Journal of Chromatography, 193 (1980) 345–356

CHROM. 12,715

ANOMALOUS PARTITION BEHAVIOUR OF SODIUM DODECYL SULPHATE MONOMER IN SEPHADEX GELS WITH HIGH DEXTRAN CONCENTRATIONS

POSSIBLE ROLE OF WATER OF HYDRATION IN THE GEL PHASE

MASANOBU JANADO, YUKI YANO and HIROKO NAKAMORI
Biochemical Laboratory, Department of Food Science, Kyoto Women's University, Kyoto 605 (Japan)
and
TOSHIRO NISHIDA
Burnsides Research Laboratory, University of Illinois, Urbana, IL 61801 (U.S.A.)
(First received November 1st, 1979; revised manuscript received January 23rd, 1980)

SUMMARY

The partition coefficient of sodium dodecyl sulphate (SDS) monomer for Sephadex gels with high dextran concentrations were found to be abnormally high, and this was attributed to the anomalous nature of the internal water of Sephadex gel stemming from strong hydration. The thermodynamic parameters relevant to the transfer of SDS from external water to internal water indicate that the preferential partition of SDS monomer is a hydrophobic free energy driven process. "Iceberg" formation is partially prevented in the water of hydration, thereby allowing the preferential partition of SDS without an excessive increase in free energy.

INTRODUCTION

The internal water of concentrated hydrophilic gel matrices may acquire anomalous properties because of extensive hydration. Strongly bound water molecules may impose orientational restrictions on neighbouring water molecules by virtue of the greater polarity, leading to so-called cooperative hydration[1]. It is possible that in dense gel matrices all water molecules are more or less immobilized and display physical properties distinct from those of normal water.

We have previously reported the preferential partition of SDS monomer in a swollen Bio-Gel, which was interpreted in terms of transfer free energy pertaining to the external and internal water of the gel beads[2]. It was concluded that the overall transfer process was primarily governed by hydrophobic free energy derived from the anomalous nature of the water of hydration in the gel matrices. That is, in water cooperatively hydrated in a hydrophilic gel matrix (polyacrylamide), "iceberg" formation as a consequence of the introduction of hydrophobic molecules into water[3] is partially

prevented and hence the hydrophobic free energy is reduced, thereby resulting in the preferential partition of SDS in the internal water phase. This contention was supported by our subsequent finding that internal water cooperatively hydrated in Sephadex G-10 was capable of dissolving appreciable amounts of water-insoluble dyes, azobenzene and dimethylaminoazobenzene[4]. The relevant thermodynamic parameters indicated that the solubilization of these dyes is a hydrophobic free energy driven process.

This anomalous property of cooperatively hydrated water may have important biological implications in the metabolism of hydrophobic compounds. Undoubtedly it bears directly upon the fractionation mechanism in gel chromatography and on hydrophobic ligand–macromolecular acceptor interactions, including that of the SDS–protein system, which is still not well understood[2,5–7]. In a previous paper[2], we presented a non-specific binding model for SDS–protein and SDS–amylose complexes, in which SDS was considered to be dissolved preferentially in internal water cooperatively hydrated in the macromolecular structures. It was also predicted that the anomaly of internal water must be increased in Sephadex gels which are more hydrophilic than polyacrylamide gel (Bio-Gel). In this study, the anomalous nature of the internal water of swollen Sephadex gels was further studied with special reference to its consequences in the gel chromatography of hydrophobic compounds.

EXPERIMENTAL

Sodium dodecyl sulphate (SDS) was purchased from Nakarai Chemical (Kyoto, Japan) and used as supplied. A highly purified SDS sample (a gift from Dr. T. Takagi of the Protein Research Institute, Osaka University, Osaka, Japan) was also used for the critical assessment and confirmation of the results obtained. A dextran fraction (T 500) and Sephadex gels (fine) were obtained from Pharmacia (Uppsala, Sweden). All other reagents used were of analytical-reagent grade.

