Molecular Weight and the Dodecyl Sulphate Binding
of a Thylakoid Membrane Polypeptide Involved in a Reaction
on the Oxygen-Evolving Side of Photosystem II

Hans Craubner, Friederike Koenig, and Georg H. Schmid

Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), Abteilung Menke,
Köln-Vogelsang

Thylakoid Membrane Polypeptide, Molecular Weight, Dodecyl Sulphate

The molecular weight of a thylakoid membrane polypeptide with the apparent molecular weight 11 000 was determined by measurement of the sedimentation velocity, the diffusion and the effective partial specific volume. The molecular weight was found to be 6300 and that of the polypeptide-dodecyl sulphate micelle was found to be 11 200. The frictional ratio was 1.35. In addition, we determined the binding of dodecyl sulphate onto the polypeptide by equilibrium dialysis. We found that 1 g polypeptide binds 0.77 g sodium dodecyl sulphate which corresponds to 17 molecules dodecyl sulphate bound per polypeptide chain. In the absence of dodecyl sulphate the polypeptide aggregates. The molecular weights of the aggregates are in 0.01 M sodium phosphate buffer pH 7.2 150 000 and in a 1 : 1 mixture of 0.01 M phosphate buffer and 96% ethanol 365 000. The frictional ratios were 1.07 and 1.16 respectively which points at a spherical shape.

The experimental conditions for the determination of the dodecyl sulphate binding were critically scrutinised.

In an earlier publication we have reported on the molecular weight determination of a polypeptide fraction in the presence of dodecyl sulphate [1]. As the polypeptide occurs in solution as polypeptide-dodecyl sulphate micelle, we combined determinations of the sedimentation velocity, the diffusion coefficient and the density with measurements of the dodecyl sulphate binding. The molecular weight was found to be 25 000 and was within the error width identical to the apparent molecular weight obtained by dodecyl sulphate polyacrylamide gel electrophoresis. However, these two methods do not necessarily yield identical results with all polypeptides [2-6]. In the present paper we report on the molecular weight and dodecyl sulphate binding of a polypeptide fraction with the apparent molecular weight 11 000. This polypeptide is involved in reactions on the oxygen-evolving side of photosystem II [7, 8]. An antiserum to this polypeptide fraction inhibits photosynthetic electron transport between the sites of electron donation of tetramethyl benzidine and diphenylcarbazide [7].

The binding measurements were carried out by equilibrium dialysis using dodecyl [35S]sulphate. With this method the dialysis equilibrium was reached within 10 hours whereas the literature reports on times up to 3 weeks. In the present paper we attempted to clarify the questions arising from this difference.

Materials and Methods

Isolation of the polypeptide fraction 11 000. The starting material was stroma-freed chloroplasts from *Antirrhinum majus* prepared according to Kreutz and Menke [9]. The procedure of dissolving these chloroplasts in sodium dodecyl sulphate and mercaptoethanol containing buffers as well as the general isolation of polypeptide fractions has been described in earlier publications [10-12]. As the method of isolation of the polypeptide fraction 11 000 differs somewhat in this paper from the earlier procedure we shall briefly describe: Fractions which after gel filtration of the thylakoid polypeptide mixture on Sepharose 6B (Pharmacia) exhibited in the analytical dodecyl sulphate polyacrylamide gel electrophoresis the apparent molecular weight 11 000 were pooled and concentrated. In order to remove lipids, 5-times the volume of cold acetone was added. The precipitate was washed several times with acetone and dried. Subsequently, the residue was redissolved with such an amount of 0.01 M sodium phosphate buffer pH 7.2 or 0.1 M Tris/HCl buffer pH 9.2 which contained 2.2% dodecyl sulphate and 1% mercaptoethanol as to give a final concentration of 1% protein in the solution. In order to remove aggregates from this solution a

further gel filtration with Sepharose CL − 6B (Pharmacia) was carried out with four in series connected columns. The total length of the connected columns was 370 cm, the diameter of the columns was 5 cm. The elution buffer was 0.1 M Tris/HCl buffer pH 9.2, containing 0.25% dodecyl sulphate and 0.1% mercaptoethanol. Again, fractions were isolated which exhibited in the dodecyl sulphate polyacrylamide gel electrophoresis the apparent molecular weight 11 000. For the determination of the molecular weight in the ultracentrifuge and for the determination of the dodecyl sulphate binding, these fractions were dialysed against 0.01 M sodium phosphate buffer pH 7.2, which contained 0.25% dodecyl sulphate and 0.1% mercaptoethanol and were subsequently concentrated (Amicon PM 10 membrane).

