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The Use of the Laser Light Scattering for the Molecular Weight Determination of Membrane Proteins in Surfactants

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A method is described for the determination of the molecular weight of the protein moiety of protein surfactant complexes using of low angle laser light (633 nm) scattering in combination with high performance porous silica gel chromatography, precision differential refractometry and differential UV absorption was described. The calibration curves in sodium dodecyl sulfate (SDS) and in a non-ionic surfactant, octaethyleneglycol dodecyl ether, using several reference proteins yielded linear lines. Molecular weight of porin oligomers and monomers, an intrinsic membrance protein that forms the permeability channel in the outer membrane of *Escherichia coli*, were calculated and were found to be 109,000 and 36,300, respectively in SDS and that of oligomers in octaethy leneglycol dodecyl ether was 114,200. Similarly, the molecular weights of maltoporin oligomers and monomers, which form maltose-maltodextrin-specific channels, appeared to be 148,500 and 48,200, respectively, in SDS and that of oligomers in octaethyleneglycol dodeclyl ether was 149,000.

(Key Words: Molecular weight, Membrane protein, Surfactant, Light scattering, Gel filtration)

INTRODUCTION

Determination of molecular weight and subunit structure of membrane proteins is often troublesome, as the most intrinsic membrane proteins are insoluble in water. Molecular weights of membrane proteins are often determined empirically by SDS-acrylamide-gel electrophoresis and by sedimentation equilibrium analysis in SDS or in the density adjusted aqueous solution of non-ionic surfactants (7, 9, 10). The molecular weight determination of the protein moiety of SDS-protein complexes in SDS, no matter which of the above method was used, often leads to erroneous results and, in most cases, the higher protein structures were loosen. Sedimentation equilibrium analysis of membrane proteins in the density adjusted non-ionic surfactants seems to work for the proteins that form comicelles with the surfactants. However, the method can not be applied to the denatured membrane protein, that is derived from protein oligomer by treating with strong reagents, such as SDS. It is, therefore, highly desirable to develop a new method by which one can determine the molecular weights of protein moiety of surfactant-protein complexes.

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An assesment study on the use of a low angle laser light scattering photometer (LS) in combination with a precision differential refractometer (RI) for the determination of the molecular weights of membrane proteins were carried out in ionic and non-ionic surfactants.

EXPERIMENTAL

Molecular weight of a protein was calculated from the following equation (see ref. 8 for detail), $n_0^2(dn^*/dc)^2 \cdot (I_0/I_i)KC = \frac{1}{M} + 2B$ where; n_0 is refractive index increment; I_0 and I_i are the intensity of incident beam and the intensity of scattered light; K, C, M, and B are instrument constant, protein concentration, molecular weight and the second virial coefficient.