All of the measurements were carried out in 0.1 *M* sodium chloride solution unless otherwise specified. The partition coefficient of SDS for Sephadex gels was measured by the equilibrium method[2]. The volume occupied by a given amount of swollen gel beads (V_G), or the volume not available to non-penetrating solutes, was determined as follows[8]. A weighed amount of dried gel beads (*ca.* 0.5 g) in a 10-ml volumetric flask was allowed to swell to equilibrium in 0.1 *M* sodium chloride solution. An aliquot of Blue Dextran solution was then added to the flask and the final volume was brought to 10 ml with 0.1 *M* sodium chloride solution. The mixture was allowed to equilibrate for 6 h with occasional shaking. When the final volume remained constant (10 ml), a portion of the supernatant solution was carefully pipetted into a small glass filter and the concentration of Blue Dextran in the filtrate was obtained from the absorbance at 625 nm. V_G was calculated from the dilution of Blue Dextran. It should be noted that no significant difference in V_G was observed in the temperature range employed (20–40 °C). The values of V_G per gram of dried Sephadex gels were as follows: G-10, 1.74 ml; G-15, 2.19 ml; G-25, 3.43 ml; G-50, 7.75 ml and G-100, 17.32 ml. These values were used for the evaluation of the partition coefficient (K_{sv}), which is given by

$$K_{sv} = \frac{C_G}{C_M} \tag{1}$$

where C_G and C_M are the equilibrium concentrations of SDS in the gel phase and in the external phase, respectively. SDS was determined as described by Takagi *et al.*[9]. The critical micellar concentration (*CMC*) of SDS in 0.1 *M* sodium chloride solution was determined by frontal gel chromatography on a Bio-Gel P-2 column[10]. To examine the effect of hydration by dextran in the external solution on the *CMC* of SDS, the partition coefficient of SDS for Sephadex G-10 was also measured in dextran solutions (20% and 40%). While it is known that the V_G of Sephadex bead changes in response to the external osmotic pressure exerted by non-penetrating or partially penetrating solutes[11–13], it does not interfere with the aim of the experiment in so far as the external dextran concentration is kept constant.

In order to evaluate the thermodynamic parameters pertaining to the transfer of SDS from the external water to the internal water of swollen Sephadex gel, it is necessary to know the actual concentration (G_G^0) of SDS monomer in the volume fraction of the gel phase accessible to this solute. The solute concentration (C_G^0) in this volume element (V_G^0) is equal to that in the external phase (C_M) only if there is no interaction between the solute and gel matrix and no differential interaction of the solute with internal and external water. Under such ideal conditions[14], V_G^0 is related to V_G by

$$V_G^0 = K_{av}^0 V_G \tag{2}$$

where K_{av}^0 is the partition coefficient pertinent to the ideal case. The experimental determination of K_{av}^0 is, however, subject to some uncertainty as there is no method that fully guarantees such a condition. This will be discussed later. As $G_G^0 = C_G(V_G/V_G^0)$, combination of eqns. 1 and 2 yields

$$\frac{C_G^0}{C_M} = \frac{K_{av}}{K_{av}^0} \tag{3}$$

From eqn. 3 it directly follows that

$$\Delta G^0 = -RT \ln\left(\frac{K_{av}}{K_{av}^0}\right) \tag{4}$$

where ΔG^0 is the free energy change involved in the transfer of SDS monomer from the external phase to the restricted region of the gel phase that is accessible to SDS monomer, and if the interaction of SDS monomer with the dextran chain of Sephadex gel is negligible, then ΔG^0 represents the transfer free energy pertaining to the passage of SDS monomer from external water to internal water. The enthalpy change (ΔH^0) of the transfer process was evaluated from the van 't Hoff plot and then the entropy change (ΔS^0) was obtained from the relationship

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{5}$$

As our primary interest was to investigate the differential properties of external and internal water, it may be more informative to include the contribution of the mixing entropy in ΔG^0, and therefore the thermodynamic parameters were calculated from the partition data given on molar concentration basis rather than on mole fraction basis

RESULTS AND DISCUSSION

Effect of pore size of Sephadex gels on K_{av} of SDS monomer

Fig. 1 shows the internal concentration (C_G) *versus* the external concentration (C_M) of SDS for various Sephadex gels. In the low range of C_M, C_G increases linearly with C_M, but as C_M increases over *CMC* (*ca.* 0.04%), C_G gradually approaches a constant value, and forms a plateau on further increase in C_M. K_{av} was calculated from the slope at $C_M = 0$ and is given in Table I together with C_f, the dextran-chain concentration of Sephadex gel. In contrast to normal gel permeation chromatography, the K_{av} of SDS monomer increases with decrease in the pore size of Sephadex gel and when Sephadex G-10 was employed K_{av} is 11.3, which is abnormally high. It should be stressed that we examined possible interactions between SDS monomer and dextran (Dextran T 500) by frontal gel chromatography on Bio-Gel P-50, but the results showed that no interaction occurred at dextran concentration in the range 50–200 m*M*. Accordingly, the preferential partition of SDS monomer in swollen Sephadex gel is attributed to a large extent, if not entirely, to the anomalous nature of internal water stemming from cooperative hydration in Sephadex gels with high dextran concentrations. This will be further discussed later.