As the dodecyl sulphate concentration is increased in the sample during the concentration a gel permeation chromatography on Biogel A − 0.5 m (Bio-Rad) was carried out in order to bring the dodecyl sulphate concentration in the sample down again. In this case the elution buffer was 0.01 M sodium phosphate buffer pH 7.2, containing only 0.25% dodecyl sulphate. Subsequently, the polypeptide preparation was dialyzed for 48 hours against 0.01 M sodium phosphate buffer pH 7.2 which contained 0.25% dodecyl sulphate. An occasional turbidity was removed by filtration (Sartorius Membrane Filter, pore diameter 0.45 μm) or by centrifugation.

The dodecyl sulphate-free polypeptide preparation was prepared from the detergent-containing preparation according to the method of Weber and Kuter [13]. The exchange of the solvents was carried out by gelfiltration with Sephadex G-10 (Pharmacia). The protein concentration was determined according to Lowry *et al.* [14], using a standard curve made by dry weight determinations of the purified polypeptide preparation.

Densities and partial specific volumes were determined using a precision densimeter Model DMA 02 (Paar), according to Kratky, Leopold and Stabinger [15]. The temperature was maintained constant to $20 \pm 0.01\ ^\circ$C. Calibration was done with water ($\varrho = 0.99823$ g/cm^3) [16].

Molecular weight determinations in the presence of dodecyl sulphate were made by *sedimentation velocity* (30 000 and 48 000 rpm) and *diffusion measurements* at 20 °C with a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner at a wavelength of 280 nm. The solvents used were 0.25 wt.% sodium dodecyl sulphate in 0.01 M sodium phosphate buffer pH 7.2, a 1 : 1 v/v mixture of 96% ethanol and 0.01 M so-

dium phosphate buffer, pH 7.2 and 0.01 M sodium phosphate buffer pH 7.2.

The results were calculated and corrected for bound detergent by making use of the equivalent of the Svedberg equation for multiple component systems as described previously [1]. For this, measurements of the sedimentation velocity and the diffusion coefficient were combined with the measurement of the intrinsic density increment

$$[\partial\varrho/\partial c_\mathrm{P}] = \lim_{c_\mathrm{P}\to 0}(\partial\varrho/\partial c_\mathrm{P})_{p,\mu} = 1 - \Phi_0' \varrho_\mathrm{s} \quad (1)$$

to yield the buoyant weight $M_\mathrm{P}[\partial\varrho/\partial c_\mathrm{P}] = M_\mathrm{P}(1 - \Phi_0' \varrho_\mathrm{s})$. Here ϱ and ϱ_s are the solution and the solvent densities in g/cm^3, c_P the protein concentration in g/cm^3, μ the chemical potential of any diffusible component (d$\mu = 0$), p the hydrostatic pressure (d$p = 0$), Φ_0' the effective partial specific volume in cm^3/g and M_P the molecular weight of the protein component of the sedimenting particles (excluding bound solvent and detergent). The contribution of bound detergent (δ_D g of detergent per g of protein) can be accounted for by the relation

$$M_\mathrm{P}(1 - \Phi_0' \varrho_\mathrm{s}) = M_\mathrm{P}[(1 - \bar{v}_\mathrm{P}\varrho_\mathrm{s}) + \delta_\mathrm{D}(1 - \bar{v}_\mathrm{D}\varrho_\mathrm{s})] \quad (2)$$

where $\bar{v}_\mathrm{P}$ and $\bar{v}_\mathrm{D}$ are the partial specific volumes of pure protein and detergent. The average molecular weight M^* of the protein-dodecyl sulphate micelles is equivalent to $M_\mathrm{P}(1 + \delta_\mathrm{D})$ and their partial specific volume $\bar{v}^*$ is $(\bar{v}_\mathrm{P} + \delta_\mathrm{D}\bar{v}_\mathrm{D})/(1 + \delta_\mathrm{D})$. Hereby, it is assumed that no significant interactions with buffer ions occur. Values of $\bar{v}_\mathrm{P}$ and $\bar{v}_\mathrm{D}$ were determined from solution density measurements as described earlier [1].

