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The Stabilization Mechanism of Acylcholinesters in Aqueous Solution by Sodium Lauryl Sulfate[1,2]

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Acylcholinesters (C_3 to C_{12}) were synthesized and the stabilization effect by sodium lauryl sulfate (SLS) against hydrolysis were studied at pH 7.7.

1) The apparent distribution of cholinesters between aqueous and micellar phases of SLS solution was measured by condensation method. The correlation between the apparent distribution and the magnitude of stabilization effect was observed.

2) The hydrolytic rate of cholinesters was suppressed from 1/2 to 1/250 by the addition of 0.5% SLS. The longer the acyl chain length of cholinesters, resulted the greater the stabilization effects of SLS. There was no apparent relation between chain length and rate constant without SLS.

3) When cholinesters were mixed with SLS in aqueous solution, transient turbidity formation was observed, which was considered to be due to the metathesis. A complete dissociation of three salts concerning to the metathesis was proved by the determination of conductivity.

4) The solubility of acylcholine iodides in water was high and that of in organic solvents was limited by the polarity of the cholinesters regardless of their acylchain length. Therefore, the mechanism of stabilization from hydrolysis was considered *via* the solubilization of acylcholine–lauryl sulfate ion pair into micellar phase.

5) From the correlation mentioned above, it was presumed that the site of solubilization and/or the dimentional change of micelle might affect the stabilization from hydrolysis.

It may be convenient to discuss the degradation of drugs in aqueous solution classifing into two major groups of reaction *i.e.*, oxidation and hydrolysis. It is possible to protect drugs from oxidation by the elimination of oxygen in the system and/or by the addition of stabilizer to stop the initiation or propagation of reaction. The hydrolytic reactions are quite contrally to the oxidation. The presence of water is inevitable as solvent. Therefore, it is very difficult to suppress hydrolysis in aqueous solution, but many methods for stabilization, though not as effective as in oxidation, have been proposed, as follows: 1) adjustment of pH, 2) complex formation by the addition of third substance, 3) reactivity change through adjustment of dielectric constant, and 4) the addition of organic solvents. The replacement of the water with organic solvent partially and to lower the concentration of water is the principle of the last method. One variation of this method is the addition of surfactants into the system proposed by Riegelman[4] or Nogami, *et al.*[5] The point is that surfactants form micelles corresponds to the dispersed organic solvent in aqueous solution. Inside of micelles are lipophilic because they are formed by the aggregation of hydrophobic part of surfactants. If the solubilization of substance into micellar phase would occur, the environments of solu-

1) This paper forms Part XVII of "Studies on Decomposition and Stabilization of Drugs in Solution," Part: XVI H. Nogami, J. Hasegawa, and M. Iwatsuru, *Yakuzaigaku*, **30**, 160 (1970).

2) Taken in part from the thesis of Motoharu Iwatsuru for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1966.

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4) S. Riegelman, *J. Am. Pharm. Assoc. Sci. Ed.*, **49**, 339 (1960).

5) H. Nogami, S. Awazu, K. Watanabe, and K. Sato, *Chem. Pharm. Bull.* (Tokyo), **8**, 1136 (1960).

bilizate could be similar to the one in organic solvent and hydrolytic reaction would never occur or occur more reluctantly.

The drug stabilization employed with surfactants has been studied under various combinations of drugs and surfactants.

From the results reported,[6] it has been supposed that the magnitude of the stabilization effect depends upon the amount of solubilized drugs in the micellar phase, but the detailed mechanism has not been discussed.

It has been postulated in solubilization experiments[7] that the solubility of a substance in water is constant, and the increase of solubility is attributed to solubilization into micellar phase. The relation mentioned may be true in an equilibrium system provided that the excess of solute exists. When the solute is in undersaturation in aqueous phase, it would be much more rational to consider that the stabilization effects depend upon the distribution of solute between aqueous and micellar phases.

It was shown in the previous report[1] that the critical micelle concentration (c.m.c.) of surfactant could be determined from volume change of the surfactant solution and decrement of surfactant using a semi-permeable membrane and dry, powdered Sephadex G-25. When a substance is added to surfactant solution, the substance may exist in micellar phase as well as in aqueous phase, but only the substance in aqueous phase can move through the membrane during the condensation procedure and the solubilizate in micellar phase will remain in one side of the membrane. Therefore it is possible to calculate the apparent distribution of a solute between aqueous and micellar phases. Before the condensation,

$$C_0 = C_{m_0} + C_{aq_0}$$

and after the condensation,

$$C_1 = C_{m_1} + C_{aq_1}$$

where C_0, C_1 are total concentration of the solute, C_{m_0}, C_{m_1}: the concentration of the solubilizate in the micellar phase per unit volume of the solution, C_{aq_0}, C_{aq_1}: the concentration of the solute in the aqueous phase per unit volume of the solution. Subscripts 0 and 1 indicate before and after the condensation, respectively.