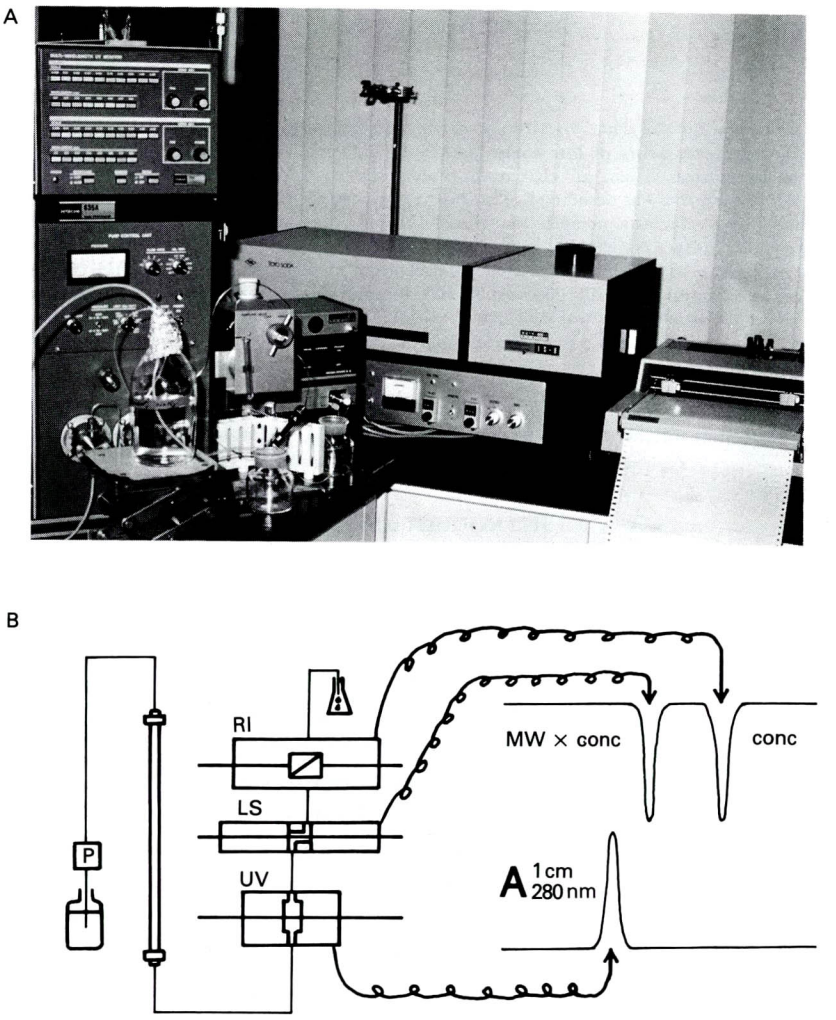

Fig. 1 Photographic and illustrative presentation of the lines. A, photography of instrumentations. B, Schematic representation of lines for the actual experiment. For the determination of LS and RI, the line was connected as shown in figure. (dn*/dc) was determined through a UV-detector-RI line without a LS photometer.

From the signals of a light scattering photometer, a precision differential refractometer and a differential UV detector, informations about (I_0/I_i), $n_0^2(dn^*/dc)^2$ and C will be obtained, respectively. Thus, the molecular weight, M, can be computed.

In practical experiment, the protein sample in surfactant was injected into a high performance porous silica gel (Toyo Soda, TSK-GEL G3000SW) connected with a low angle laser light scattering photometer (Toyo Soda, LS-8, using He-Ne laser light at 633 nm) and a precision differential refractometer (Shodex RI-11), or a differential UV detector (Hitachi 635M LC detector) and a differential refractometer as shown in Fig. 1. Thus, the former and the latter lines provided the information LS/RI and $RI/A_{280nm,}^{1\,cm}$ respectively. Thus, LS/RI·1/(dn*/dc) could be obtained for the several reference proteins and the outer membrane proteins (Fig. 2).

Porins and maltoporins (λ-receptor proteins) were purified from *E. coli* B and K-12 strains, respectively, as reported earlier (6). SDS and octaethyleneglycol dodecyl ether ($G_{12}E_8$) were purchased from Bio Rad and Nikko Chemicals, respectively.