Measurements of the binding of the dodecyl sulphate onto the polypeptide were made by self-diffusion equilibrium dialysis as described previously [1]. Sodium dodecyl [35S]sulphate was purchased from Amersham Radiochemical Centre (U.K.). A dialyzing system Dianorm GD, according to Weder, Schildknecht, and Kesselring [17] with Visking membranes was used. The polypeptide (conc. about 1 mg/cm^3) dissolved in a solution of unlabelled sodium dodecyl sulphate in 0.01 M sodium phosphate buffer (pH 7.2) was dialyzed at $20 \pm 0.05\ ^\circ$C and 10 rpm against the same concentration of 0.25% by weight dodecyl sulphate which contained dodecyl [35S]sulphate. Dialysis with initially different concentrations of 35S-labelled and unlabelled sodium dodecyl sulphate (between 0 and 0.5% by weight) were carried out at $20 \pm 0.05\ ^\circ$C and 10 rpm in 0.01 M sodium phosphate buffer (pH 7.2) [18−22]. For the determination of unlabelled dodecyl sulphate the methylen blue method by Rey-

nolds *et al.* was used (conc. 0.7 g methylen blue/liter water) [19, 20, 23].

Viscosity determinations of the solvents were carried out in a suspended level Ubbelohde viscometer (Schott, Modell I). The temperature was maintained constant at $20 \pm 0.02\,°C$. Kinetic energy corrections were applied. Calibration measurements were carried out with water ($\eta = 1.002$ centipoise) [16].

The properties of the aqueous solvent mixtures and the rotor speeds are given in Table I.

Results and Discussion

The molecular weight of the polypeptide fraction 11 000

The ultracentrifugal measurements in sodium dodecyl sulphate solutions were carried out at 0.25% by weight of dodecyl sulphate above the critical micelle concentration. As the dependence of logarithmus r and of the apparent diffusion coefficient (D_{app}) on time is linear, it appears that no perturbing interactions occur during centrifugation (Figs 1 and 2). r is the radial distance of the moving boundary from the centre of rotation. Sedimentation (S_c in s) and diffusion coefficients (D_c in cm²/s) were calculated from the initial slopes and the intercepts at zero time (polypeptide concentration of the samples $c_P = 0.50$ mg/cm³). The sedimentation coefficients and the diffusion coefficients were plotted against the polypeptide concentrations (Figs 3 and 4). The graphs are linear. The values $S_0 = 1.14 \times 10^{-13}$ s and $D_0 = 13.0 \times 10^{-7}$ cm²/s were obtained by linear regression analysis from the intercepts. As seen in Fig. 5, the course of the density (in g/cm³) plotted versus the polypeptide concentration is linear at higher concentra-
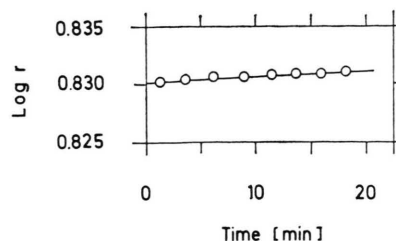

Fig. 1. Plot of log r *vs* time. r is the distance in cm of the boundary from the axis of rotation. The solution contained 0.50 mg/cm³ polypeptide, 0.25% dodecyl sulphate in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C.
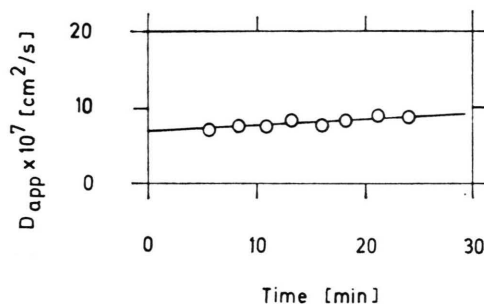

Fig. 2. Plot of the apparent diffusion coefficient *vs* time. The solution is the same as in Fig. 1.
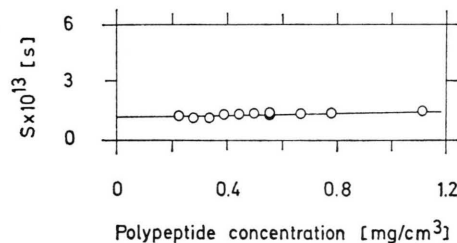

Fig. 3. Dependence of the sedimentation coefficient on the polypeptide concentration. The solutions contained 0.25% sodium dodecyl sulphate in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C.

Table I. Properties of the aqueous solvent mixtures at 20 °C and ultracentrifugal conditions (ϱ_s = density; η_s = viscosity).

