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# Quantitative micellar chromatographic analysis of interaction between peptides and sodium dodecyl sulfate micelles

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#### Abstract

Interaction between short, water-soluble peptides and sodium dodecyl sulfate (SDS) micelles was analyzed by chromatography of the peptides on a Sephadex G-50 M el bed in an eluent containing the micelles. Peptides that interacted with the micelles showed intermediate elution volumes. In the absence of micelles in the eluent, the peptides (7-16 amino acid residues) were eluted near the total volume, whereas micelles applied as a sample appeared at the void volume. The association constants,  $K_A$ , were calculated from the capacity factors k' obtained at different SDS micelle concentrations. The range of the  $K_A$  values was  $(0.5-11)\times10^4~M^{-1}$ . As a rule, the longest peptides and the positively charged ones interacted most strongly with the micelles, but the amino acid sequences also affected the interaction. Partially hydrophobic peptides that may correspond to interfacial segments of a transmembrane protein, the glucose transporter Glut1, showed relatively weak interactions with SDS micelles. The peptide interaction with sodium 1-decane sulfonate micelles supplemented with SDS was similar to that with SDS micelles, whereas none of several peptides tested interacted significantly with micelles of the non-ionic detergent n-dodecyl octaoxyethylene. The peptide–micelle interaction was proposed to occur mostly at the micelle surface as in the protein-decorated micelle structure for SDS–protein complexes.

Keywords: Micellar liquid chromatography; Peptides; Sodium dodecyl sulfate micelles; Glucose transport

### 1. Introduction

The anionic detergent, sodium dodecyl sulfate (SDS), forms complexes with both water-soluble proteins and membrane proteins. The SDS-protein complexes can be separated by electrophoretic and chromatographic methods for analytical and preparative purposes. Analyses of the hydrodynamic and electrophoretic behavior and the neutron and small-angle X-ray scattering (SAXS) of SDS complexes with water-soluble proteins have led to the proposal

of several structural models. In the "necklace model", SDS clusters or micelle-like clusters are arranged along the polymer chain [1,2], whereas in the "protein-decorated micelle structure", based on neutron scattering determinations, the dodecyl chains of the detergent form hydrophobic micelle cores, surrounded by shells consisting of the hydrophilic and amphiphilic stretches of the polypeptide chain and the sulfate head-groups [3,4]. Similarly, SAXS results showed a few micelle-like SDS clusters as the major substructure of the complex [5]. SAXS and cryo-electron microscopic data indicated a protein-decorated micelle structure, which was called a necklace structure [6]. Transmembrane proteins in

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SDS-complexes may retain the tertiary structure of their hydrophobic regions.

Even short peptides interact with SDS micelles, as shown by effects on the electrophoretic mobility of the micelles [7]. The affinity of a hydrophilic polypeptide to SDS micelles presumably depends on the polypeptide length. The aim of the present work was to quantitatively analyze interactions between short hydrophilic peptides and SDS micelles by micellar liquid chromatography (MLC, see Ref. [8]). Suitable model peptides were studied. Some of these corresponded to segments of a transmembrane protein, the human red cell glucose transporter (Glut1). Most peptides had earlier been used in a study of peptideliposome interactions [9]. Two peptides were synthesized to have different lengths but zero net charge and zero net water-to-oil transfer free energy  $(\Delta G_{\rm W/O})$  for the side-chains [10] to test the hypothesis that the association becomes stronger with increasing peptide length, possibly owing to hydrogen-bonds between the sulfate head-groups and the peptide bonds [11]. The association constants  $(K_{\Delta})$ for the SDS-peptide complexes were determined by MLC, by use of the mathematical model described by Foley [8]. The strengths of peptide interaction with micelles of SDS, sodium 1-decane sulfonate and the non-ionic detergent n-dodecyl octaoxyethylene  $(C_1, E_8)$  were compared.