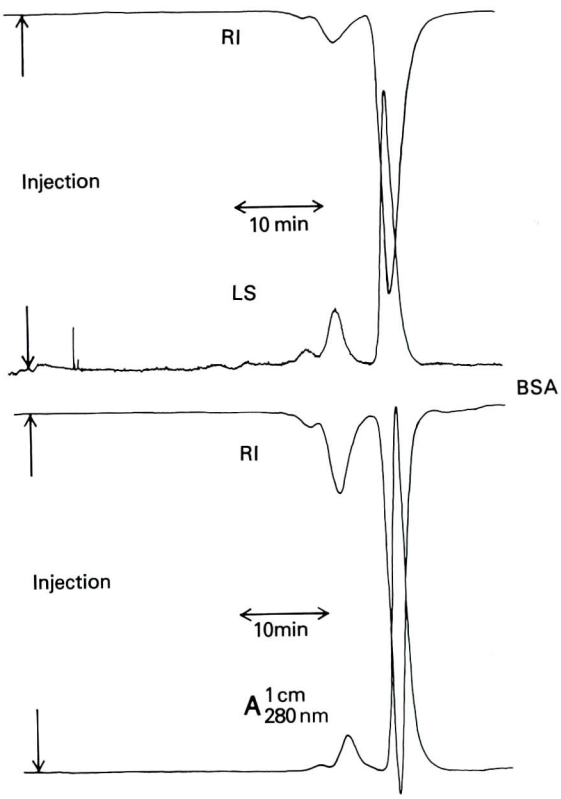

Fig. 2 Typical tracings of the detector responses. About 1 mg of BSA was dissolved in 200 μl of the SDS-buffer (see the text), injected into a TSK-GEL G3000SW and the column was eluted with the same buffer at 15 kg/cm², and 0.4 ml/min. Note that BSA-monomers, dimers and trimers were clearly separated.

RESULTS AND DISCUSSION

Experiments in SDS.

Protein samples were dissolved in a buffer containing 0.25% SDS-100 mM NaCl-3 mM NaN_3 and 50 mM Tris-HCl, pH 7.0. LS, RI and (dn*/dc) of several reference proteins such as RNAse B ($M_r = 14,700$), myoglobin ($M_r = 16,890$), chymotrypsinogen ($M_r = 25,400$), alcohol dehydrogenase ($M_r = 37,000 \times 4$), bovine serum albumin (BSA, $M_r = 68,000$) and glutamic dehydrogenase ($M_r = 53,000 \times 6$) were recorded as shown in Table 1. The values of LS/RI increased roughly as the molecular weights of reference proteins increased. The (dn*/dc) of SDS-protein complexes appeared to be around 0.3 to 0.4 (protein conc. ml/g) suggesting that the amounts of bound SDS to the unit protein weight are more or less constant. If LS/RI was divided by (dn*/dc), normalizing the refractivity increment of SDS-protein complexes, the values became a function of the molecular weights of protein moiety of SDS-protein complexes, Fig. 3.

The outer membrane of *E. coli* harbors several pore-forming proteins such as porins (4) and maltoporins (λ-receptor proteins) (6). Both of these proteins are known to exist as trimeric aggregates in native form and as denatured monomers by heating in SDS (3, 5). We studied these proteins as model membrane proteins. As shown in Table 1, (dn*/dc) of SDS-porins-oligomers- and -monomers-complexes appeared to be 0.266 and 0.389 respectively, suggesting a low SDS binding to the oligomers. Maltoporin oligomers and monomers were apt to behave more or less similar to porin (Table 1).

Table 1 The values obtained from LS, RI and UV detectors in SDS. Proteins were dissolved in 200 μl of the SDS-buffer (see the text) and injected into a silica gel column connected with either LS-RI line or UV-RI line, and the column was eluted with the same buffer as described in legend to Fig. 2.

Protein	M_r	LS/RI $\times 10^4$	(dn*/dc)[b]	$\frac{\text{LS/RI}}{\text{(dn*/dc)}} \times 10^5$
cytochrome c	12,385	10.9	0.421	2.58
ribonuclease B	14,700	18.2	0.451	4.03
myoglobin	16,890	15.9	0.370	4.29
trypsin	23,800	21.1	0.318	6.64
chymotrypsinogen	25,400	31.7	0.392	8.03
alcohol DH[a]	37,000	34.5	0.369	9.34
glutamic DH[a]	53,000	62.7	0.414	15.14
BSA	68,000	61.4	0.337	18.22
porin oligomer		83.1	0.266	31.24
monomer		39.7	0.389	10.22
maltoporin oligomer		95.1	0.225	42.29
monomer		45.8	0.331	13.84

