaCl, $NaClO_4$ and NaI, were added to the systems to study the salt effect (Fig. 2). NaCl had only a small effect in lowering the partition coefficient of poly(lysine, tryptophan). With $NaClO_4$ and NaI the *K* values were strongly decreased. This can be explained by the partitioning of the ClO_4^- and I^- ions to the valeryl dextran phase. In a dextran–benzoyl dextran system composed of 4.75% dextran T500 and 6.0% benzoyl dextran (DS=0.17), the partition coefficients of NaCl, NaI and $NaClO_4$ are 1.20, 1.17 and 1.13, respectively [10]. The lower partition coefficient of NaI and $NaClO_4$ compared with NaCl indicates higher affinity for the HM-dextran phase of I^- and ClO_4^- compared with Cl^-. This results in decreased *K* values for the positively charged poly(lysine, tryptophan) in systems containing NaI and $NaClO_4$.

3.3. Partitioning of salts

Partition coefficients of sodium phosphate buffer (pH 7.0), Na_2HPO_4, NaH_2PO_4, NaCl, $NaClO_4$ and NaI in PEG–benzoyl dextran and PEG–valeryl dextran aqueous two-phase systems are shown in Table 6. The systems were composed of 6% PEG 8000, 6% benzoyl dextran (or valeryl dextran) with different degrees of substitution, and 100 m*M* salts. In the PEG–dextran system, sodium phosphate buffer, Na_2HPO_4 and NaH_2PO_4 were partitioned towards the bottom phase which shows the hydrophilicity of these salts. NaCl had a *K* value of ~1,

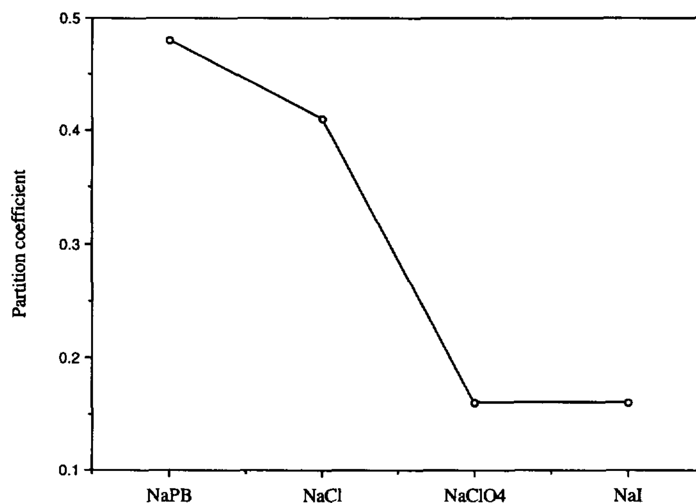

Fig. 2. Partition coefficient of poly(lysine, tryptophan) in systems of dextran–valeryl dextran. The system composition was: 3.6% dextran T500, 3.3% valeryl dextran (DS=0.20), 10 m*M* sodium phosphate buffer, pH 7.0. The concentration of salts was 100 m*M*. Room temperature (22 °C).

NaI and $NaClO_4$ were partitioned to the top phase. The partition coefficients of NaPB, Na_2HPO_4, NaH_2PO_4 and NaCl were increased by increasing the DS of benzoyl (or valeryl) dextran. In contrast, the partition coefficients of NaI and $NaClO_4$ were decreased by increasing DS of benzoyl dextran or valeryl dextran. This is in accordance with the ions position in the Hofmeister series, where I^- and ClO_4^- are chaotropic ions (more hydrophobic) compared with Cl^- and $HPO_4^{2-}/H_2PO_4^-$. The partition coefficients of NaCl, $NaClO_4$ and NaI were earlier measured in the dextran–benzoyl dextran system (Table 4 in [10]). To complement these measurements the K value for sodium phosphate (pH 7.0) was determined in the same two-phase system (4.75% dextran, 6.0% benzoyl dextran). The obtained K value was 1.34, which was higher than the K values for NaCl, NaI and $NaClO_4$, also in accordance with the Hofmeister series.