### 2. Experimental

### 2.1. Materials

Sephadex G-50 M was bought from Pharmacia Biotech (Uppsala, Sweden), sodium dodecyl sulfate (SDS, No. 13760) from Merck (Darmstadt, Germany) and C<sub>12</sub>E<sub>8</sub> from Fluka (Buchs, Switzerland). Sodium 1-decane sulfonate (D 3412), dithioerythritol (DTE), Lys-Tyr-Lys (KYK) and Glu-Asn-Gly (ENG) were obtained from Sigma (St. Louis, MO, USA). Triton X-100 was purchased from Serva (Heidelberg, Germany). Other chemicals were of analytical grade.

The amino acids (W, Y, S, C, G, D, N, R and M, calibration standards from Sigma) were kindly provided by Dr. David Eaker at our Department. The peptides YGSTWPG (P<sub>1</sub>), YGSTWPGC (P<sub>1</sub>C),

 $(P_1S),$  $(P_1CC),$ **YGSTWPGS** YGSTWPGCC  $(P_1SS)$ . MPSWTGG  $(P_2)$ , YGSTWPGSS (P,C), **MPSWTGGS** (P,S),**MPSWTGGC MPSWTGGCC** (P,CC),**MPSWTGGSS** (P,SS),**IPCCPVHLKR KPSSPARLLK**  $(P_3),$  $(P_4)$ , MGSTWPG (Ps), YPSWTGG (Ps), MSWTGGSSP  $(P_7)$ ,  $a_{51-64C}$ ,  $b_{125-134}$ ,  $c_{201-210}$ ,  $d_{293-307C}$ ,  $f_{421-429}$ , g<sub>478-492YC</sub> and h<sub>218-232YC</sub> were synthesized as described in Refs. [9,12]. Peptides YTTNYTTS (P<sub>8</sub>) and YTTNYTTSYTTNYTTS (P.P.) were synthesized by Dr. Åke Engström, Department of Medical and Physiological Chemistry, Uppsala University, solid-phase  $N^{\alpha}$ -q-fluorenylmethyloxycarbonyl (Fmoc) chemistry, purified by reversed-phase HPLC and analyzed by plasma desorption mass spectrometry [13] using a Bion 20 instrument (Bio-Ion Nordic AB, Uppsala, Sweden). Peptides P<sub>3</sub> and P<sub>4</sub> correspond to hydrophilic segments of chondromodulin-I from fetal bovine cartilage [14]. Peptides a<sub>51-64C</sub>-h<sub>218-232YC</sub> correspond to the segments 51-64 etc (Fig. 6, below) of the human HepG2 hepatoma glucose transporter, which is similar or identical to the human red cell glucose transporter Glut1 [15]. In some cases, C-terminal Cys or Tyr-Cys were added as indicated in the above peptide denotations [12].

#### 2.2. Eluents and micelle concentrations

Eluent A contained 50 mM sodium phosphate (pH 7.1) and 1 mM Na<sub>2</sub>EDTA; eluent B was eluent A supplemented with 3 mM NaN<sub>3</sub>. Detergent was included in eluent A and B as described below. Upon analysis of peptides containing Cys, 0.2 mM DTE was also included.

The values of critical micelle concentration (CMC) and aggregation number (N) used for calculation of micelle concentrations in the eluents were as follows. For SDS in eluent A or B, CMC=1.8 mM at 23°C [3,16,17] and N=89 (estimated from data in Fig 7, [18], dashed line, 0.1 M NaCl, which has the same ionic strength as the eluents used); for  $C_{12}E_8$ , CMC=0.11 mM at 25°C [19] and N=120 [20]; and for sodium decane sulfonate in eluent B, CMC=28 mM at 23°C (measured by weighing of thermostated drops) and N≈89 (estimated). The effect of the presence of SDS in mixed decane sulfonate/dodecyl sulfate micelles was neglected.