[a]DH, dehydrogenase

[b](dn*/dc) was expressed by the protein concentration, ml/g

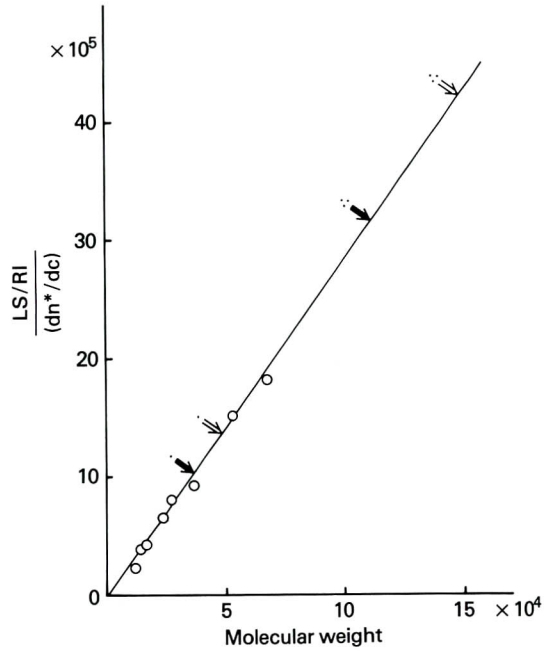

Fig. 3 Calibration curve in the SDS-buffer solution. The values LS/RI·1/(dn*/dc) of reference proteins (see, table 1) were plotted against the molecular weights of respective proteins. Arrows indicated the values of LS/RI·1/(dn*/dc) of porin oligomers (∴ ➡), monomers (•➡), λ-receptor oligomers (∴⇨) and monomers (•⇨).

The values LS/RI·1/(dn*/dc) of the reference proteins in SDS were plotted against the molecular weights of respective proteins. As shown in Fig. 3, the plots yielded a linear line and the linearity was fairly good over the range tested. The LS/RI·1/(dn*/dc) of porin oligomers and monomers appeared to be 31.24 and 10.22×10^5, and the molecular weights of these were calculated to be 109,000 and 36,300, respectively. Since the molecular weight of porin monomer was computed to be 37,200 from amino acid sequencing study (1), the errors for the oligomers and monomers appeared to be −2.3% and −2.4%. The subunit number of the oligomers was thus calculated to be 3.0. Similarly, the molecular weights of maltoporin oligomers and monomers were calculated to be 148,500 and 48,200. Errors of the calculated molecular weights of the oligomers and the monomers were found to be +4.4% and +1.6%, against the reported molecular weights, 47,400, that was deduced from DNA sequencing study (2). Thus, the subunit number of maltoporin was computed to be 3.08. As the experiment shows, the present method can be applied to the molecular weight determination of protein moiety of SDS-protein complexes, regardless of bound SDS.

Experiments in non-ionic surfactant.

Similar experiments were carried out under milder condition in the solution containing non-ionic surfactant. The surfactant used was homogeneous polyoxyethyleneglycol dodecyl ether ($C_{12}E_8$). Reference proteins as

well as membrane proteins were dissolved in the buffer solution containing 5mM $C_{12}E_8$-100mM NaCl-3mM NaN_3- and 10mM HEPES buffer pH 7.0. Since the micellar size of non-ionic surfactants is larger than that of ionic surfactants, the laser light scattering showed a little noise to compare with the buffer only or SDS-buffer. Table 2 shows that (dn*/dc) of the most reference proteins stayed very close from each other around 0.16 to 0.19 in spite of the fact that LS/RI increased according to the size increment of the proteins. The (dn*/dc) of the reference proteins were more or less similar to the (dn*/dc) of such proteins in aqueous solution without the surfactant, suggesting that the proteins did not form comicelle with the surfactant. Membrane proteins such as porin oligomers and maltoporin oligomers showed their (dn*/dc) values about 1.5 times higher than that of the reference proteins. This observation suggests that the membrane proteins used in this experiment interact with the surfactant.

Table 2 The data obtained from LS, RI and UV detectors in $C_{12}E_8$. Experimental details were similar to Table 1, except that $C_{12}E_8$-buffer (see the text) was used.

