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Water-soluble proteins do not bind octyl glucoside as judged by molecular sieve chromatographic techniques

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ABSTRACT

It is well known that the non-ionic detergent octyl glucoside (1-O-*n*-octyl-β-D-glucopyranoside) solubilizes biological membrane components. It forms complexes with membrane-spanning proteins by hydrophobic interactions and it forms mixed micelles with membrane lipids. In contrast, non-ionic detergents usually do not bind to water-soluble proteins. According to a recent report, substantial and cooperative binding of octyl glucoside to several water-soluble proteins does occur near the critical micelle concentration. However, data have been obtained that contradict this report. No decrease was found in the elution volumes of five water-soluble proteins on molecular sieve chromatography on two Superose columns in tandem when 35 m*M* octyl glucoside was included in the eluent. No binding of the detergent to these proteins was observed at 20 or 22.5 m*M* octyl glucoside on molecular sieve chromatography on a TSK SW guard column as determined by differential refractometry and UV spectrophotometry of the proteins in the absence or presence of octyl glucoside. The experiments were done with the same buffer system and with six of the proteins used in the reported study. It is concluded that, as expected, there is no binding of octyl glucoside to water-soluble proteins above the detection limit (0.1 g detergent/g protein) of the refractometric method. The binding of, on average, 1.3 ± 0.2 g of detergent per gram of water-soluble protein that was observed at 20 m*M* octyl glucoside in the reported study is not consistent with the present results.

INTRODUCTION

Octyl glucoside (1-O-*n*-octyl-β-D-glucopyranoside) has a high solubilizing capacity for some membrane proteins [1–7] and does not usually affect the biological acitivity of membrane proteins and water-soluble enzymes [8–11]. However, the reconstituted activity of monosaccharide transporters may decrease if essential lipids are displaced from the protein on solubilization or fractionation [9,10,12]. The relative micellar mass of octyl glucoside at room temperature and near the critical micelle concentration is 21 000–25 000 [13–15]. The value of 8000 reported earlier [16] is an underestimate. The micelle size seems to become heterogeneous as the octyl glucoside concentration is increased [14]. In water, at 22°C, the micellar hydrodynamic radius is 2.35 nm [15]. The high critical micelle concentration of 23–25 m*M* [15–17] makes

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octyl glucoside easy to remove by molecular sieve chromatography or dialysis, for the preparation of proteoliposomes in reconstitution experiments [10,18]. The critical micelle concentration of octyl glucoside decreases with increasing ionic strength [17] and increases as the temperature is decreased from 25°C [15]. Some membrane proteins have been crystallized in the presence of octyl glucoside [13,19–21]. Results from X-ray crystallographic studies of the octyl glucoside–bacteriorhodopsin complex [20] were interpreted as an interaction of the detergent only with the hydrophobic parts of the protein, whereas the hydrophilic ends of the protein molecules bind to each other and thus build up the crystal lattice [21, 22]. Octyl glucoside has also been reported to show effects on the crystallization of water-soluble proteins [23].

A knowledge of the structures of the complexes between amphiphilic proteins and octyl glucoside, in addition to other non-ionic or zwitterionic detergents, is important in membrane biochemistry. Essential aspects are the state of association of the solubilized proteins and the sizes of their monomeric complexes with the detergent, which can be determined by molecular sieve chromatography [10,24]. Quantitative determinations can be made by low-angle laser light-scattering (LALLS) photometry, differential refractometry and UV absorbance photometry of eluates from high-performance gel chromatographic columns [25,26].

Non-ionic detergents do not bind to water-soluble proteins according to the few reports that we have found [27–30]. This is not surprising as the hydrophobic hydrocarbon moieties of the detergent molecules probably do not interact with the mainly hydrophilic surfaces of water-soluble proteins, at moderate ionic strengths. However, results suggesting substantial binding of octyl glucoside to several water-soluble proteins, at low ionic strength, below the critical micelle concentration and in proportion to the molecular weights of the proteins, have recently been reported [11]. Enzymes nevertheless had the same activities in the absence as in the presence of octyl glucoside [11]. The results were interpreted as indicating incorporation of each protein molecule into an octyl glucoside micelle. This seems unlikely as no denaturation occurred and as the protein surfaces are mainly hydrophilic, whereas the interior of an octyl glucoside micelle is hydrophobic.