# 2.3. MLC analysis of interactions between micelles and peptides or amino acids

The interactions between peptides or amino acids and SDS micelles were analyzed by MLC at 23±1°C on a 0.5 (diameter)×20-cm Sephadex G-50 M gel bed (HR 5/20 glass column, Pharmacia Biotech) which was equilibrated overnight at 0.1 ml/min with SDS (concentration 1.8, 3, 5, 7, 10, 50 or 100 mM) in eluent A (for analysis of peptides without aromatic residues, detection at 220 nm) or eluent B (for analysis of peptides with aromatic residues, detection at 280 nm). The peptides (0.3 mg/ml) were dissolved in the eluent (A or B), containing 10 mM SDS. Amino acids (0.5 mg/ml) were dissolved in eluent A containing 10 mM SDS. The peptides or amino acids were run at each of the above concentrations of SDS in the eluent, except that 7 mM SDS was used only in some cases. Analyses were also done with sample and eluent containing sodium decane sulfonate (32 mM) supplemented with SDS (1.6 mM, to enhance the solubility), or  $C_{12}E_8$  (4.5 mM) (see Table 2 below). The total  $(V_t)$  and void  $(V_0)$  volumes of the gel bed were determined by use of NaN3 and tobacco mosaic virus, respectively. SDS micelles (supplemented with Triton X-100 at the monomer molar ratio 100:1 for absorbance detection) were eluted slightly after  $V_0$ . The small difference was neglected in the calculations below. Peptide-micelle samples (50  $\mu$ l) were applied and run at a flow-rate of 0.2 ml/min (HPLC pump No. 2248, Pharmacia Biotech). The elution was monitored by a tunable UV photometer (Waters 486, Millipore, Milford, MA). The elution positions of amino acids and peptides of low absorptivity were confirmed by ninhydrin staining [1 mg/ml in ethanol-acetic acid (3:1)] of eluent drops dried on a filter paper.

## 3. Theory

# 3.1. The peptide-SDS micelle association constant $K_A$

MLC was used for the analysis of the peptidemicelle interactions (Fig. 1) and the SDS-peptide association constants,  $K_A$ , were calculated essentially

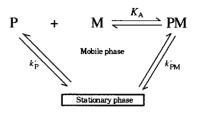


Fig. 1. Retention model for peptide-micelle chemical equilibrium in MLC with P, peptide; M, SDS micelle; PM, peptide-micelle complex;  $k'_{\rm P}$ , the chromatographic capacity factor for the free peptide;  $k'_{\rm PM}$ , the chromatographic capacity factor for the peptide-micelle complex, and  $K_{\rm A}$ , the association constant for interaction between peptides and micelles.

as described in Ref. [8]. The presence of an equilibrant M (micelles) in the mobile phase allows an analyte P (peptide) to exist in two forms: as free analyte and in an analyte—equilibrant complex PM. The rapid interchange between these forms results in elution of the analyte in a single zone. The elution volume decreases with increasing micelle concentration in the eluent, as shown below. The peptide retention, expressed as the capacity factor k' [see also Eq. (8)], is given by

$$k' = F_{\rm PM}k'_{\rm PM} + F_{\rm p}k'_{\rm P} \tag{1}$$

where  $F_{PM}$ , the stoichiometric fraction of the PM complex, is:

$$F_{PM} = [PM]/\{[PM] + [P]\}$$
 (2)

and  $F_{\rm p}$ , the fraction of P in free form, is:

$$F_{\rm P} = [P]/\{[PM] + [P]\}$$
 (3)

and where  $k'_{PM}$  and  $k'_{P}$  are the "limiting" capacity factors of the corresponding entities. In the present work, a small-pore gel was used and the micelles and peptide-micelle "complexes" did not enter the gel beads, i.e.,  $k'_{PM}$  is zero. Eq. (1) thus becomes

$$k' = F_{\mathbf{p}} k_{\mathbf{p}}' \tag{4}$$

Furthermore,

$$K_{A} = [PM]/[P][M] \tag{5}$$

where [M], the concentration of SDS micelles, can be expressed as  $[M] = \{[SDS] - CMC\}/N$ , where [SDS] is the SDS concentration and N is the micellar aggregation number. We assumed that the interaction

between peptides and micelles did not change the average micellar size. Eq. (4) and Eq. (5) give

$$k' = \{1/(1 + K_{A}[M])\}k'_{P} \tag{6}$$

which can be rearranged to read

$$1/k' = (K_{\rm A}/k_{\rm P}')[M] + 1/k_{\rm P}' \tag{7}$$

in which k' can be expressed as

$$k' = (V_{\rm e} - V_{\rm 0})/(V_{\rm t} - V_{\rm 0}) \tag{8}$$

where  $V_{\rm e}$  is the retention volume of the peptide at the micelle concentration [M] in the eluent;  $V_{\rm t}$  is the total volume and  $V_{\rm 0}$  is the void volume. A plot of 1/k' versus [M] yields a straight line with the intercept  $1/k_{\rm P}$ , and the slope  $K_{\rm A}/k_{\rm P}$ , according to Eq. (7). In the calculations,  $k_{\rm P}$  was taken to equal 1 (see the legend to Fig. 2).