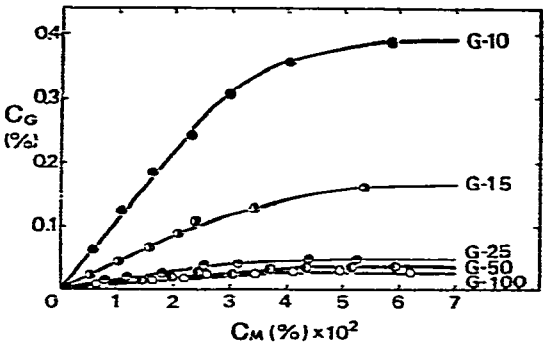

Fig. 1. Effect of pore size of Sephadex gels on K_{av} of SDS monomer. C_G = concentration of SDS in the gel phase; C_M = concentration in the external solution. K_{av} is obtained from the slope of the curves at $C_M = 0$. The measurements were made in 0.1 *M* NaCl solution at 25°C.

TABLE I

EFFECT OF THE INNER DEXTRAN-CHAIN CONCENTRATION (C_f) OF SEPHADEX GELS ON K_{av} OF SDS MONOMER AT 25°C

The measurements were made in 0.1 *M* NaCl solution.

Sephadex	C_f (*g per 100 ml*)	K_{av}
G-10	57.4	11.3
G-15	45.6	4.3
G-25	29.2	1.5
G-50	12.9	1.1
G-100	5.8	1.0

Effect of urea on K_{av} of SDS monomer

As with Bio-Gel[2], the preferential partition of SDS monomer is nullified in the presence of urea. Fig. 2 shows that the K_{av} of SDS monomer for the various Sephadex gels decreases asymptotically with increase in urea concentration. While the disruptive action of urea is not fully explainable, it is conceivable that the hydration effect of the dextran chain is swamped by the overwhelming hydration effect of urea, which must be freely accessible to the internal space of the gel matrices. Nevertheless, as the preferential partition of SDS disappears at higher urea concentration, it seems reasonable to take the asymptotic value of each curve as the K^0_{av} of SDS monomer for the various Sephadex gels employed. Accordingly, K_{av} was plotted against the reciprocal of urea concentration ($1/C$) and the linear part of the resulting curve was extrapolated to $1/C = 0$ to obtain K^0_{av}. The values of K^0_{av} thus obtained were as follows: 0.37 for G-10, 0.45 for G-15, 0.56 for G-25, 0.60 for G-50 and 0.86 for G-100. The variation of K^0_{av} with temperature was examined only for Sephadex G-10 and was found to be within experimental error in the temperature range examined (20–40 °C).

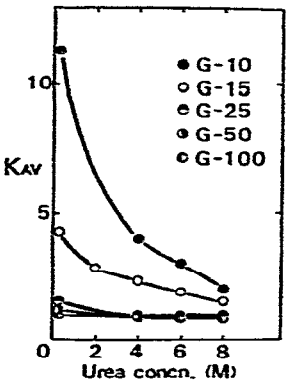

Fig. 2. Effect of urea concentration on K_{av} of SDS monomer. The data were obtained at 25°C.

Effect of salt concentration and temperature on K_{av} of SDS monomer

The effect of salt concentration on the K_{av} of SDS monomer was examined with Sephadex G-10 in sodium chloride solutions of various concentrations (pH = 5.35 ± 0.04 at 35 °C). The results (Table II) clearly show that K_{av} increases markedly with increase in salt concentration. It was also found that the K_{av} of SDS monomer for Sephadex G-10 increased appreciably with increase in temperature (Fig. 3), indicating that the transfer of SDS monomer from the external phase to the internal phase of Sephadex G-10 is an endothermic process. K_{av} values calculated from the slope of the curves at $C_M = 0$ were 9.7 at 20 °C, 11.3 at 25 °C, 12.2 at 30 °C, 12.6 at 35 °C and 13.2 at 40 °C. The mode of the salt-concentration dependence of K_{av} and the endothermicity of the partition process are indicative of the hydrophobic interaction. Morris[15] contended that chromatographic retention can be ascribed mainly to hydrophobic interaction only if all three of the following criteria are met: (1) hydrophobic sites can be identified on the stationary phase; (2) the solute is more firmly bound at higher rather than lower temperatures, so that the free energy of