To verify or disprove these data, we have now studied the effect of octyl glucoside on the elution volumes of some water-soluble proteins on molecular sieve chromatography on Superose columns. If a globular protein binds a large amount of octyl glucoside (as reported by Cordoba *et al.* [11]), the corresponding decrease in elution volume can easily be detected [31]. We have also attempted to detect octyl glucoside binding to some water-soluble proteins by differential refractometry combined with UV spectrophotometry for monitoring the proteins on high-performance molecular sieve chromatography, in the absence and presence of octyl glucoside, on a TSK SW guard column. This procedure allows the determination of binding by use of the ratios between the refractometer and spectrophotometer signals [26]. The results of the above two methods are internally consistent but they contradict the results of Cordoba *et al.* [11]. By use of equilibrium dialysis and infrared spectrometry, about fifteen binding sites for octyl glucoside monomers per bovine serum albumin molecule have also been found [32]. However, this value is below our detection limit of 0.1 g detergent/g protein, or about 20 octyl glucoside monomers per albumin molecule.

EXPERIMENTAL

Materials

For elution volume determinations, octyl glucoside (No. O-8001), ribonuclease A (Type 1-A; No. R 4875), ovalbumin (Grade V; No. A 5503), bovine serum albumin ("essentially fatty acid free"; No. A 7030), fibrinogen from human plasma (Type 1; No. F 3879), bovine catalase (No. C 100) and *Aspergillus niger* catalase (No. C 3515) were bought from Sigma (St. Louis, MO, U.S.A.). All chemicals were of analytical-reagent grade. Solutions were passed through 0.2-μm filters (SM 11107; Sartorius, Göttingen, F.R.G.) and simultaneously degassed.

For binding measurements, octyl glucoside was bought from Dojin Chemicals (Kumamoto, Japan) and bovine serum albumin preparations (No. 001, "fatty acid free" and No. 002, "reagent grade") from Chiba Chikusan Kogyo (Chiba, Japan). Other proteins were from the same source as above. Solutions were filtered through 0.3-μm filters (PHWP 04700; Nihon Millipore Kogyo, Yonezawa, Japan).

Methods

Molecular sieve chromatographic experiments. These experiments, for comparison of protein elution volumes in the absence or presence of octyl glucoside, were done on prepacked 23-ml (28 × 1 cm I.D.) columns of Superose-12 and Superose-6 cross-linked agarose gels connected in tandem, unless stated otherwise. A mixture containing all of the six proteins studied was usually applied. For peak identifications the proteins were also applied separately. In a series of experiments on the possible time dependence of binding of octyl glucoside, only the Superose-6 column was used. In these latter experiments bovine serum albumin and bovine catalase were studied. The proteins were fractionated separately on the Superose-6 column. All experiments were done at room temperature (23 ± 1°C).

The columns were equilibrated with 25 m*M* sodium phosphate buffer (pH 6.4) containing 0.1 *M* NaCl (as used in ref. 11) in the presence or absence of 35 m*M* octyl glucoside, with at least five column volumes of the solution to be used. The sample volume was 500 μl. The flow-rate was 0.3 ml/min. The chromatographic equipment [31] used for the above experiments was provided by Pharmacia LKB Biotechnology (Uppsala, Sweden).

Differential refractometric measurements. The proteins were applied, one by one, to a 3.3-ml (7.5 × 0.75 cm I.D.) TSK SW guard column, at room temperature (25 ± 1°C). The column was connected to a high-speed liquid chromatograph (Type CCPD) equipped with a degasser (ERC-3522). The eluate was monitored by a UV spectrophotometer (UV-8010) and by the LALLS instrument LS-8000, which contains a light-scattering detector and a differential refractometer (RI-8011). All the above pieces of equipment were obtained from Tosoh (Tokyo, Japan), except the degasser, which was from Erma Optical Works (Kawaguchi, Japan).

The solutions used were the same as for molecular sieve chromatography except that 3 m*M* sodium azide and 0, 20 or 22.5 m*M* octylglucoside were included. The sample volume was 100 μl and the flow-rate was 0.2 ml/min.