## 4. Results and discussion

### 4.1. Interaction of peptides with SDS micelles

Several peptides (Table 1) were run on Sephadex G-50 M with SDS micelles in the eluent. The reciprocals of the k' values increased linearly with

the SDS micellar concentration, at least up to 0.1 mM micelles, with a correlation  $r^2$  above 0.99. Some examples are illustrated in Fig. 2 and Fig. 3. The peptide-SDS micelle association constants,  $K_{\Delta}$ , were calculated according to Eq. (7) as described in the Section 3 and in the legend to Fig. 2. The values are given in Table 1. The five P2 peptides showed much stronger interaction with the SDS micelles than did the P<sub>1</sub> peptides. The sequences of the P<sub>1</sub> and P<sub>2</sub> peptides differed, but the amino acid composition was the same, except for an exchange of Tyr in P<sub>1</sub> for Met in P<sub>2</sub> between the two series of peptides. The sequence effect is further described in Section 4.3. An exchange of the N-terminal Tyr in P<sub>1</sub> to Met to obtain the more hydrophobic peptide P5 did not increase the affinity for SDS micelles, and substitution of N-terminal Met in P<sub>2</sub> for Tyr to obtain peptide P<sub>6</sub> did not decrease the affinity (Table 1). However, the association constants of the peptides with 7-9 residues in Table 1 increased with decreasing  $\Delta G_{\mathrm{W/O}}$  values, although the linear correlation was weak  $(r^2 = 0.65)$  (not shown). For these peptides of about the same length, the hydrophobicity of the side chains thus affected the association between the peptides and the micelles.

Table 1  $K_A$  for peptide-SDS micelle interaction and  $\Delta G_{W/O}^a$  of the peptides at pH 7.1 b

Peptide		$\begin{array}{c} K_{\rm A} \\ (10^4 \ M^{-1}) \end{array}$	$\Delta G_{ m w/o}$ (kcal/mol)
Denote	Sequence	(10 M )	(KCal/Inol)
$P_1$	YGSTWPG	0.9	4.1
$P_iS$	YGSTWPGS	1.4	3.5
$P_1C$	YGSTWPGC	1.5	2.1
$P_1SS$	YGSTWPGSS	2.4	2.9
P <sub>1</sub> CC	YGSTWPGCC	2.3	0.1
$\mathbf{P}_{2}$	MPSWTGG	5.9	0.4
$P_2S$	MPSWTGGS	11.0	0.2
$P_2C$	MPSWTGGC	8.9	-1.6
P <sub>2</sub> SS	MPSWTGGSS	9.8	-0.8
P <sub>2</sub> CC	MPSWTGGCC	10.3	-3.6
$P_5$	MGSTWPG	0.7	0.4
$P_6$	YPSWTGG	5.9	4.1
$P_8$	YTTNYTTS	0.5	9.3
$P_8P_8$	YTTNYTTSYTTNYTTS	8.7	9.3

<sup>&</sup>lt;sup>a</sup> Water-oil transfer free energies of side chains of peptides, calculated according to the values given in Table 1 of [10]. The  $\Delta G_{\text{W/O}}$  value for each peptide is the sum of  $\Delta G_{\text{W/O}}$  values for every amino acid residue of the peptide. This calculation included the amino and carboxylic groups at the termini. The  $\Delta G_{\text{W/O}}$  values of Y, G, S, T, W, P, C, N, M, N- and C-terminal are 0.3 (0.7 is given in Ref. [10]; it is not correct), -1.0, -0.6, -1.2, -1.9, 0.2, -2.0, 4.8, -3.4, 5.0 and 4.3 respectively [10].

