

## Kinetics of Sodium Dodecyl Sulfate Solubilization of *Mycoplasma laidlawii* Plasma Membranes

(critical micelle concentration/stopped-flow spectrophotometer/light scattering)

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**ABSTRACT** The kinetics of sodium dodecyl sulfate solubilization of aqueous suspensions of *Mycoplasma laidlawii* membranes have been investigated by light scattering in a stopped-flow apparatus. There was evidence of direct interaction between the membranes and sodium dodecyl sulfate micelles above the critical micelle concentration, although of lower order kinetically than with monomeric dodecyl sulfate anions below the critical micelle concentration. The activation energy remained the same in either case, about 10 kcal/mol. Static light-scattering studies at higher resolution showed that the solubilized membranes are in the form of small aggregates.

Since the isolation of "structural protein" from various membrane sources (1), the use of the anionic detergent sodium dodecyl sulfate (SDS) for membrane solubilization has been an established method for the investigation of membrane substructure. There has recently been a flurry of interest in the "miniproteins" (molecular weight about 5,000), discovered in SDS-solubilized membranes from mammals (2, 3). Many other peptide fractions are detected by SDS-acrylamide gel and SDS-Sepharose 6B columns (2, 4-7).

Few membrane peptides have been purified from SDS solutions to date. We have performed kinetic studies of the interaction between aqueous membrane suspensions and SDS in order to better understand the mechanism of SDS action. We have chosen to work with *Mycoplasma* because of their special advantages (8, 9), and the extensive work with SDS disaggregation of these membranes (8-13).

The present study with stopped-flow and light-scattering apparatus brings to light the following information about the SDS solubilization of *M. laidlawii* (strain A) membranes. (a) There is evidence for direct interaction between SDS micelles and membranes above the critical micelle concentration (cmc) for the detergent. (b) Although a higher order of interaction occurs with monomeric SDS anions below the cmc, the activation energy for the solubilization remains the same, about 10 kcal/mole, indicating that a similar rate-determining step occurs in the interaction of membranes with either micelles or monomeric detergent anions. (c) The membranes disaggregate to an average molecular weight of slightly more than 70,000 when solubilized in SDS at room temperature. (d) Heat treatment of the solubilized membranes causes a considerable decrease in light-scattering ability. Thus, solubilized membranes are in the form of small aggregates, perhaps dimers to tetramers.

Abbreviations: SDS, sodium dodecyl sulfate; cmc, critical micelle concentration.

## MATERIALS AND METHODS

### Membranes

Membranes were prepared from *Mycoplasma laidlawii* (strain A) by osmotic lysis as described (14). The washed membranes were suspended in twice-distilled, demineralized water. The suspension was diluted 1:10 with 2-chloroethanol and absorbance was measured at 280 nm in a Beckman DB spectrophotometer. An extinction coefficient of 1.82 for 1 mg/ml of membrane protein in a 1-cm cell was used (14). The pH of a 1 mg/ml aqueous membrane suspension was typically 7.2. As reported (14), *M. laidlawii* membranes do not contain either cysteine or cystine, therefore, reducing agents were not added to the dissociation media. Electrophoresis studies with SDS-acrylamide gels show no difference in banding patterns, with or without mercaptoethanol treatment.

### Detergent

99% pure SDS was obtained from Sigma and used without further purification. A 100 mM solution has a pH of 9.2. Experiments were also performed with Eastman SDS, which had been recrystallized several times from 95% ethanol. These highly purified SDS solutions had a pH of 7.3 at a concentration of 100 mM. Kinetic experiments have shown no significant differences between the two SDS preparations.