TABLE II

EFFECT OF THE SALT CONCENTRATION ON K_{av} OF SDS MONOMER FOR SEPHADEX G-10

Solvent	K_{av}	
	25°C	*35°C*
Water	n.d.*	2.0
0.01 *M* NaCl	6.6	7.1
0.05 *M* NaCl	9.5	10.6
0.10 *M* NaCl	11.3	12.6
0.20 *M* NaCl	13.5	15.0

* n.d. = not determined.

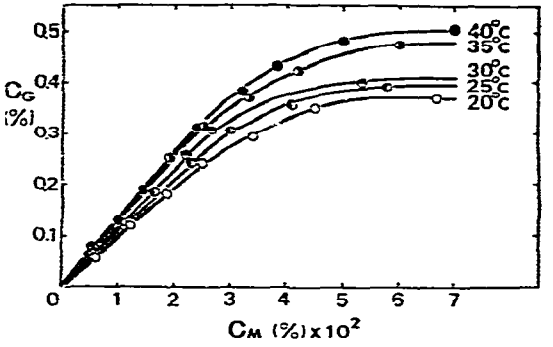

Fig. 3. Effect of temperature on K_{av} of SDS monomer for Sephadex G-10. C_G = concentration of SDS in the gel phase; C_M = concentration in the external solution (0.1 *M* NaCl). The slope of the curves at $C_M = 0$ represents K_{av} at the temperatures indicated.

binding is primarily entropic in nature; and (3) binding occurs at relatively high salt concentrations and elution at lower salt concentrations. As will be discussed later, we consider that cooperatively hydrated water in the gel matrix acts as the acceptor of hydrophobic solutes and therefore the above criteria seem to be met fully in the present system.

At all temperatures examined, C_G increases linearly with C_M up to *ca.* 0.03% of C_M, but above this concentration it curves gradually and forms a plateau. Within the region of curvature there should be found the *CMC* of SDS, above which SDS forms non-penetrating micelles in the external phase. Therefore, in the plateau region, multi-equilibria operate between internal SDS monomer and external SDS monomer, and between SDS monomer and its micelles in the external solution. As the external monomer concentration at the plateau is equal to the *CMC* of SDS at a given temperature, K_{av} can also be evaluated from the relationship

$$K_{av} = \frac{\bar{C}_G}{CMC} \tag{6}$$

where $\bar{C}_G$ is the C_G value at the plateau region at a given temperature. To evaluate eqn. 6, we measured the *CMC* of SDS at various temperatures by frontal gel chromato-

graphy in 0.1 *M* sodium chloride solution and obtained 0.041% at 20 °C, 0.039% at 25 °C, 0.041% at 30 °C, 0.046% at 35 °C and 0.051% at 40 °C. Substitution of these values together with $\bar{C}_G$ values relevant to the respective temperatures into eqn. 6 gave K_{av} values of 9.8 at 20 °C, 10.3 at 25 °C, 11.3 at 30 °C, 11.0 at 35 °C and 10.8 at 40 °C, which are comparable to those obtained from the C_G *versus* C_M plot (Fig. 3). Considering some uncertainty in $\bar{C}_G$ imposed by the osmotic effect exerted by non-penetrating micelles[10–12], the agreement between the two sets of K_{av} values may be regarded as satisfactory. The above results lead to the important conclusion that in the internal phase of Sephadex G-10, where water molecules are cooperatively hydrated by the concentrated dextran-chain, SDS monomer can be concentrated to levels far exceeding the *CMC*, in spite of the restricted translational freedom in the condensed gel matrix. This translational entropy loss, as will be discussed later, must be outweighed by an entropy increase due to the diminished "iceberg" formation in the water of hydration.