<sup>&</sup>lt;sup>b</sup> The net charge (calculated as described in [21,22]) of all peptides was  $-0.24-0.25\times1.6\times10^{-19}$  As.

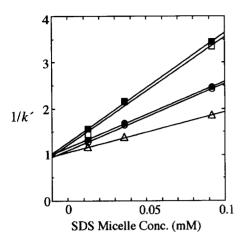


Fig. 2. Dependence of the reciprocal of the capacity factor on the SDS micelle concentration in the mobile phase for five peptides:  $P_1$  (triangles),  $P_1$ C (filled circles),  $P_1$ S (open circles),  $P_1$ CC (filled squares) and  $P_1$ SS (open squares). The  $K_A$  values were calculated as described in Section 3 and were approximated to equal the slopes  $K_A/k_P'$ , for the reason that the intercepts  $1/k_P'$  were expected to be 1.0. The experimental values of  $(V_c - V_0)/(V_1 - V_0)$  were near to 1.0 in the absence of SDS. The use of the intercept values in the figure was found to be less reliable, as the position [M] = 0 was not precisely defined, since the peptide samples contained 10 mM SDS in all runs, and since the CMC and aggregation number of SDS are difficult to define with sufficient accuracy.

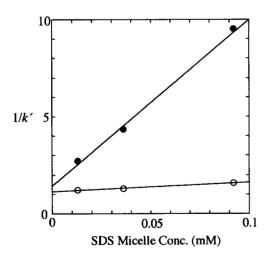


Fig. 3. Dependence of the reciprocal of the capacity factor on the SDS micelle concentration in the mobile phase for  $P_g$  (open circles) and  $P_g P_g$  (filled circles).  $K_A$  values were calculated as described in the Section 3, further details being given in the legend to Fig. 2.

### 4.2. Effect of peptide length

As the P<sub>1</sub> peptide length (seven residues) was increased to eight and nine residues by addition of one or two C-terminal Ser or Cys residues, the interaction strength increased (Fig. 2 and Table 1). Similar results were obtained for the peptides P<sub>2</sub>, P<sub>2</sub>SS and P<sub>2</sub>CC (Table 1). The effect of Cys was the same as that of Ser, contrary to the specific effect of C-terminal Cys in peptide-liposome interaction [9]. In an attempt to distinguish whether the proposed hydrogen-bonds between peptide-bond nitrogen and sulfate-group oxygens [11] participated in the interaction of peptide to SDS micelles or not, the hydrophilic peptides P<sub>8</sub> and P<sub>8</sub>P<sub>8</sub> were chosen to have the same charge and  $\Delta G_{W/O}$  (calculated as the transfer free energy for transfer of side-chains and terminal groups from water-to-oil [10]). Only the length of the peptide differed. The monomer interacted only weakly with the SDS micelles, whereas the sixteen-residue dimer exhibited strong interaction (Fig. 3 and Table 1). Longer peptides thus showed stronger interaction with SDS micelles than did shorter ones. This may be explained both by hydrogen-bonding and by other cooperative interactions between the peptides and the micelles.

### 4.3. Effect of peptide sequence

The sequence of peptide P<sub>1</sub> (YGSTWPG) differs from that of peptide P<sub>6</sub> (YPSWTGG) although the amino acid composition is the same. P<sub>6</sub> interacted much more strongly with SDS micelles than did P<sub>1</sub>, and similarly, P<sub>2</sub> (MPSWTGG) interacted more strongly than did P<sub>5</sub> (MGSTWPG) (Table 1 and Fig. 4). This shows the importance of the peptide sequence. Peptides P<sub>1</sub> and P<sub>6</sub> in a mixture could be separated by MLC with 10 mM SDS in the eluent (Fig. 4A), as could peptides P<sub>2</sub> and P<sub>5</sub> (Fig. 4B). Another nonapeptide, P<sub>7</sub>, was chosen to have the same sequence as P<sub>2</sub>SS, except that the Pro residue was moved from position 2 to the C-terminus. This sequence modification weakened the interaction between the peptide and the micelles (k'=0.4 for  $P_7$ and 0.2 for P<sub>2</sub>SS at 5 mM SDS). Also this result shows that the peptide sequence can affect the interaction with the SDS micelles.