### Kinetic experiments

Rapid mixing experiments were performed in a Durrum-Gibson stopped-flow spectrophotometer, equipped with a fluorescence attachment. The 435.8-nm mercury line was used for illuminating the sample, with the monochromator slits opened as far as possible. Fluorescence filters were not used. The absorbance due to carotenoid in the membrane suspension was small (less than 0.01 at 0.5 mg/ml) and did not interfere with light-scattering measurements in the stopped-flow cuvette, where we were concerned only with the large amount of light scattered from the membranes before solubilization. We calibrated the instrument by measuring the scattered light intensity of aqueous membrane suspensions without SDS; a linear concentration dependence was observed from 0 to 1.0 mg/ml. The scattered light intensity from SDS-solubilized membranes in the stopped-flow apparatus was insignificant and taken as zero on the concentration scale.

### Static light-scattering experiments

High resolution studies of static light-scattering of the solubilized membranes were performed with a Brice-Phoenix

light-scattering photometer under single beam operation with galvanometer output. The 546-nm mercury line was used for illumination, in order to avoid interference from carotenoid absorbance in these more sensitive measurements. A test for fluorescence at this wavelength was negative for *Mycoplasma* membranes in aqueous SDS. Dissymmetry was also negligible. All values here reported are derived from 90° scattering.

Washed membranes were suspended in distilled water and dry SDS was added to bring the solution to the appropriate concentration. Individual dilutions were made from the initial solution. Each solution was prefiltered through a oil-un pore Sartorius filter, then filtered again directly into a 10 × 10 mm "micro-cell" for light-scattering measurements. Protein concentrations were determined after light-scattering measurements were completed.

Refractive index measurements were made in an Abbe-3L Bausch and Lomb refractometer. A circulating water bath maintained the temperature at 25°C ± 0.02°C. The refractive index  $\eta^{25}$  was 1.3340 for 1% SDS, and  $\Delta\eta/\Delta c$  for membranes in 1% SDS was 0.375/gm of protein per ml. Correspondingly for 5% SDS,  $\eta^{25}$  was 1.3384 and  $\Delta\eta/\Delta c$  for membranes was 0.260/gm of protein per ml.

Molecular weight calculations were made using the relation

$$\frac{Hc}{\tau} = \frac{1}{M} + 2Bc + 3Cc^2 + \dots$$

where  $H$  is a function of the solvent refractive index  $\eta^{25}$  and the refractive increment of solute  $\Delta\eta/\Delta c$ ,  $\tau$  is the measured turbidity corrected for refractive index and various calibration parameters,  $c$  is the concentration of solute, and  $M$  is the average molecular weight.

## RESULTS AND DISCUSSION

### Kinetics

Equal volumes of aqueous SDS solution and membrane suspension were mixed in stopped-flow experiments. Concentrations are expressed as their values after mixing. Initial reaction velocities were determined by drawing a tangent to the oscilloscope trace of scattered light intensity versus time

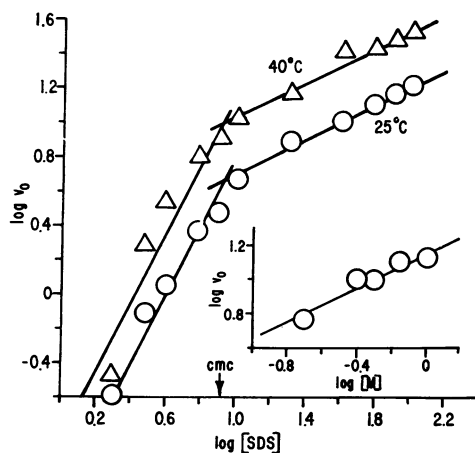


FIG. 1. Logarithm of initial membrane solubilization velocity  $v_0$  (mg/ml per sec) vs logarithm of membrane concentration  $[M]$  (mg/ml) in 1% SDS at 25°C (insert). Logarithm of initial membrane solubilization velocity vs. logarithm of SDS concentration  $[SDS]$  (mM) at 0.5 mg/ml membrane concentration at 25°C (O) and 40°C ( $\Delta$ ).