Effect of dextran concentration on CMC of SDS

If SDS monomer can indeed be concentrated above the *CMC* in the internal phase of Sephadex G-10, the same should apply to SDS in highly concentrated dextran solutions. Accordingly, the use of a highly concentrated dextran solution as the external solution of Sephadex G-10 would give the C_G *versus* C_M plot with a linear response extending above the *CMC* (0.039% at 25 °C) and breaking only at the point where SDS starts to form micelles in the external solution. Indeed, when 20% dextran was used as an external solution, the linearity extended to a C_M value of 0.04%, as shown in Fig. 4 (in which arrows indicate the points of deviation from linearity). With 40% dextran as the external solution, no deviation from linearity occurred on increasing C_M even up to 0.1%. On the other hand, in the absence of external dextran, the linear response was limited only to a C_M value of 0.03%. This parallels the result given in Table I, which indicates that the striking anomaly of SDS partition starts at a dextran-chain concentration (C_f) between 29.2% and 45.6%. Hence, provided that the surface properties of Sephadex G-10 remain unchanged in the dextran solutions, the results presented in Fig. 4 indicate that SDS tends to exist as a monomer in highly concentrated dextran solutions, and this is in

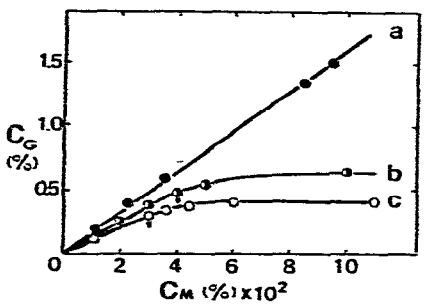

Fig. 4. Effect of the external dextran (Dextran T 500) concentration on the *CMC* of SDS. The data were obtained with Sephadex G-10 at 25°C. C_G = concentration of SDS in the gel phase; C_M = concentration in the external solution. (a) In 40% dextran solution; (b) in 20% dextran solution; (c) in the absence of dextran (0.1 *M* NaCl).

accord with our previous contention. That is, unlike in free water, in cooperatively hydrated water "iceberg" formation is partially hindered, thereby resulting in the preferential partition of SDS and also an increased solubility of hydrophobic compounds in swollen gels[2-4]. However, it should be borne in mind that substantial alterations of the gel surface can be brought about, *e.g.*, by strong adsorption of dextran molecules forming the concentric layers of dextran matrices around the gel surface. If this is the case, the gel beads will behave like large-pore gels, allowing the partition of SDS micelles and hence leading to phenomena similar to Fig. 4, even in the presence of SDS micelles. Another point to be noted in Fig. 4 is that the slope of the C_G *versus* C_M plot increases with an increase in the external dextran concentration, thus leading to an apparent increase in K_{av} at high dextran concentrations. This may be reasonably explained in terms of the steric exclusion effect of external dextran molecules, which in effect increases the external concentration of SDS (C_M).

Thermodynamic parameters pertaining to the transfer of SDS monomer from external water to internal water

The free energy change involved in the transfer of SDS monomer from the external phase to the gel phase is given by

$$\Delta G^0 = -RT \ln K_{av} \tag{7}$$

where K_{av} is the apparent equilibrium solute concentration in the gel phase (C_G) divided by equilibrium solute concentration in the external phase (C_M). ΔG^0, which is considered to be a composite of several component terms[16-18], may be conveniently written as a sum of the two terms describing the steric exclusion effect (ΔG^0_s) and all types of interaction between the solute and the gel phase (ΔG^0_{int}), *i.e.*,

$$\Delta G^0 = \Delta G^0_s + \Delta G^0_{int} \tag{8}$$

The term "gel phase" refers to all of the components of the swollen gel phase including the gel fibre, water of hydration, free water and inorganic salt. Accordingly, ΔG^0_{int} includes a contribution of differential interactions of the solute with external and internal water of the gel phase that may arise from different water structures in the two phases. In the ideal case of gel permeation chromatography (GPC), where the solute is free from any type of interaction with the gel phase, the steric exclusion effect is the sole factor determining the partition coefficient, K^0_{av}[14]. In so far as the volume of the gel phase (V_G) remains constant, the steric exclusion effect or the volume fraction of the gel phase accessible to the solute ($K^0_{av}V_G$) may be assumed to remain constant without regard to solute–gel phase interactions, and therefore it follows that $-RT \ln(K_{av}/K^0_{av}) = \Delta G^0_{int}$, which is identical with eqn. 5. As the interaction between SDS and the dextran chains of Sephadex gel does not occur to an appreciable extent, as indicated by frontal gel chromatographic analysis, ΔG^0 in eqn. 5 refers essentially to the free energy change involved in the transfer of SDS monomer from external water to internal water. However, the validity of eqn. 5 depends on the accurate measurement of K^0_{av}, which is not always possible. Addition of urea to the SDS–Sephadex G-10 system decreases the K_{av} value of SDS from 11.3 to 0.37, which