Protein	M_r	LS/RI $\times 10^4$	(dn*/dc)[a]	$\frac{\text{LS/RI}}{\text{(dn*/dc)}} \times 10^5$
ribonuclease B	14,700	5.2	0.195	2.7
chymotrypsinogen	25,400	12.5	0.194	6.4
BSA	68,000	38.0	0.178	21.3
alcohol DH[b]	148,000	79.8	0.160	49.8
glutamic DH[b]	280,000	188.0	0.198	95.1
porin oligomer	Exp 1	109.8	0.290	37.8
	2	110.5	0.282	39.2
maltoporin oligomer	Exp 1	121.9	0.242	50.4
	2	121.6	0.244	49.8

[a](dn*/dc) was expressed as ml/g of protein
[b]DH, dehydrogenase

The data, LS/RI·1/(dn*/dc), were plotted against the molecular weight of the respective reference proteins. As shown on Fig. 4, a line is fairly linear over the range of molecular weights tested. The linearity was slightly poor at low molecular weight range which might be due to a high noise to signal ratio in this particular surfactant. We believe that this could be improved by the use of an alternative surfactant of smaller micellar size or by the reduction of surfactant concentrations. The molecular weight of porin oligomers was computed to be 114,200. The error was +2.3%. Similarly, the molecular weight of maltoporins appeared to be 149,000 and the error to the reported molecular weight was +4.7%.

As we have shown here, the molecular weights of membrane proteins are nicely determined by the use of high performance porous silica gel chromatography, low angle laser light scattering, precision differential refractometry and UV absorption. The advantages of the present method are; (i) Molecular weights of membrane proteins can be determined in both denatured and non-denatured conditions. (ii) Minor contamination of

foreign materials can be separated out by gel filtration and the molecular weight from only the desired protein peak can be determined. (iii) This method gives rather accurate estimation of the molecular weights of protein moiety of surfactant-protein complexes. (iv) Prior knowledge of surfactant binding to the protein is not required and, hence, time consuming equilibrium dialysis and the determination of bound surfactants can be eliminated. (v) The last importance, the present method is time saving. Once the calibration curves were made, one can determine a molecular weight of protein within two hours. Needless to mention, this system works for the molecular weight determination of water soluble proteins, non-proteinous materials and for the size determination of complex materials such as liposomes.

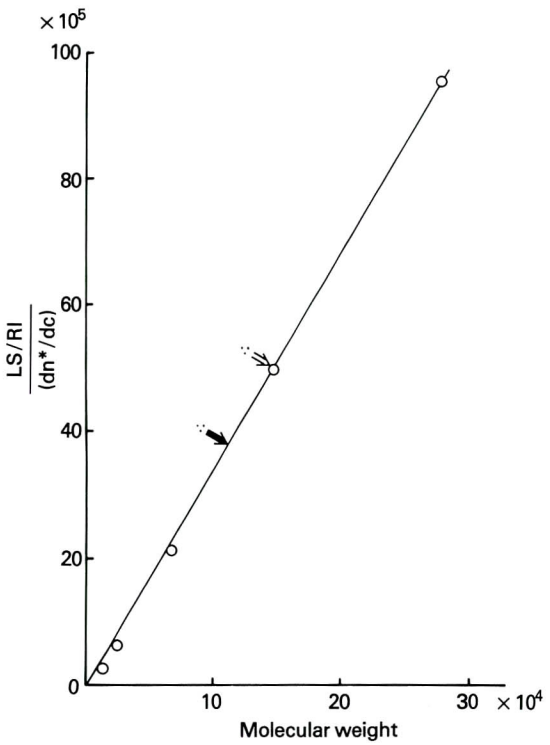

Fig. 4 Calibration curve in the $C_{12}E_8$-buffer solution. Data in Table 2 were plotted as in Fig. 3. Symbols were similar to the legend to Fig. 3.

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