The TSK SW guard column was chosen as it affords a resolution high enough for the present purpose, and as its small volume gives short run times. The amount of the expensive octyl glucoside consumed is also minimized.

A procedure similar to that described above has recently been developed for the determination of sodium dodecyl sulphate (SDS) binding to proteins above the critical micelle concentration [33]. This procedure is based on the decrease in the refractometer signal from the micelles in samples with known amounts of SDS when part of the micellar detergent binds to protein molecules. It gave higher binding ratios than those obtained by earlier methods.

Sample preparation

Experiments with Superose columns. The proteins were dissolved together in 25 m*M* sodium phosphate buffer, (pH 6.4), prepared from Na_2HPO_4 and NaH_2PO_4, containing 100 m*M* NaCl, unless stated otherwise. The protein concentrations were ribonuclease A 3, ovalbumin 3, bovine serum albumin 3.2, fibrinogen 1.4, bovine catalase 1.6 and *Aspergillus niger* catalase 1.6 mg/ml. The protein solutions were passed through 0.2-μm Acrodisc-13 filters (Gelman, Ann Arbor, MI, U.S.A.).

The protein stability and octyl glucoside binding to bovine catalase and bovine serum albumin during incubations with octyl glucoside for several days were also tested. Separate samples of the proteins (concentrations as above) were incubated at 25°C in the absence or presence of 50 m*M* octyl glucoside in the above solution. This detergent concentration is sufficient to obtain the binding levels reported in ref. 11. After 20, 90 and 120 h, 500-μl aliquots of the samples were applied separately to a Superose-6 column.

Binding measurements. The proteins were dissolved separately at a concentration of 1.0 mg/ml in 25 m*M* sodium phosphate buffer (pH 6.4) containing 100 m*M* NaCl and 3 m*M* NaN_3 or, for experiments in the presence of octyl glucoside, in the above solution containing 22.5 m*M* octyl glucoside. The samples for runs at 20.0 or 22.5 m*M* octyl glucoside were dialysed overnight against buffer solution containing 22.5 m*M* octyl glucoside (the dialysis volume was ten times the sample volume). All samples were filtered through 0.22-μm filters (Type SLGV025LS; Nihon Millipore Kogyo).

Evaluation

Elution volumes. A difference in the elution volume (ΔV_e) of 30 μl can be detected in the Superose chromatographic experiments. According to a graph of V_e *vs.* log M_r for the proteins studied (not shown), $\Delta V_e = 30$ μl corresponds to an increase in molecular weight of 1% and an increase in radius for a globular protein of 0.3%. Massive binding of octyl glucoside, as reported by Cordoba *et al.* [11], can thus easily be detected as a decrease in V_e in the molecular sieve chromatographic experiments.

Binding. The refractometer signal, $(\text{Output})_{RI}$, can be expressed as

$$(\text{Output})_{RI} = k_1(dn/dc)c \tag{1}$$

where k_1 is a constant, dn/dc is the specific refractive index increment and c is the weight concentration of the protein. The UV absorbance signal, $(\text{Output})_{UV}$, can be expressed as

$$(\text{Output})_{UV} = k_2Ac \tag{2}$$

where k_2 is a constant and A is the absorption coefficient, based on weight concentration. This gives

$$dn/dc = k_3\{(\text{Output})_{RI}/[(\text{Output})_{UV}/A]\} \quad (3)$$

where k_3 is a constant.

The refractive index increment for a solution of the complex between detergent (D) and protein (P) with respect to the protein concentration, at constant chemical potential of diffusible components, $(\partial n/\partial c_P)_\mu$, can be expressed as [34]

$$(\partial n/\partial c_P)_\mu = (\partial n/\partial c_P)\{1 + \xi[(\partial n/\partial c_D)_c/(\partial n/\partial c_P)_c]\} \quad (4)$$

where ξ is the binding ratio (w/w), $(\partial c_D/\partial c_P)_\mu$, of detergent to protein, assuming that the system can be regarded as a three-component system (protein, detergent, aqueous solution). Thus,