3.4. Partitioning of β-galactosidase and lysozyme

The properties of the two proteins are shown in Table 7, where it can be seen that β-galactosidase and lysozyme have relatively high contents of tryptophans, 3 and 4.6%, respectively. The effect of salts on the partition coefficient of β-galactosidase and lysozyme in aqueous two-phase systems composed of 6% PEG 8000 and 6% benzoyl dextran (DS=0.14) is shown in Fig. 3. NaCl, $NaClO_4$ and NaI were added to the systems at 100 m*M* to study the salt effect. The salts studied showed different effects on the partitioning of β-galactosidase compared with lysozyme. For comparison, K values for

Table 7
Properties of lysozyme and β-galactosidase

	Lysozyme	β-galactosidase
Molecular mass	13 900 [29]	465 400 [18,26]
p*I*	11.0 [30]	4.6 [32]
Net charge at pH 7.1	7 [31]	Negative
Total content of Trp	4.6% (total 6) [19]	3% (total 156) [18,26]
Number of Trp exposed on the surface	2 totally exposed and 4 partly exposed	-

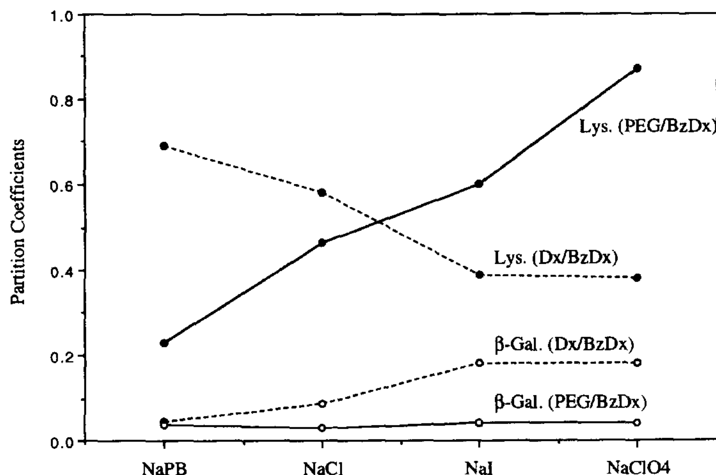

Fig. 3. Partition coefficients of β-galactosidase (○) and lysozyme (●) in system of 6% PEG 8000 and 6% benzoyl dextran (DS=0.14). Sodium phosphate buffer 56 m*M* (pH 7.4), for β-galactosidase and sodium phosphate buffer 67 m*M* (pH 7.0), for lysozyme. NaCl, NaI and $NaClO_4$ were added to 100 m*M* with 10 m*M* NaPB. Room temperature (22 °C). The concentrations of sodium phosphate buffer were calculated to give total ionic strength equal to the systems which contained 10 m*M* NaPB and 100 m*M* salts. The dashed lines show the *K* values in two-phase systems composed of 3.6% dextran T500, 3.3% benzoyl dextran (DS=0.17). The data on the dextran–benzoyl dextran system are from Lu et al. [10].

the two enzymes in the system 3.6% dextran T500 and 3.3% benzoyl dextran (DS=0.17) (from [10]) were also plotted in Fig. 3.

In the PEG–benzoyl dextran two-phase system containing only sodium phosphate buffer, the partition coefficient of β-galactosidase was 0.036 and the *K* values were not significantly affected by the addition of salts (Fig. 3). In this case the hydrophobic interaction between β-galactosidase and benzoyl dextran dominated in the partitioning of the enzyme to the bottom phase. β-Galactosidase from *E. coli* contains a total of 156 of tryptophan residues [18]. The partitioning results indicate that the strong interaction between β-galactosidase and benzoyl groups is due to the interaction between tryptophans on the β-galactosidase surface and the benzoyl groups.