Aqueous solvent	ϱ_s [g/cm³]	η_s [centipoise]	Rotor speed [rpm]
water	0.99823 [a]	1.002 [b]	—
0.01 M sodium phosphate buffer (pH 7.2)	0.99944 ± 0.00001	1.009 ± 0.001	30 000
sodium dodecyl sulphate (0.25%) in 0.01 M sodium phosphate buffer (pH 7.2)	0.99979 ± 0.00001	1.020 ± 0.001	48 000
1 : 1 v/v mixture of 0.01 M sodium phosphate buffer (pH 7.2) and 96% ethanol	0.92714 ± 0.00001	2.870 ± 0.001	30 000

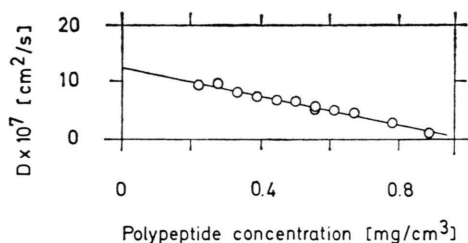[a] Density of water [16]. [b] Viscosity of water [16].

Fig. 4. Dependence of the diffusion coefficient on the polypeptide concentration. The aqueous solvent mixture is the same as in Fig. 3.
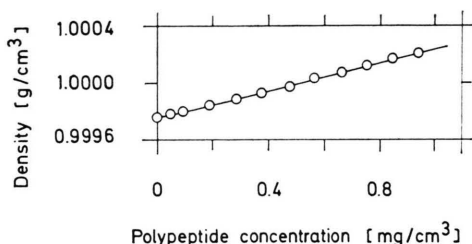

Fig. 5. Dependence of the solution density on the concentration of the polypeptide. The solvent mixture is the same as in Fig. 3.

tions but slightly curved at lower concentrations ($c_P < 0.15$ mg/cm^3). The intrinsic density increment $[\partial \varrho / \partial c_P] = 0.332$ and the effective partial specific volume of the polypeptide $\Phi_0' = 0.668$ cm^3/g were obtained from the initial slope of the density concentration curve at infinite dilution. Hence, an average molecular weight of $M_P = 6300$ is obtained for the polypeptide in dodecyl sulphate solutions.

Because the molecular weight depends on small differences of the effective partial specific volume this value is verified by a second method. According to Eqn (2), the effective partial specific volume $\Phi_0' = \bar{v}_P - \delta_D(\varrho_s^{-1} - \bar{v}_D)$ is related to the partial specific volume of the polypeptide, to the partial specific volume as well as to the amount of detergent bound to the polypeptide and to the density of the solvent. Hence, Φ_0' can be independently calculated. This permits the determination of the buoyant density factor of Eqn (2) as well as the determination of the molecular weight without direct measurement of Φ_0' and $[\partial \varrho / \partial c_P]$. The partial specific volume of the polypeptide was found to be 0.779 cm^3/g as determined by extrapolation of the slope of the density concentration curve to infinite dilution (Fig. 6). In the absence of dodecyl sulphate the polypeptide was dispersed in aqueous 0.01 M phosphate buffer (pH 7.2) at 20 °C. In Fig. 7 the density of solutions of sodium dodecyl

sulphate in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C is plotted as a function of the dodecyl sulphate concentration. The graph shows that the density of sodium dodecyl sulphate undergoes a change in slope at $cmc = 1.0$ mg/cm^3. The partial specific volume above the critical micelle concentration is a few per cent higher ($\bar{v}_D = 0.855$ cm^3/g; Table II) than it is below this concentration ($\bar{v}_D = 0.835$ cm^3/g).

From the binding value $\delta_D = 0.77$ g sodium dodecyl sulphate per g polypeptide (Table II) follows $\Phi_0' = 0.667$ cm^3/g for the effective partial specific volume and $\bar{v}_P = 0.780$ cm^3/g for the partial specific volume of the polypeptide. These values agree within the error limits with the data obtained by density measurements (Table II). With the binding value δ_D the average molecular weight of the polypeptide-sodium dodecyl sulphate micelles was found to be $M^* = 11\,200$. For the corresponding partial specific volume $\bar{v}^*$ a value of 0.81 cm^3/g was obtained. This value applied to the Svedberg equation yields the same molecular weight for the polypeptide-dodecyl sulphate micelles as the calculation from the molecular weight of the polypeptide moiety and the amount of sodium dodecyl sulphate bound
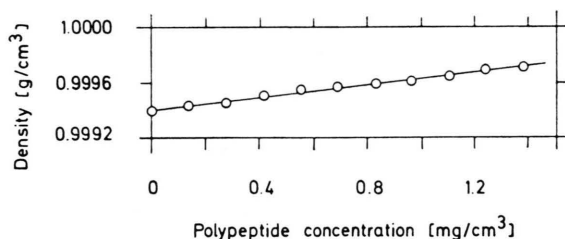

Fig. 6. Dependence of the solution density on the concentration of the polypeptide in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C. The solution contained no sodium dodecyl sulphate.
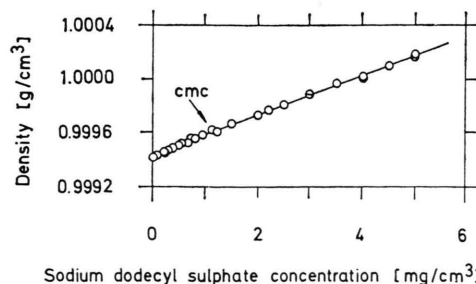

Fig. 7. Dependence of the solution density on the sodium dodecyl sulphate concentration in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C below and above the critical micelle concentration ($cmc = 1$ mg/cm^3).