$$\xi = (R-1)(\partial n/\partial c_P)_c/(\partial n/\partial_D)_c \quad (5)$$

where $R = (\partial n/\partial c_P)_\mu/(\partial n/\partial c_P)_c$, $(\partial n/\partial c_P)_c = 0.187$ ml/g [26] and $(\partial n/\partial c_D)_c = 0.138$ ml/g, as determined for octyl glucoside micelles [15]. According to eqn. 3,

$$R = [(\text{Output})_{RI}/(\text{Output})_{UV}]_{DP} / [(\text{Output})_{RI}/(\text{Output})_{UV}]_P \quad (6)$$

where DP denoting values in the presence of octyl glucoside and P values in the absence of detergent. The peak heights were measured. R was calculated according to eqn. 6 and finally the binding values ξ (g octyl glucoside/g protein) were calculated according to eqn. 5, which, with the above numerical values, can be written as

$$\xi = 1.355\,(R-1)$$

The limits of error (see Table I) are based on the sums of the standard deviations (σ_{n-1}) for the peak-height ratios in eqn. 6.

For a complete description of this methodology, see ref. 26.

RESULTS

Elution volumes

Fractionation on the tandem Superose-12 and Superose-6 columns (see *Methods*) showed that the presence of 35 mM octyl glucoside (*i.e.*, well above the critical micelle concentration) did not detectably affect the elution volumes of five of the six proteins studied, compared with fractionation in the absence of the detergent. The elution volumes were constant (Fig. 1). The only exception was fibrinogen, which was eluted earlier in the presence of 35 mM octyl glucoside than in the absence of detergent. The decrease in elution volume was small, about 300 μl (*cf.*, Fig. 1). This corresponds to an increase in M_r of *ca.* 17 000 for a globular protein or to about 60 octyl glucoside molecules to one protein molecule. However, as no binding to fibrinogen was detected by the refractometric method (see below), a plausible explanation

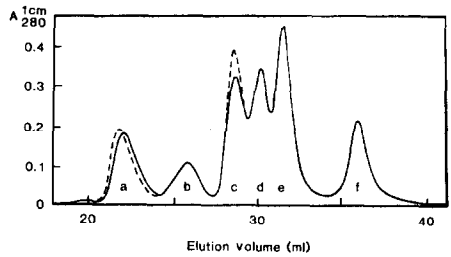

Fig. 1. Fractionation of six water-soluble proteins on a Superose-12 and a Superose-6 column in tandem, equilibrated with 25 m*M* sodium phosphate buffer (pH 6.4), containing 0.1 *M* NaCl, without detergent (solid line) or with 35 m*M* octyl glucoside (dashed line). Flow-rate, 0.3 ml/min; sample volume, 500 μl. The columns were equilibrated with at least five column volumes of the solution to be used. After equilibration in the detergent-free solution, ten 500-μl aliquots of the protein sample were applied one by one. No change in the elution pattern was observed. After equilibration with at least five column volumes of solution containing 35 m*M* octyl glucoside, six 500-μl aliquots of the protein sample were chromatographed as above with about two column volumes of eluent for each run. The elution patterns were identical in all six runs, within the narrow limits set technically by the sample application device, the monitor and the recorder. Finally, the columns were again equilibrated with detergent-free solution and four 500-μl aliquots of the protein sample were fractionated. The elution patterns were identical with the ten previous patterns in the absence of detergent. The studied proteins were (a) fibrinogen, (b) *Aspergillus niger* catalase, (c) bovine catalase, (d) bovine serum albumin, (e) chicken ovalbumin and (f) ribonuclease A. The elution peaks of these proteins are indicated. The decrease in elution volume for fibrinogen (a) with octyl glucoside may reflect decreased retardation rather than detergent binding (*cf.*, Results, *Elution volumes*). The reason for the increase in peak height for bovine catalase (c) in the presence of octyl glucoside is not known.

may be that fibrinogen was slightly retarded, by weak adsorption effects, in the Superose columns, in the absence of octyl glucoside.

Chromatography on Superose-6 of bovine catalase and bovine serum albumin after long incubation times in the presence of 50 m*M* octyl glucoside showed no differences in the elution volumes of these proteins compared with chromatography after incubations in the absence of detergent (not illustrated). The elution volumes were also the same whether the fractionations were done in the presence of octyl glucoside or not.