In the dextran–benzoyl dextran two-phase system containing only sodium phosphate buffer, the partition coefficient of β-galactosidase was 0.046. The *K* value was slightly increased by addition of NaCl, NaI and $NaClO_4$. The p*I* of β-galactosidase is 4.6 (Table 7) and in a pH 7.4 system the protein is negatively charged. Sodium phosphate salts (Na_2HPO_4 and NaH_2PO_4) are hydrophilic salts. In a dextran–benzoyl dextran system these salts are partitioned to the hydrophilic dextran top phase ($K=$ 1.34). The partitioning of negatively charged phosphate ions to the top phase will lead to β-galactosidase partitioning to the bottom phase. Our previous study showed that in a dextran–benzoyl dextran system composed of 4.75% dextran and 6.0% benzoyl dextran (DS=0.17), the partition coefficient of the salts was decreased in the order NaCl, NaI and $NaClO_4$ (Table 4 in [10]). The decreased partition coefficient of salts resulted in increased *K* values for β-galactosidase in the corresponding phase systems. However, even by adding 100 m*M* NaI or $NaClO_4$ the highest *K* value of β-galactosidase obtained was only 0.18. The strong partitioning of β-galactosidase to the benzoyl dextran bottom phase, and the low sensitivity to added salts, show also in this system the hydrophobic interaction between the protein and the benzoyl groups on the dextran.

The p*I* of lysozyme is 11 (Table 7) and in a pH 7.0 system lysozyme is positively charged. In a PEG–benzoyl dextran two-phase system sodium phosphate was partitioned to the bottom phase (Table 6). The partitioning of phosphate ions leads to partitioning of lysozyme to the bottom phase (Fig. 3). Also, the hydrophobic interaction between lysozyme and benzoyl dextran is favourable for lyso-

zyme partitioning to the benzoyl dextran bottom phase. Lysozyme has a relatively high content of tryptophans and several are located on the surface (Table 7). The addition of NaCl, NaI and $NaClO_4$ in the PEG–benzoyl dextran system led to increased K values for lysozyme. This can be correlated with the increased K values for these salts in the same phase system (Table 6).

The salt effects on K values for lysozyme showed opposite behaviour in dextran–benzoyl dextran systems compared with PEG–benzoyl dextran systems (Fig. 3). For the system containing only sodium phosphate buffer the partition coefficient of lysozyme was 0.69 in the dextran–benzoyl dextran system which is higher than in PEG–benzoyl dextran system. This difference can be correlated with the different K values for the phosphate salts, where a higher K value was obtained in the dextran–benzoyl dextran systems ($K=1.34$) compared with the PEG–benzoyl dextran system ($K=0.91$). Decreased K values for lysozyme in dextran–benzoyl dextran system were obtained by addition of NaCl, NaI and $NaClO_4$, which reflects the increasing affinity of the corresponding anions for the hydrophobic bottom phase (Table 4 in [10]). The increasing affinity of lysozyme counterions to the benzoyl dextran phase leads to increased partitioning of the protein to this phase.

4. Conclusions

Tryptophan residues show hydrophobic interaction with benzoyl dextrans and valeryl dextrans. The interaction was stronger with increasing numbers of tryptophan in the molecule. Benzoyl groups, compared with valeryl groups, showed stronger interaction with tryptophan containing molecules. The partitioning of tryptophan, tryptophan–tryptophan and $(tryptophan)_3$ in systems with the modified dextrans was affected very little by the addition of salts. The partitioning of poly(lysine, tryptophan), β-galactosidase and lysozyme was affected by salt addition and the salt effects could be correlated with the partitioning of the salts in systems containing hydrophobically modified dextrans. The results from partitioning of β-galactosidase and lysozyme in phase systems with benzoyl dextran indicate that the relatively high number of tryptophans in these proteins strongly affects the partitioning.

Acknowledgments

This work was supported by the Swedish Research Council for Engineering Sciences (TFR).