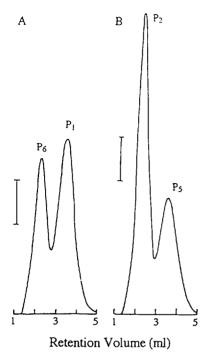


Fig. 4. Separation of (A)  $P_1$  mixed with  $P_6$ , and (B)  $P_2$  mixed with  $P_5$ , by MLC in eluent A supplemented with 10 mM SDS. The bars correspond to an absorbance of 0.02 at 1-cm light path.

# 4.4. Interaction of peptides corresponding to segments of Glut 1 with SDS micelles

Peptides a-d and f-h correspond to segments of the glucose transporter from human HepG2 hepatoma cells [15] as indicated in Fig. 5. The hydrophilic peptides, a, h and d (with 12-16 residues) interacted most strongly with SDS (Fig. 6). Even at the CMC these peptides formed peptide-micelle complexes (Fig. 6). The short peptide c, which is negatively charged, interacted less strongly with the micelles (Fig. 6). This peptide contained two Pro residues: according to the hydrogen-bonding hypothesis model of the interactions and hydrogen-bonding in SDS-protein complexes [11], the Pro-Phe-Cys-Pro (see Fig. 5B) segment in peptide c cannot form hydrogen bonds with the head group of SDS, however the two Pro residues may allow adjacent hydrophobic residues Phe and Cys to insert into the hydrophobic micelle core. The peptide g showed weak interaction (see graphs for 1.8 mM and 10 mM SDS in Fig. 6), presumably because it was repelled

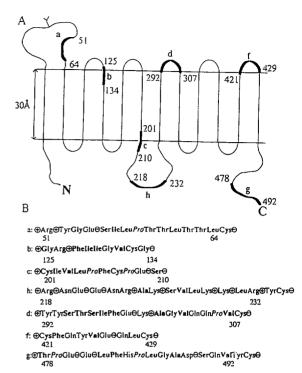


Fig. 5. Position and sequence of selected Glut1 segments. (A) Position of the polypeptide segments (thick lines) a-d and f-h in the topology model of [15]. (B) Sequence of the synthesized polypeptides corresponding to the above segments. Charges at pH 7 are indicated, residues with hydrophobic side chains are given in bold-face and Pro is shown in italics.

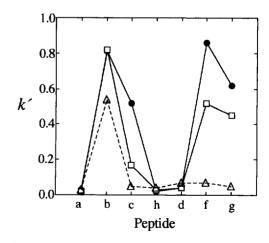


Fig. 6. The capacity factors of peptides corresponding to segments of Glut1 (see Fig. 5) at the SDS concentrations; 1.8 mM (the CMC, filled circles), 10 mM (squares) and 50 mM (triangles), in eluent B.

from the sulfate head-groups of the micelles by four negative charges, only the N-terminal being positively charged. Also the short peptide f showed weak interaction, perhaps owing to a negatively charged side chain. Peptide b showed the weakest interaction among the Glut1 peptides tested, despite a positive net charge, perhaps because the hydrophobic stretch FIIGVCG is situated between a positive and a negative charge in the peptide and is too short to become inserted into the micelle core with hydrogen bonding between the NH- and C=O groups. Peptides b and c correspond to segments that are partially inserted in the membrane of the 12-helical model (Fig. 5, above). Interestingly, several peptides that interacted strongly with liposomes upon immobilized-liposome chromatography [9] associated weakly with the micelles, and vice versa, as illustrated in Fig. 7. Presumably the interactions of peptides with the SDS micelles were mostly of an electrostatic nature (attraction of positively charged groups and, possibly, hydrogen bonding), whereas the peptide interactions with the non-charged liposomes were mostly of hydrophobic character. The two positively charged and hydrophilic peptides, P<sub>2</sub> and P4, interacted very strongly with SDS micelles

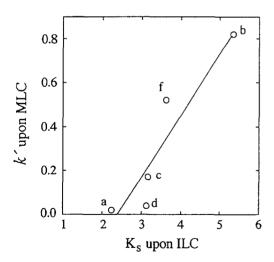


Fig. 7. The capacity factors of the peptides a-d and f that correspond to segments of Glut1 (see Fig. 5 and Fig. 6) on the MLC column with 10 mM SDS (y-axis), versus the specific capacity factors on an immobilized-phosphatidylcholine-phosphatidylethanolamine (4:1) liposome column (data from Fig. 8B in [9]).