Binding

A series of high-performance gel chromatographic experiments with differential refractometric and UV spectrophotometric monitoring failed to detect any binding of octyl glucoside to any of the water-soluble proteins studied (Table I). The high resolution of the TSK SW guard column is illustrated in Fig. 2. Only with ribonuclease A was the refractometric peak sometimes slightly disturbed by minor total-volume valleys or peaks. A binding of, on the average, 1.3 g of octyl glucoside per gram of protein at an equilibrium concentration of 20 m*M* octyl glucoside (as calculated from the $\bar{\nu}$ values in Table I in ref. 11) is more than ten times higher than our detection limit of 0.1 g detergent/g protein.

DISCUSSION

Binding of detergents to proteins can be divided into two main categories, (I)

TABLE I

ATTEMPT TO DETECT OCTYL GLUCOSIDE BINDING TO WATER-SOLUBLE PROTEINS BY HIGH-PERFORMANCE GEL CHROMATOGRAPHY[a] MONITORED BY DIFFERENTIAL REFRACTOMETRY AND UV SPECTROPHOTOMETRY[b]

Protein[c]	Bound octyl glucoside (g/g protein)	
	C_{OG} = 20 mM[d]	C_{OG} = 22.5 mM[d]
Ribonuclease A	−0.01 ± 0.02	–
Ovalbumin	−0.04 ± 0.02	−0.04 ± 0.02
Bovine serum albumine[e]	0.00 ± 0.02	0.02 ± 0.02
Bovine serum albumin[f]	0.00 ± 0.02	0.00 ± 0.03
Bovine catalase	−0.03 ± 0.02	−0.03 ± 0.01
Fibrinogen[g]	0.00 ± 0.01	0.01 ± 0.02
Average: −0.01 ± 0.02 (n = 11)		

[a] Three to five runs for each protein on a TSK SW guard column for each of the octyl glucoside concentrations 0, 20 and 22.5 mM.

[b] Details in *Methods*.

[c] See *Materials*.

[d] Equilibrium concentration in the chromatographic column. For runs in 20 and 22.5 mM octyl glucoside the samples were dialysed against 22.5 mM octyl glucoside (in buffer).

[e] "Fatty-acid free".

[f] "Reagent grade".

[g] Fibrinogen was partly adsorbed on the prefilter or column in the first two runs in the absence of octyl glucoside. Only the two following runs were used for the calculations.

non-denaturing binding of non-ionic or zwitterionic detergents to amphiphilic proteins, by hydrophobic interaction, and (II) denaturing binding of ionic detergents to amphiphilic and hydrophilic proteins.

Examples of the Type-I binding are complexes between octyl glucoside and the human red cell glucose transporter [10,24] and the octaethylene glycol *n*-dodecyl ether complex with canine renal Na^+/K^+-ATPase [35]. Both of these proteins are integral membrane proteins.

Type-II binding is found in SDS complexes of water-soluble and membrane proteins. It is still not clear why SDS and other ionic detergents bind not only hydrophobically to amphiphilic proteins but also to water-soluble proteins. Binding of SDS dramatically affects the protein structure. A hypothesis on the course of events during interactions between SDS and water-soluble proteins is outlined in ref. 31.

Non-ionic detergents, on the other hand, can presumably not generally bind to the surfaces of water-soluble, hydrophilic proteins. The hydrophobic interactions that may occur with some grooves or patches of the surface of a hydrophilic protein are probably insufficient to allow cooperative binding of detergent.

The glucose moiety of octyl glucoside is large (M_r = 179) compared with the the alkyl chain (M_r = 113). The number of monomers in a micelle is also large (87 according to ref. 15). This indicates a compact structure in the hydrophilic shells of the octyl glucoside micelle, as illustrated in Fig. 3A. In reality the alkyl chains are probably staggered [15]. Octyl glucoside micelles may interact with surfaces of water-soluble proteins, mainly by hydrogen bonding (Fig. 3A). Such interactions can hardly lead to