References

[1] P.-Å. Albertsson, Partition of Cell Particles and Macromolecules, Wiley, New York, NY, 3rd edn., 1986.
[2] H. Walter and G. Johansson (Editors), Methods Enzymol. 228 (1994).
[3] V.P. Shanbhag and G. Johansson, Eur. J. Biochem., 93 (1979) 363–367.
[4] G. Kopperschläger and G. Johansson, Anal. Biochem., 124 (1982) 117–124.
[5] A. Cordes and M.-R. Kula, J. Chromatogr., 376 (1986) 375–384.
[6] G. Johansson and M. Andersson, J. Chromatogr., 303 (1984) 39–51.
[7] G. Johansson and M. Joelsson, J. Chromatogr., 393 (1987) 195–208.
[8] M. Lu, F. Tjerneld, G. Johansson and P.-Å. Albertsson, Bioseparation, 2 (1991) 247–255.
[9] M. Lu, P.-Å. Albertsson, G. Johansson and F. Tjerneld, J. Chromatogr. A, 668 (1994) 215–228.
[10] M. Lu, G. Johansson, P.-Å. Albertsson and F. Tjerneld, Bioseparation, 5 (1995) 351–358.
[11] M. Lu, P.-Å. Albertsson, G. Johansson and F. Tjerneld, J. Chromatogr. B, 680 (1996) 65–70.
[12] C.C. Bigelow, J. Theoret. Biol., 61 (1967) 187–211.
[13] E. Keshavarz and S. Nakai, Biochim. Biophys. Acta, 576 (1979) 269–279.
[14] J.A. Asenjo, A.S. Schmidt, F. Hachem and B.A. Andrews, J. Chromatogr. A, 668 (1994) 47–54.
[15] V.P. Shanbhag and C.G. Axelsson, Eur. J. Biochem., 60 (1975) 17–22.
[16] K. Köhler, C. Ljungquist, A. Kondo, A. Veide and B. Nilsson, Bio/Technology, 9 (1991) 642–646.
[17] C. Hassinen, K. Köhler and A. Veide, J. Chromatogr. A, 668 (1994) 121–128.
[18] A. Kalnins, K. Otto, U. Rüther and B. Müller-Hill, EMBO J., 2 (1983) 593–597.
[19] T. Imoto, L.N. Johnson, A.C. North, D.C. Phillips and J.A. Rupley, in P.D. Boyer (Editor), The Enzymes, Vol. 7, Academic Press, New York, NY, 1972, pp. 665–868.
[20] M. Carlsson, K. Berggren, P. Linse, A. Veide and F. Tjerneld, J. Chromatogr. A, 756 (1996) 107.
[21] A. Veide, A.L. Smeds and S.-O. Enfors, Biotechnol. Bioeng., 25 (1983) 1789–1800.

[22] D. Shugar, Biochim. Biophys. Acta, 8 (1952) 302–309.
[23] L.A. Decker (Editor), Worthington Enzyme Manual, 1977.
[24] G. Johansson, Biochim. Biophys. Acta, 221 (1970) 387–390.
[25] M.H. Klapper, Biochem. Biophys. Res. Commun., 78 (1977) 1018–1024.
[26] A.V. Fowler and I. Zabin, J. Biol. Chem., 253 (1978) 5521–5525.
[27] K. Köhler, A. Veide and S.O. Enfors, Enzyme Microb. Technol., 13 (1991) 204–209.
[28] P.-Å. Albertsson, A. Cajarville, D.E. Brooks and F. Tjerneld, Biochim. Biophys. Acta., 926 (1987) 87–93.
[29] G. Zubay, Biochemistry, Macmillan, New York, NY, 2nd edn., 1988, p. 88.
[30] M. Wahlgren, Ph. D. Thesis, University of Lund, Lund, 1992.
[31] C. Tanford and M.L. Wagner, J. Am. Chem. Soc., 76 (1954) 3331–3336.
[32] K. Wallenfels and R. Weil, in P.D. Boyer (Editor), The Enzymes, Vol. 7, Academic Press, New York, NY, 3rd edn., 1972, p. 627.