Table II. Molecular weights and hydrodynamic properties at 20 °C. Deviations are given as mean errors of the average values.

Solvent	Sodium dodecyl sulphate (0.25%), 0.01 M sodium phosphate buffer (pH 7.2)	0.01 M sodium phosphate buffer (pH 7.2)	1:1 v/v mixture of 0.01 M sodium phosphate buffer (pH 7.2) and 96% ethanol
$\bar{M}_P$	6300 ± 150	150000 ± 11000	365000 ± 27000
M^*	11200 ± 400	—	—
$S_0 \times 10^{13}$ [s]	$1.14 \pm <0.01$	7.51 ± 0.17	9.67 ± 0.42
$D_0 \times 10^7$ [cm²/s]	13.0 ± 0.4	5.51 ± 0.07	1.46 ± 0.04
$[\partial\varrho/\partial c_P]$	0.332 ± 0.007	0.221 ± 0.009	$0.442 \pm <0.001$
Φ_0' [cm³/g]	0.668 ± 0.007	—	$0.602 \pm <0.001$
$\bar{v}_P$ [cm³/g]	—	0.779 ± 0.009	—
$\bar{v}^*$ [cm³/g]	0.81 ± 0.02	—	—
$\bar{v}_D$ [cm³/g]	$0.855^a \pm 0.002$	—	—
δ_D [g sodium dodecyl sulphate/ g polypeptide]	0.77 ± 0.02	—	—
$\bar{\nu}$ [mol sodium dodecyl sulphate/ mol polypeptide]	17 ± 1	—	—
cmc [mg/cm³]	1.0	—	—
f/f_0	1.35 ± 0.04	1.07 ± 0.04	1.16 ± 0.04

^{a} $c_D > cmc$. $\bar{M}_P$, mean molecular weight of the polypeptide. M^*, molecular weight of the polypeptide-dodecyl sulphate micelle. S_0, sedimentation coefficient. D_0, diffusion coefficient. $[\partial\varrho/\partial c_P]$, density increment. Φ_0', effective partial specific volume of the polypeptide. $\bar{v}_P$, partial specific volume of the polypeptide. $\bar{v}^*$, partial specific volume of the polypeptide-dodecyl sulphate micelles. $\bar{v}_D$, partial specific volume of the dodecyl sulphate. δ_D, g sodium dodecyl sulphate bound per g polypeptide. $\bar{\nu}$, mol sodium dodecyl sulphate bound per mol polypeptide. cmc, critical micelle concentration. f/f_0, frictional ratio.

to the polypeptide. From the sedimentation and diffusion coefficients S_0 and D_0 the frictional ratio was calculated to be $f/f_0 = 1.35$ which points at an asymmetric shape.

After removal of the sodium dodecyl sulphate, the sedimentation velocity, diffusion and density measurements were carried out in 0.01 M sodium phosphate buffer solutions at pH 7.2 as well as in a 1 : 1 v/v mixture of 0.01 M phosphate buffer of pH 7.2 and 96% ethanol. Logarithmic plots of the radial distance r of the moving boundary of the polypeptide against time as well as the time courses of the corresponding apparent diffusion coefficients were linear. In Figs 8 and 9 the sedimentation coefficients and the diffusion coefficients in 0.01 M sodium phosphate buffer were plotted versus the polypeptide concentration. The graphs are linear at low values of time and concentration. Hence, aggregation or thermodynamic perturbation during the time course of the measurements can be neglected. The values $S_0 = 7.5 \times 10^{-13}$ s, $D_0 = 5.5 \times 10^{-7}$ cm²/s for the phosphate buffer solutions and $S_0 = 9.7 \times 10^{-13}$ s, $D_0 = 1.46 \times 10^{-7}$ cm²/s for the ethanol solutions were again obtained from linear

regression analysis from the intercepts. The partial specific volume of the polypeptide in 0.01 M sodium
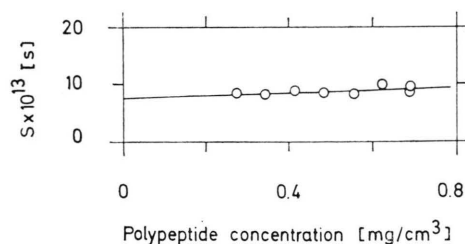