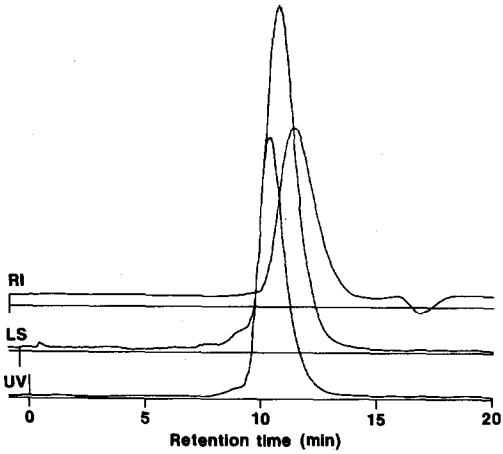

Fig. 2. Chromatography of bovine catalase on a TSK SW guard column (see *Methods*). These elution profiles were obtained at an equilibrium concentration of 22.5 m*M* octyl glucoside in the column. Essentially identical profiles were found at 0 and 20 m*M* octyl glucoside. UV, UV absorption (at 280 nm; 1 cm path length); LS, low-angle laser light-scattering; RI, differential refractive index. The UV curve indicates a high protein purity as regards low-molecular-weight proteins or protein fragments. Small amounts of high-molecular-weight proteins or protein aggregates are separated, as shown by the light-scattering curve. The main refractive index peak is well separated from the small valley approximately at the total volume of the column. The valley derives from a slight deviation of the salt and detergent concentrations of the sample from those of the eluent. The detectors were connected to the column in the order UV, LS and RI. The distances between the horizontal lines is 10% of full-scale. Similar elution profiles as in this example were obtained for the other proteins that were analysed. For a homogenous material (such as, in this instance, a pure protein) the peak positions along the time axis would coincide if the three parameters were monitored in a single cell and if the signals were recorded with a common starting point. Note that the baseline levels for the light-scattering signal and for the differential refractometric signal are the same before as after the peaks. This shows that equilibrium prevails.

the formation of micelles below the critical micelle concentration or to binding of micelles. The micellar structure probably prevents denaturation of proteins by octyl glucoside, as a polypeptide cannot easily distribute its hydrophilic and hydrophobic groups between (a) the aqueous phase outside the micelle or the glucose shell of the micelle and (b) the apolar micelle core, without severely disrupting the micelle structure. The type of octyl glucoside interaction with water-soluble proteins that was suggested in ref. 11 (Fig. 3B) seems to us thermodynamically unstable, as hydrogen bonding to serine or threonine (inward-facing monomers in Fig. 3B) will be weak and as most of the surface of a water-soluble protein is hydrophilic. The latter disfavours an outward-facing mode of binding whether the alkyl chains of octyl glucoside are staggered or not. Hence octyl glucoside can probably neither denature nor bind to non-denatured water-soluble proteins.

The sulphate groups of the ionic detergent SDS are small ($M_r = 96$) and repel each other owing to their negative charge, whereas the alkyl chains of SDS are relatively long ($M_r = 169$). The micelles of SDS allow denaturation, possibly as they give space enough for hydrophilic side-groups of an interacting polypeptide to face the aqueous medium and also space enough for hydrophobic side groups to be inserted

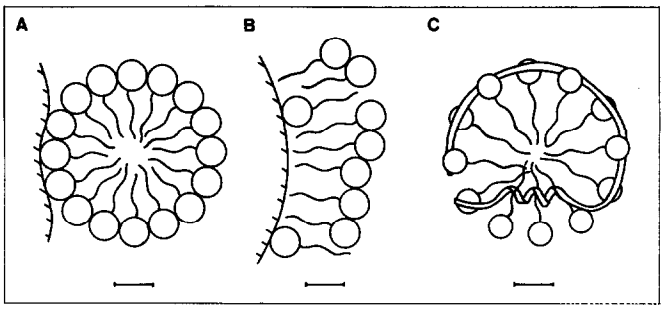

Fig. 3. Detergent–protein complexes. (A) Hypothetical interaction between an octyl glucoside micelle and the surface of a water-soluble protein (see Discussion). (B) Interpretation of the structure proposed tentatively in ref. 11 for monolayers of octyl glucoside on the surfaces of water-soluble proteins below the critical micelle concentration. These types of octyl glucoside binding to water-soluble proteins were not found in our experiments. (C) Model of a complex between SDS and the denatured polypeptide of a water-soluble protein lacking disulphide bonds, partly based on neutron-scattering data [37] (see Discussion). The length of the bars corresponds to 1 nm.