is reasonable for a solute with a molecular weight of 288 conforming to a typical GFC calibration for Sephadex G-10. A similar effect of urea on the partition coefficients of SDS, sulphanilamide and phenol for Bio-Gel P-2 have been reported[2,19,20]. These results indicate that the solute–gel phase interaction is practically abolished at high concentrations of urea and that any differential physical properties of external and internal water due to the cooperative hydration in the gel matrices is eliminated by the overwhelming hydration of urea. It seems feasible, therefore, to use 0.37, the value obtained in the presence of urea, as K_{av}^0 for a qualitative description of the transfer process. The thermodynamic parameters calculated for the transfer process at 25 °C were $\Delta G^0 = -2.0$ kcal/mole, $\Delta H^0 = 3.2$ kcal/mole and $\Delta S^0 = 17.5$ e.u. [ΔH^0 was obtained from the van 't Hoff plot (Fig. 5), which shows that the enthalpy

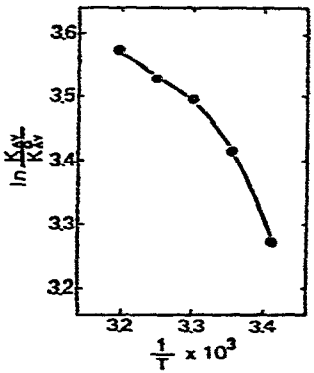

Fig. 5. Van 't Hoff plot for the transfer process of SDS monomer from external water to internal water of Sephadex G-10. The data were obtained in 0.1 *M* NaCl solution.

change is strongly temperature dependent]. Hence, the transfer process is accompanied by an increase in enthalpy and by a large increase in entropy, indicating that the preferential partition of SDS monomer is primarily governed by hydrophobic free energy arising from the anomalous nature of internal water. While it is not immediately possible to envisage the state of the hydrocarbon chain of SDS in internal water, it can be assumed that in water highly organized by extensive and cooperative hydration in dense gel matrices "iceberg" formation[3] is substantially hindered. This results in a decrease in hydrophobic free energy and thereby allows the preferential partition of SDS monomer in internal water. Indeed, the internal water of Sephadex G-10 is capable of dissolving a variety of hydrophobic compounds, including azobenzene, dimethylaminoazobenzene[4], alkylbenzenes and alkanes (unpublished data). The high *CMC* of SDS in 40% dextran solution (Fig. 4) indicates that the solubility of SDS monomer increases in concentrated dextran solutions, and this conforms to the above view.

Effect of the dextran-chain concentration (C_f) on K_{av} of SDS monomer

As shown in Fig. 6, the plot of K_{av} *versus* C_f is well described by a regression curve of the fifth power in C_f:

$$K_{av} = 1 + 1.67 \cdot 10^2 C_f^5 \qquad (9)$$

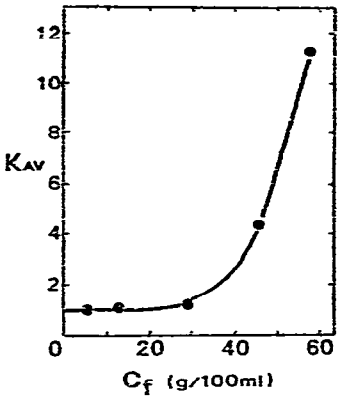

Fig. 6. K_{av} of SDS monomer as a function of the inner dextran-chain concentration (C_f) of the Sephadex gels. The data (closed circles) were obtained in 0.1 *M* NaCl solution at 25°C. The solid line represents a regression curve of the fifth power in C_f: $K_{av} = 1 + 1.67 \cdot 10^{-2} C_f^5$.