Fig. 8. Plot of the sedimentation coefficient *vs* concentration of the polypeptide in 0.01 M sodium phosphate buffer (pH 7.2) at 20 °C.
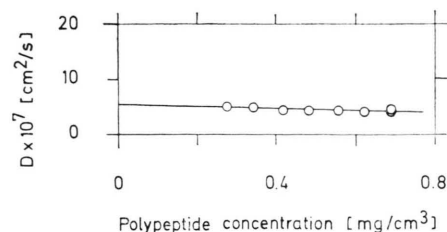

Fig. 9. Plot of the diffusion coefficient *vs* concentration of the polypeptide. The solvent is the same as in Fig. 8.

phosphate buffer (pH 7.2) is $\bar{v}_P = 0.779$ cm³/g (Table II, Fig. 6), and the effective partial specific volume of the polypeptide in the alcoholic solution is 0.602 cm³/g. From this data the molecular weights obtained for the polypeptide in the phosphate buffer solution and the polypeptide in the ethanolic solution correspond to 150 000 and 365 000 respectively (Table II). The frictional ratios were 1.07 and 1.16 which points at spherical shapes.

Measurements of dodecyl sulphate binding

The binding value of sodium dodecyl sulphate as g per g polypeptide was measured by self-diffusion equilibrium dialysis using dodecyl [35S] sulphate. As previously described [1], the term self-diffusion means that in a multiple component system without any concentration difference the diffusion of a component is followed. Decays per minute and per unit volume of sodium dodecyl [35S]sulphate on the two sides of the membrane were converted into the corresponding concentrations and plotted in Figs 10 and 11 versus dialysis

Fig. 10. Time dependence of the diffusion of sodium dodecyl [35S]sulphate through the membrane at 20 °C. Both compartments contained 0.25% dodecyl sulphate in 0.01 M sodium phosphate buffer (pH 7.2). Compartment a of the dialysis cell contained at the time onset the whole radioactivity (−○−). Compartment b contained no label at zero time (−△−).

Fig. 11. Same experiment as in Fig. 10. The compartment b did not contain radioactivity at the time onset but contained the polypeptide (1 mg/cm³).

time. Fig. 10 shows the time course of the control experiment without polypeptide. As the concentrations are the same on both sides of the membrane it is obvious that no mass transport occurs. After a dialysis time of about 5 hours the equilibrium was reached, since on both sides of the membrane 50% of the applied radioactivity was recovered which indicates that no adsorption of dodecyl sulphate onto the membrane took place. On the other hand (Fig. 11), the double plot shows that in the presence of the polypeptide more sodium dodecyl [35S]sulphate is in the compartment where the polypeptide is (curve b) than in the compartment without polypeptide (curve a). The steady state values were used for the calculation of the binding values δ_D. On the average we find that 1 g of polypeptide binds 0.77 g sodium dodecyl sulphate which corresponds to $\bar{v} = 17$ molecules dodecyl sulphate per polypeptide chain. In contrast to this 1 g of polypeptide 25 000 binds 1.15 g dodecyl sulphate which corresponds to 100 molecules of dodecyl sulphate.