( $k'\approx0.03$  at 1.8 mM SDS), indicating that the charge of the peptides affected the peptide–micelle interactions. At pH 11 (0.05 M phosphate–NaOH), where the peptides are negatively charged, the peptide–micelle interactions were very weak for P<sub>1</sub>SS, P<sub>2</sub>SS, P<sub>7</sub>, P<sub>8</sub>, and P<sub>8</sub>P<sub>8</sub> (charge between -2 and  $-1\times1.6\times10^{-19}$  A s).

# 4.5. Interaction of amino acids and tripeptides with SDS micelles

The interaction between SDS micelles and the amino acids Trp, Tyr, Ser, Cys, Gly, Asp, Asn, Arg and Met was analyzed. Trp showed stronger interaction  $(K_{\Delta} = 0.1 \times 10^4 M^{-1})$  than did the other amino acids, for which the association was very weak. In [23], amino acids were shown to have different interactions with SDS micelles at pH 2.7, at which pH the amino acids are positively charged. These interactions were probably of an electrostatic nature. At pH 6.5 the binding capacity was decreased. The negatively charged tripeptide ENG showed no interaction with SDS micelles, whereas the positively charged peptide KYK interacted fairly strongly with SDS micelles  $(K_A = 7.4 \times 10^4 \ M^{-1})$ . These results indicate that the electrostatic charge of amino acids and very short peptides strongly affect their interaction with SDS micelles.

#### 4.6. Different detergents

The interaction of peptides with micelles of the anionic detergents SDS and sodium decane sulfonate, and the non-ionic detergent  $C_{12}E_8$ , were compared. The capacity factors of the peptides were much smaller (the interaction stronger) with SDS micelles than with  $C_{12}E_8$  micelles (Table 2). No obvious difference was found in the strength of interaction of peptides with SDS micelles and the mixed sodium decane sulfonate and SDS micelles, to judge from MLC capacity factors (Table 2). This result indicated that the surface structure and the charge of the micelles are important for the interaction with peptide. The sulfate head-group showed only slightly stronger interaction with the peptides than did the sulfonic head-group.

Table 2 The capacity factors k' of peptides upon MLC with different detergents

Peptide	k ' a				
	SDS <sup>b</sup>	Sodium 1-decane sulfonate <sup>c</sup>	$C_{12}E_8^{d}$	No detergent	
P <sub>1</sub> C	0.6	0.7	1	1	
$P_2C$	0.2	0.3	0.9	0.9	
P <sub>s</sub>	0.8	0.9	0.9	0.9	
$P_8P_8$	0.2	_	0.8	0.8	
a <sub>51-64C</sub>	0.03°	_	0.7	0.7	
b <sub>125-134</sub>	0.8°	_	0.9	0.9	

<sup>&</sup>lt;sup>a</sup>  $k' = (V_c - V_0)/(V_t - V_0)$ . Average of 2-5 measurements.

#### 5. Conclusions

Even short hydrophilic peptides (7-16 residues) can associate strongly with SDS micelles. The charge, sequence, length and amphiphilic characteristics of the peptides are important for their interaction with SDS micelles. Hydrogen-bonding between the sulfate head-groups and the peptide bond nitrogens [11] may occur. Short hydrophilic peptides do not associate with non-ionic micelles (C<sub>12</sub>E<sub>8</sub>). Micelles of sodium decane sulfonate interacted with peptides nearly as strongly as did SDS micelles. Peptides corresponding to hydrophilic segments of Glut1 on the cytoplasmic and extracellular faces [9,15] behaved in SDS solution like water-soluble peptides and showed strong interaction with SDS micelles. Amphiphilic Glut1 peptides that probably corresponded to interfacial polypeptide segments of Glut1 showed weaker and more varying interactions with SDS micelles.