formally indicating that K_{av}, a measure of the solubility of SDS monomer in the internal water of the Sephadex gels, is proportional to C_f^5. Accordingly, it may be conceivable that in the gel matrices SDS monomers occupy the spaces surrounded by, on average, five dextran chains (a five-membered unit cell) in which water molecules are more or less immobilized by cooperative hydration. This may be better understood by considering the analogy between the present system and the equilibrium dialysis experiment in which Visking tubes containing dextran solutions of the same concentrations as the Sephadex gels are immersed in an SDS solution below the *CMC*, to allow the equilibrium partition of SDS. Let us consider that inside the Visking tubes SDS monomers are either dissolved in free water, as in the outside diffusate, or accommodated in the interstitial space formed in the water of hydration contained in the *n*-membered unit cells. The equilibrium dialysis system is then described by

$$S + nD = SD_n \tag{10}$$

where S and D represent SDS and dextran, respectively, and SD_n is SDS accommodated in the *n*-membered unit cell, which may well be regarded as a sort of inclusion complex. Using the usual notations, the equilibrium constant (K_{eq}) can be written as

$$K_{eq} = \frac{[SD_n]}{[S]\,[D]^n} \tag{11}$$

As $[S] = aC_M$, $[SD_n] = a(C_G - C_M)$ and $[D] = bC_f$ (a and b being the factors for conversion of the concentrations from a weight to a molar basis), eqn. 11 can be rewritten in the form

$$K_{av} = 1 + b^n K_{eq} C_f^n \tag{12}$$

where $K_{av} = C_G/C_M$. By comparison of eqns. 9 and 12, it follows that $n = 5$, indicating that one SDS molecule is accommodated in water of hydration contained in

a five-membered unit cell. It should be noted, however, that eqn. 12 may not necessarily represent a unique situation derivable from the empirical equation (eqn. 9). Further, because of the limited range and number of experimental data, the exponent 5 is not a uniquely determined value. We have recently proposed a similar tentative model based on the solubility data of water insoluble dyes, azobenzene and dimethylaminoazobenzene in the internal phase of Sephadex G-10[4]. In the assumed unit cell structure, the five dextran chains are not necessarily linked to each other by specific interactions, as at a high dextran concentration, *e.g.*, 57% in Sephadex G-10, dextran chains must be closely packed to produce a statistical unit. Goto and Isemura[21] showed that the degree of hydration of sucrose and mannite fell steeply as the temperature was increased. This may reflect a cooperative tendency toward hydration that may be easily abolished by thermal perturbation[1]. In a highly concentrated dextran solution, and hence in the unit cell structure as described above, the cooperative tendency would be further augmented and persist even at elevated temperatures owing to the restriction of the thermal motion of the solute and water molecules. The preferential partition of SDS monomer was also observed with polyacrylamide gel (Bio-Gel), but to a much lesser extent[2]. If water of hydration is indeed involved in the solubilization of hydrophobic groups, it follows that the K_{av} of SDS monomer depends not only on the gel fibre concentratoin (C_f) but also on the hydration capacity of the gel fibre. In this context, the larger K_{av} values obtained with Sephadex gels are reasonable, as dextran must be more hydrophilic than polyacrylamide. The present study is insufficient to provide a deep insight into the solubilized state of SDS monomer in water of hydration, but it seems plausible that SDS monomers fit in the interstitial spaces formed in strongly oriented water confined in the gel matrices, without altering greatly the pre-existing orientational order of cooperatively hydrated water.

Expression for elution volume of SDS monomer

The elution volume (V_e) is usually given by the equation $V_e = V_0 + K_{av}V_g$, where V_0 is the void volume of column and V_g is the volume of the gel phase. The K_{av} of SDS may be split into two terms, K^0_{av}, describing the steric exclusion effect, and K_{int}, the parameter for the differential interaction of SDS with external and internal water. To establish the empirical relationship between K_{av} and K^0_{av}, K_{av}/K^0_{av} was plotted against C_f, which resulted in a regression curve of the fifth power in C_f, *i.e.*, $K_{av}/K^0_{av} = 1 + kC_f^5$ where k (proportionality constant) $= 4.74 \cdot 10^2$ ml^5/g^5. Combination of the two equations leads to

$$V_e = V_0 + K^0_{av}(1 + kC_f^5)V_g \tag{13}$$

As kC_f^5 is constant for a given grade of Sephadex gel, it may be written as K_{int}, and accordingly eqn. 13 takes the form $V_e = V_0 + K^0_{av}V_g + K^0_{av}K_{int}V_g$. This is formally similar to the expression derived by Lecourtier *et al.*[17], who assigned a different meaning to the constant.