If the dialysis cell contains on one side of the membrane 0.5% dodecyl sulphate in phosphate buffer and phosphate buffer without detergent on the other side, the equilibrium is not reached even after 60 hours (Figs 12 and 13). This confirms the data reported in the literature [21, 24, 25]. However, if the same experiment is carried out with a dodecyl sulphate concentration below the critical micelle concentration, the equilibrium is reached within a few hours (unpublished experiments). This shows that the dodecyl sulphate micelles do not permeate through the membrane whereas below the critical micelle concentration diffusion through the membrane occurs. If the dialysis is started with 0.5% unlabelled dodecyl sulphate in phosphate buffer on one side of the membrane and with phosphate buffer containing a trace of labelled dodecyl sulphate on the other side, within half an hour the radioactivity reaches on both sides the same value. The time course of the distribution of the radioactivity is depicted in Fig. 12. Thereafter, apparently radioactivity is accumulated on the side with the unlabelled dodecyl sulphate. This means that an exchange of radioactive dodecyl sulphate occurs with the unlabelled micelles. After approximately 5 hours the permeation of the bulk dodecyl sulphate through the membrane becomes apparent. From this time onward the curves show the same time course
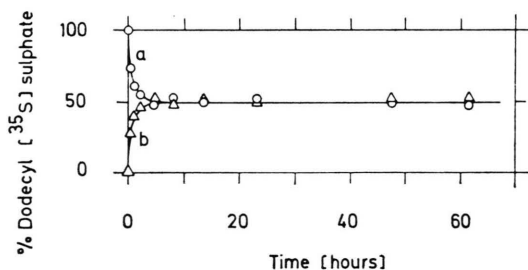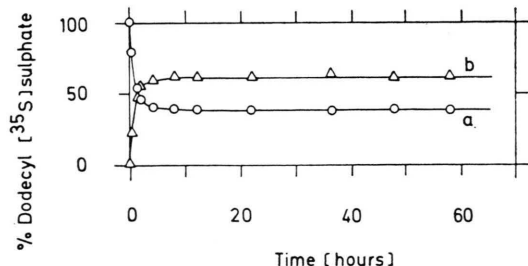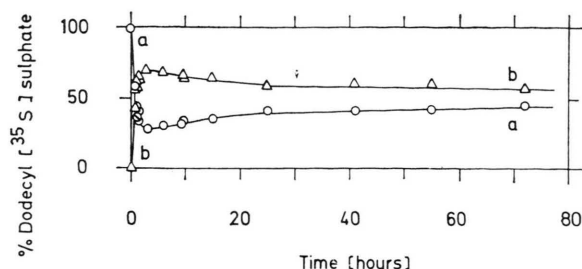
Fig. 12. Time dependence of the diffusion of sodium dodecyl [^{35}S]sulphate through the membrane at 20 °C. Compartment a contained at the time onset 0.01 M sodium phosphate buffer (pH 7.2) which was labelled with dodecyl [^{35}S]sulphate ($-\bigcirc-$). Compartment b contained at the time onset 0.5% dodecyl sulphate in 0.01 M sodium phosphate buffer (pH 7.2) but contained no label at zero time ($-\triangle-$).
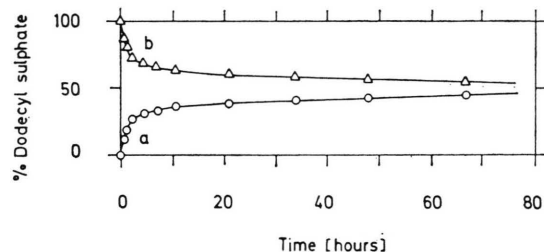

Fig. 13. Same experiment as in Fig. 12 but with unlabelled dodecyl sulphate. Compartment a contained at the time onset 0.01 M sodium phosphate buffer solution (pH 7.2; $-\bigcirc-$). Compartment b contained at the onset of time 0.5% dodecyl sulphate in 0.01 M sodium phosphate buffer solution (pH 7.2) ($-\triangle-$).

as in Fig. 13. In this plot the overall distribution of dodecyl sulphate is depicted whereas in the preceeding experiments the distribution of radioactivity was measured. In this case the dodecyl sulphate concentrations on both sides were determined with the methylene blue method. In order to check the consistency of our results, we have determined the apparent diffusion coefficients for all dialysis measurements [26]. From this it appears that the dependence of the diffusion coefficients of the monomer dodecyl sulphate on the difference of the total dodecyl sulphate concentration in the two dialysis cells is linear (Fig. 14). The high initial values of the apparent diffusion coefficients of some 10^{-5} cm^2/sec decrease fast in the course of the dialysis
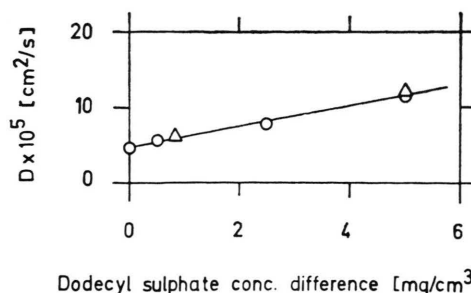

Fig. 14. Dependence of the apparent diffusion coefficients of the monomer dodecyl sulphate on the concentration difference of dodecyl sulphate. The values refer to the onset of dialysis; Temp. 20 °C; ($\bigcirc$) experiments with dodecyl [^{35}S]sulphate; ($\triangle$) experiments with unlabelled dodecyl sulphate.

to values of a few 10^{-7} cm^2/sec.