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#### References

- K. Shirahama, K. Tsujii and T. Takagi, J. Biochem. (Tokyo), 75 (1974) 309.
- [2] X.H. Guo, N.M. Zhao, S.H. Chen and J. Teixeira, Biopolymers, 29 (1990) 335.
- [3] K. Ibel, R.P. May, K. Kirschner, H. Szadkowski, E. Mascher and P. Lundahl, Eur. J. Biochem., 190 (1990) 311.
- [4] K. Ibel, R.P. May, M. Sandberg, E. Mascher, E. Greijer and P. Lundahl, Biophys. Chem., 53 (1994) 77.
- [5] S. Shinagawa, M. Sato, K. Kameyama and T. Takagi, Langmuir, 10 (1994) 1690.
- [6] M. Samsó, J.-R. Daban, S. Hansen and G.R. Jones, Eur. J. Biochem., 232 (1995) 818.
- [7] M.R. Karim, S. Shinagawa and T. Takagi, Electrophoresis, 15 (1994) 1141.
- [8] J.P. Foley, Anal. Chim. Acta, 231 (1990) 237.
- [9] Y. Zhang, S. Aimoto, L. Lu, Q. Yang and P. Lundahl, Anal. Biochem., 229 (1995) 291.
- [10] D.M. Engelman, T.A. Steitz and A. Goldman, Annu. Rev. Biophys. Biophys. Chem., 15 (1986) 321.
- [11] P. Lundahl, E. Greijer, M. Sandberg, S. Cardell and K.-O. Eriksson, Biochim. Biophys. Acta, 873 (1986) 20.
- [12] L. Andersson and P. Lundahl, J. Biol. Chem., 263 (1988) 11414.
- [13] B. Sundqvist and R.D. Macfarlane, Mass. Spectrom. Rev., 4 (1985) 421.
- [14] Y. Hiraki, H. Tanaka, H. Inoue, J. Kondo, A. Kamizono and F. Suzuki, Biochem. Biophys. Res. Commun., 175 (1991) 971.

<sup>&</sup>lt;sup>b</sup> At 5 mM SDS (0.036 mM micelle concentration).

<sup>&</sup>lt;sup>c</sup> With 32 mM sodium 1-decane sulfonate mixed with 1.6 mM SDS (≈0.04 mM micelle concentration).

 $<sup>^{\</sup>rm d}$  At 4.5 mM  $C_{12}E_8$  ( $\approx 0.04$  mM micelle concentration).

<sup>&</sup>lt;sup>e</sup> At 10 mM SDS (0.072 mM micelle concentration).

- [15] M. Mueckler, C. Caruso, S.A. Baldwin, M. Panico, I. Blench, H.R. Morris, W.J. Allard, G.E. Lienhard and H.F. Lodish, Science, 229 (1985) 941.
- [16] E. Mascher and P. Lundahl, J. Chromatogr., 476 (1989) 147.
- [17] T. Takagi, J. Miyake and T. Nashima, Biochim. Biophys. Acta, 626 (1980) 5.
- [18] N.A. Mazer, G.B. Benedek and M.C. Carey, J. Phys. Chem., 80 (1976) 1075.
- [19] M.J. Rosen, A.W. Cohen, M. Dahanayake and X.-y. Hua, J. Phys. Chem., 86 (1982) 541.
- [20] C. Tanford, Y. Nozaki and M.F. Rohde, J. Phys. Chem., 81 (1977) 1555.
- [21] A.-K. Englund and P. Lundahl, Biochim. Biophys. Acta, 1065 (1991) 185.
- [22] L. Lu, E. Brekkan, L. Haneskog, Q. Yang and P. Lundahl, Biochim. Biophys. Acta, 1150 (1993) 135.
- [23] F. Maley and D.U. Guarino, Biochem. Biophys. Res. Commun., 77 (1977) 1425.