Biological implications of water of hydration

It seems remarkable that cooperatively hydrated water can dissolve various hydrophobic compounds. In a preliminary examination we found that a 40% solu-

tion of Dextran T 500, but not of glucose, dissolves a variety of hydrophobic molecules to an appreciable extent, including azobenzene, dimethylaminoazobenzene, alkylbenzenes and alkanes. This anomalous property of water of hydration suggests its possible involvement in the metabolic transport of hydrophobic compounds. It is generally believed that in the macromolecular acceptor–hydrophobic ligand interactions the latter is bound to specific hydrophobic sites or buried in the hydrophobic domain of the former so as to minimize the hydrophobic free energy level. In view of the present finding, it also seems plausible that hydrophobic ligands are solubilized in cooperatively hydrated water in the hydrophilic region of macromolecular structures. Indeed, it has been suggested that naphthalene dissolved in dodecylammonium chloride micelles resides at the micelle–water interface[22], *i.e.*, in close proximity to the polar head where water molecules must be more or less immobilized by cooperative hydration. We have recently observed that Oil Red O, a water-insoluble dye, solubilized in serum high-density lipoprotein is transferred within a few minutes to low-density lipoprotein upon mixing to establish equilibrium. This may indicate that the dye is dissolved in the interstices of the surface polar groups of the lipoproteins and not in the internal lipid core. A similar mechanism may operate in an initial stage of the membrane transport, resulting in a local concentration of hydrophobic ligands at the surface of cell membranes, which in turn facilitates subsequent transfer to their specific receptors.

ACKNOWLEDGEMENT

This study was supported in part by grant HL 17597 from the National Institute of Health, U.S.A.

REFERENCES

1 H. Noguchi, *Nippon Nogeikagaku Kaishi*, 52 (1978) R39.
2 M. Janado, R. Nakayama, Y. Yano and H. Nakamori, *J. Biochem.*, 86 (1979) 795.
3 W. Kauzman, *Advan. Protein Chem.*, 14 (1959) 1.
4 M. Janado, K. Takenaka, H. Nakamori and Y. Yano, *J. Biochem.*, 87 (1980) 57.
5 J. A. Reynolds and C. Tanford, *J. Biol. Chem.*, 245 (1970) 5161.
6 K. Shirahama, K. Tsujii and T. Takagi, *J. Biochem.*, 75 (1974) 309.
7 E. S. Rowe and J. Steinhardt, *Biochemistry*, 15 (1976) 2579.
8 M. Janado, R. Nakayama, Y. Yano and H. Nakamori, *J. Biochem.*, 84 (1978) 965.
9 T. Takagi, K. Tsujii and K. Shirahama, *J. Biochem.*, 77 (1975) 939.
10 M. Janado, Y. Yano, H. Nakamori and T. Nishida, *J. Biochem.*, 86 (1979) 177.
11 E. Edmond, S. Farquhar, J. R. Dunstone and A. G. Ogston, *Biochem. J.*, 86 (1968) 755.
12 A. G. Ogston and J. D. Wells, *Biochem. J.*, 119 (1970) 67.
13 L. W. Nichol, M. Janado and D. J. Winzor, *Biochem. J.*, 133 (1973) 15.
14 T. C. Laurent and J. Killander, *J. Chromatogr.*, 14 (1964) 317.
15 C. J. O. R. Morris, *Trends Biochem. Sci.*, 2(1) (1977) N16.
16 S. Hjertén, *J. Chromatogr.*, 50 (1970) 189.
17 J. Lecourtier, R. Audebert and C. Quivoron, *J. Chromatogr.*, 121 (1976) 173.
18 A. Heyraud and M. Rinaudo, *J. Chromatogr.*, 166 (1978) 149.
19 M. Janado, K. Shimada and T. Nishida, *J. Biochem.*, 79 (1976) 513.
20 M. Janado, K. Shimada, N. Horie and T. Nishida, *J. Biochem.*, 80 (1976) 69.
21 S. Goto and T. Isemura, *Bull. Chem. Soc. Jap.*, 37 (1964) 1697.
22 Riegelman, N. A. Allawala, M. K. Hrenoff and L. A. Strait, *J. Colloid Sci.*, 13 (1958) 208.