individually or in stretches (probably as α-helices) into the micelle core(s). The backbone of the polypeptide may even be hydrogen bonded to sulphate groups [36]. Most of the polypeptide of a non-disulphide-bonded protein in a complex with SDS is probably situated at the surface of SDS micelles [37]. The repulsion between the sulphate head groups keeps the polypeptide segments apart and prevents refolding. This type of complex is illustrated schematically in Fig. 3C. The properties of SDS contribute to a spacious and stable structure of the micelles in a complex with a polypeptide, a type of structure which cannot be attained by octyl glucoside.

We therefore consider it unlikely that octyl glucoside can bind, at moderate ionic strengths, to water-soluble proteins, except possibly at a few sites where stable binding on the protein surface is possible. Our results show that octyl glucoside does not bind to water-soluble proteins, at least not in amounts exceeding 0.1 g per gram of protein. However, hydrophobic interactions between water-soluble proteins and hydrophobic groups do occur at high concentrations of salts, as evidenced by hydrophobic interaction chromatography, where water-soluble proteins are adsorbed on hydrophobic ligands at 2–5 *M* salt concentrations.

Interestingly, SDS analogues with six or eight oxyethylene ($-CH_2CH_2O-$) groups inserted between the sulphate group and the dodecyl chain do not denature bovine serum albumin [38]. The reason for this is presumably that the hydrophilic moiety becomes much larger ($M_r = 360$ or 448), which leads to steric effects similar to those proposed for octyl glucoside (above). The weight proportion between the hydrophilic and hydrophobic moieties is 0.6 for dodecyl sulphate, 2.1 for the dodecyl sulphate analogue with six oxyethylene groups and 1.6 for octyl glucoside.

We cannot exclude that some subtle differences in conditions cause the discrepancy between our results and those of Cordoba *et al.* [11]. However, as the octyl glucoside concentrations were determined only in the protein-free compartment of the dialysis cell and as we do not find the proposed micellar binding of octyl glucoside around the proteins convincing, there are reasons to suspect that some unknown factor in the experimental procedures used by Cordoba *et al.* [11] may be the cause of

the inconsistency. One possibility is that the binding observed was not to the native proteins but to amphiphilic contaminants such as polypeptide fragments (formed on proteolysis). Even small fragments would remain in the protein compartment if they formed complexes with octyl glucoside and induced formation of micelles, as the relative micellar mass is 21 000–25 000 [13–15] and the exclusion limit of the dialysis membrane used in ref. 11 was 6 000–8 000. Small proteolytic fragments and octyl glucoside binding to such fragments will not easily be detected on molecular sieve chromatography.

A method suitable for studying this aspect further may be small-angle neutron scattering [22,36].

CONCLUSION

The non-ionic detergent octyl glucoside does not bind in micellar form to the water-soluble proteins ribonuclease A, ovalbumin, bovine serum albumin, fibrinogen and bovine catalase or *Aspergillus niger* catalase at an ionic strength of 0.14 *M* and at equilibrium concentrations of 20–35 m*M* octyl glucoside. This is contrary to the model used by Cordoba *et al.* [11] for the analysis of binding data. The binding of octyl glucoside to the studied water-soluble proteins fell below the detection limit of 0.1 g detergent/g protein in refractometrically monitored chromatography at 20 m*M* octyl glucoside. The substantial binding at 20 m*M* octyl glucoside reported by Cordoba *et al.* [11] is thus not consistent with our results.