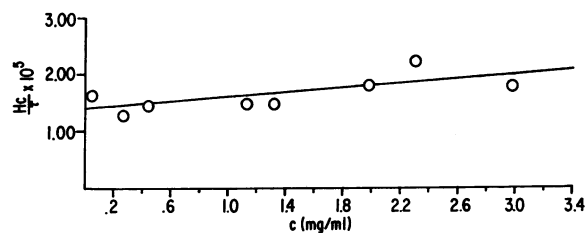


FIG. 2. Static light-scattering data for *M. laidlawii* membranes in 5% SDS after standing at room temperature for several hours.

immediately after mixing and determining the slope of the tangent. The initial rate of disappearance of 0.5 mg/ml membranes in 1% (34.7 mM) SDS was typically determined to be 10 mg/ml per sec.

The insert in Fig. 1 shows the logarithm of initial solubilization velocity versus the logarithm of membrane concentration at 25°C in 1% SDS. The slope of this line indicates that the reaction is half order with respect to membrane concentration.

The larger graph in Fig. 1 is a similar plot at a constant 0.5 mg/ml membrane concentration with various concentrations of SDS at 25 and 40°C. Note the break in these curves at the detergent cmc. Instead of flattening out as expected, the curves continue to rise with a smaller, but still appreciable, concentration dependence. Since the concentration of dissociated SDS anions remains constant above the cmc, a direct interaction between micelles and membranes is indicated. The slope of these curves above the cmc indicates that the solubilization is half order with respect to SDS micelles. The concentration dependence is much greater below the cmc, and the slope of the curves suggests second-order kinetics with respect to dissociated SDS anions. The plots at 25 and 40°C remain parallel above and below the cmc, with the same vertical separation, showing that the activation energy for membrane solubilization is the same for micelles as for dissociated anions. A quantitative treatment of this data yields an activation energy of 10 kcal/mol for each type of solubilization.

### Reaction products

The above kinetic data are valid only for the initial rapid solubilization of the membranes. The intensity of the scattered light rapidly drops below the sensitivity of the detection system in the stopped-flow spectrophotometer. Therefore, a highly sensitive light-scattering photometer was used to study the solubilized membranes. Measurements were performed on membranes that had been exposed to SDS at room temperature for several hours to insure that the initial dissociation reaction was complete (11).

Light-scattering data for membranes in various concentrations from 0.1 to 3 mg/ml in 5% SDS are shown in Fig. 2. These data indicate an average molecular weight of 71,400 for the solubilized membranes. Similar data in 1% SDS yield a molecular weight of 76,300.

SDS-solubilized membrane solutions were heat treated in a boiling-water bath for up to 10 min to find out if further dissociation was possible. The solutions were cooled to room temperature after heat treatment and light-scattering measurements were repeated. These results are summarized in Table 1. The turbidity was decreased by as much as 50% by heat treatment, and the 5% SDS membrane solutions re-

TABLE 1. Static light-scattering data on *Mycoplasma laidlawii* membranes solubilized in aqueous sodium dodecyl sulfate before and after heat treatments in boiling water.

SDS Conc. (%)	Protein Conc. (mg/ml)	Unheated ( $\tau$ )*	Heat treatment ( $\tau$ )		
			No. 1 (2 min)	No. 2 (2 min)	No. 3 (5 min)
1	3.5	0.00317	0.00151	0.00135	0.00144
5	3.5	0.00362	0.00178	0.00182	—

\*  $\tau$  denotes turbidity at a 90° angle using 546-nm light.

quired fewer heat treatments to equilibrate than did the solutions in 1% SDS.

Preliminary experiments with Sepharose 6B gel filtration and SDS-gel electrophoresis show that most of the heat-treated fractions have molecular weights in the range from 20,000 to 40,000. We therefore conclude that the materials solubilized at room temperature are in the form of small aggregates, perhaps dimers to tetramers. The question remains, however, as to the degree of aggregation of the heat-treated materials.

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