For the understanding it should be borne in mind that above the critical micelle concentration the monomer concentration is nearly independent on the total dodecyl sulphate contentration (Fig. 14) [27, 28]. Therefore, despite large concentration differences the equilibrium is only slowly reached. In addition, our experiments show that the dodecyl sulphate micelles do not permeate through the membrane, whereas the diffusion of monomer dodecyl sulphate through the membrane is not appreciably hindered. Moreover, we conclude from our experiments, that the exchange between monomer dodecyl sulphate and dodecyl sulphate micelles is slower than the exchange between monomer dodecyl sulphate and dodecyl sulphate protein micelles [29].

For binding measurements this means that the dodecyl sulphate concentrations on both sides of the membrane should be nearly the same at the beginning of the dialysis. Only then the equilibrium is reached within a reasonable time. In this context it should be noted that we have observed that it does not matter whether the experiment is carried out with labelled or unlabelled dodecyl sulphate as also shown in Fig. 14.

[1] H. Craubner, F. Koenig, and G. H. Schmid, Z. Naturforsch. **30 c**, 615 [1975].

[2] A. K. Dunker and R. R. Rueckert, J. Biol. Chem. **244**, 5074 [1969].

[3] J. G. Williams and W. B. Gratzer, J. Chromatogr. **57**, 121 [1971].

[4] K. Weber and M. Osborn, The Proteins, 3rd ed., **Vol. I,** p. 179 (H. Neurath and R. L. Hill, eds.), Academic Press, New York, San Francisco, London 1975.

[5] A. K. Wright, M. R. Thompson, and R. L. Miller, Biochemistry **14**, 3224 [1975].

[6] C. Tanford and J. A. Reynolds, Biochim. Biophys. Acta **457**, 133 [1976].

[7] G. H. Schmid, W. Menke, F. Koenig, and A. Radunz, Z. Naturforsch. **31 c**, 304 [1976].

[8] G. H. Schmid, G. Renger, M. Gläser, F. Koenig, A. Radunz, and W. Menke, Z. Naturforsch. **31 c**, 594 [1976].

[9] W. Kreutz and W. Menke, Z. Naturforsch. **15 b**, 402 [1960].

[10] W. Menke, F. Koenig, A. Radunz, and G. H. Schmid, FEBS Letters **49**, 372 [1975].

[11] F. Koenig, G. H. Schmid, A. Radunz, B. Pineau, and W. Menke, FEBS Letters **62**, 342 [1976].

[12] W. Menke, A. Radunz, G. H. Schmid, F. Koenig, and R.-D. Hirtz, Z. Naturforsch. **31 c**, 436 [1976].

[13] K. Weber and D. J. Kuter, J. Biol. Chem. **246**, 4504 [1971].

[14] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193**, 265 [1951].

[15] O. Kratky, H. Leopold, and H. Stabinger, Methods in Enzymol. **27**, 98 [1973].

[16] Handbook of Chemistry and Physics (R. C. Weast, ed.), The Chemical Rubber Co., Cleveland 1975.

[17] H. G. Weder, J. Schildknecht, and P. Kesselring, Amer. Lab. No. **10**, 15 [1971].

[18] R. M. Rosenberg and I. M. Klotz, Analytical Methods of Protein Chem., **Vol. 2,** p. 131 (P. Alexander and R. J. Block, eds.), Pergamon Press, Oxford, London, New York, Paris 1960.

[19] A. Ray, J. A. Reynolds, H. Polet, and J. Steinhardt, Biochemistry **5**, 2606 [1966].

[20] J. A. Reynolds, S. Herbert, H. Polet, and J. Steinhardt, Biochemistry **6**, 937 [1967].

[21] R. Pitt-Rivers and F. S. A. Impiombato, Biochem. J. **109**, 825 [1968].

[22] J. Steinhardt and J. A. Reynolds, Multiple Equilibria in Proteins, p. 45, Academic Press, New York, London 1969.

[23] K. Hayashi, Anal. Biochem. **67**, 503 [1975].

[24] J. A. Reynolds and R. H. Simons, J. Biol. Chem. **249**, 3937 [1974].

[25] N. C. Robinson and C. Tanford, Biochemistry **14**, 369 [1975].

[26] W. Jost, Diffusion in Solids, Liquids, Gases, Academic Press, New York 1970.

[27] J. A. Reynolds and C. Tanford, Proc. Nat. Acad. Sci. U.S. **66**, 1002 [1970].

[28] C. Tanford, The Hydrophobic Effect, J. Wiley, New York, London, Sydney, Toronto 1973.

[29] A. L. Shapiro and J. V. Maizel, Jr., Anal. Biochem. **29**, 505 [1969].