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REFERENCES

1 C. Baron and T. E. Thompson, *Biochim. Biophys. Acta*, 382 (1975) 276.
2 G. W. Stubbs and B. J. Litman, *Biochemistry*, 17 (1978) 215.
3 P. Rosevear, T. VanAken, J. Baxter and S. Ferguson-Miller, *Biochemistry*, 19 (1980) 4108.
4 R. J. Gould, B. H. Ginsberg and A. A. Spector, *Biochemistry*, 20 (1981) 6776.
5 P. K. Werner and R. A. F. Reithmeier, *Biochemistry*, 24 (1985) 6375.
6 Z. Markovic-Housley and R. M. Garavito, *Biochim. Biophys. Acta*, 869 (1986) 158.
7 L. Corazzi and G. Arienti, *Biochim. Biophys. Acta*, 875 (1986) 362.
8 A. Helenius and J. Kartenbeck, *Eur. J. Biochem.*, 106 (1980) 613.
9 S. A. Baldwin, J. M. Baldwin and G. E. Lienhard, *Biochemistry*, 21 (1982) 3836.
10 E. Mascher and P. Lundahl, *Biochim. Biophys. Acta*, 945 (1988) 350.
11 J. Cordoba, M. D. Reboiras and M. N. Jones, *Int. J. Biol. Macromol.*, 10 (1988) 270.
12 C.-C. Chen and T. H. Wilson, *J. Biol. Chem.*, 259 (1984) 10150.
13 W. Kühlbrandt, *Q. Rev. Biophys.*, 21 (1988) 429.
14 R. W. Roxby and B. P. Mills, *J. Phys. Chem.*, 94 (1990) 456.
15 K. Kameyama and T. Takagi, *J. Colloid Interface Science*, 137 (1990) 1.
16 T. VanAken, S. Foxall-VanAken, S. Castleman and S. Ferguson-Miller, *Methods Enzymol.*, 125 (1986) 27.
17 K. Shinoda, T. Yamaguchi and R. Hori, *Bull. Chem. Soc. Jpn.*, 34 (1961) 237.

18 G. D. Eytan, *Biochim. Biophys. Acta*, 694 (1982) 185.
19 R. M. Garavito and J. P. Rosenbusch, *J. Cell Biol.*, 86 (1980) 327.
20 H. Michel and D. Oesterhelt, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 1283.
21 H. Michel, *Trends Biochem. Sci.*, 8 (1983) 56.
22 M. Roth, A. Lewit-Bentley, H. Michel, J. Deisenhofer, R. Huber and D. Oesterhelt, *Nature (London)*, 340 (1989) 659.
23 A. McPherson, S. Koszelak, H. Axelrod, J. Day, R. Williams, L. Robinson, M. McGrath and D. Cascio, *J. Biol. Chem.*, 261 (1986) 1969.
24 E. Mascher and P. Lundahl, *J. Chromatogr.*, 397 (1987) 175.
25 S. Makino, S. Maezawa, R. Moriyama and T. Tagaki, *Biochim. Biophys. Acta*, 874 (1986) 216.
26 Y. Hayashi, H. Matsui and T. Takagi, *Methods Enzymol.*, 172 (1989) 514.
27 A. Helenius and K. Simons, *J. Biol. Chem.*, 247 (1972) 3656.
28 S. Makino, J. A. Reynolds and C. Tanford, *J. Biol. Chem.*, 248 (1973) 4926.
29 A. Helenius and K. Simons, *Biochim. Biophys. Acta*, 415 (1975) 29.
30 S. Clarke, *J. Biol. Chem.*, 250 (1975) 5459.
31 E. Mascher and P. Lundahl, *J. Chromatogr.*, 476 (1989) 147.
32 Z. Wasylewski and A. Kozik, *Eur. J. Biochem.*, 95 (1979) 121.
33 P.-F. Rao and T. Takagi, *Anal. Biochem.*, 174 (1988) 251.
34 H. Eisenberg, *Biological Macromolecules and Polyelectrolytes in Solution*, Clarendon Press, Oxford, 1976.
35 Y. Hayashi, K. Mimura, H. Matsui and T. Takagi, *Biochim. Biophys. Acta*, 983 (1989) 217.
36 P. Lundahl, E. Greijer, M. Sandberg, S. Cardell and K.-O. Eriksson, *Biochim. Biophys. Acta*, 873 (1986) 20.
37 K. Ibel, R. P. May, K. Kirschner, H. Szadkowski, E. Mascher and P. Lundahl, *Eur. J. Biochem.*, 190 (1990) 311.
38 K. Ohbu, J. Jona, N. Mizushima and I. Kashiwa, *Yukagaku*, 29 (1980) 866.