As C_{aq_0} and C_{aq_1} are not affected by the condensation,

$$C_{aq_0} = C_{aq_1}$$

C_{m_0} increases to C_{m_1} while the volume of the solution V_0 decreases to V_1 by the condensation,

$$V_0 \cdot C_{m_0} = V_1 \cdot C_{m_1}$$

where V_0 and V_1 are the volume of the solution before and after the condensation, respectively.

From these equations

$$C_{aq_0} = (C_0 \cdot V_0 - C_1 \cdot V_1)/(V_0 - V_1)$$

Thus, C_{aq} and C_m can be determined.

Acylcholinesters were chosen as the simplified forms of methantheline bromide[5] and in the relation to the previous report[8] to be studied the effects of acyl chain length on the stabilization or the apparent distribution between aqueous and micellar phases.

6) *a*) N. Nakajima, *Yakugaku Zasshi*, **81**, 1684 (1961); *b*) K. Watanabe, *Yakuzaigaku*, **29**, 124 (1969).
7) M.E.L. McBain and E. Hutchinson, "Solubilization and Related Phenomena," Acad. Press, New York, 1955.
8) H. Nogami, S. Awazu, and M. Iwatsuru, *Chem. Pharm. Bull.* (Tokyo), **11**, 1251 (1963).

Experimental

Materials——1) Sodium Lauryl Sulfate: A commercial products of Nikko Co., Ltd., recrystallized from EtOH after extraction of higher alcohols with ether.

2) Cellulose Membrane: A commercial products of Visking Co., Ltd. The diameter of the casing was 15.9 mm. The pore size was reported to be 24 Å.

3) Sephadex G-25: A commercial products of Pharmacia Co., Ltd., Uppsala, Sweden. Fine grade.

4) Acylcholinesters were synthesized according to Loury's method.[9] Propionylchloine odide, mp[10] 130°. *Anal.* Calcd. for $C_8H_{18}O_2NI$: C, 33.46; H, 6.30; N, 4.88. Found: C, 33.82; H, 6.51; N, 4.81. Butyrylcholine iodide, mp 92°. *Anal.* Calcd, for $C_9H_{20}O_2NI$: C, 35.89; H, 6.69; N, 4.65. Found: C, 35.98; H, 7.04; N, 4.22. Hexanoylcholine iodide, mp 126°. *Anal.* Calcd. for $C_{11}H_{24}O_2NI$: C, 40.13; H, 7.35; N, 4.26. Found: C, 40.19; H, 7.38; N, 4.29. Octanoylcholine iodide, mp 146°. *Anal.* Calcd. for $C_{13}H_{28}O_2NI$: C, 43.70; H, 7.90; N, 3.92. Found: C, 43.74; H, 7.87; N, 3.46. Dodecanoylcholine iodide, mp 164°. *Anal.* Calcd. for $C_{17}H_{36}O_2NI$: C, 49.39; H, 8.78; N, 3.39. Found: C, 49.55; H, 8.56; N, 3.31.

5) Acylcholine–lauryl sulfates were obtained from acylcholine iodide and silver lauryl sulfate[11] and recrystallized from acetone. Octanoylcholine–lauryl sulfate, mp 157°. *Anal.* Calcd. for $C_{25}H_{53}O_6NS$: C, 60.57; H, 10.78; N, 2.82. Found: C, 59.63; H, 10.27; N, 2.32. Dodecanoylcholine–lauryl sulfate, mp 155°. *Anal.* Calcd. for $C_{29}H_{61}O_6NS$: C, 63.11; H, 11.14; N, 2.54. Found: C, 63.41; H, 11.24; N, 2.56.

All reagents used were of guaranteed reagents grade.

Procedure——1) Measurement of Dissociation Constant by Electroconductivity: The electroconductivities were measured with Yanagimoto Conductivity Outfit: Model No. 55, and a cell having the cell constant 0.5446 at 25.0±0.05° in a thermostatically controlled water bath.

2) Measurement of Distributions of Cholinesters between Aqueous and Micellar Phases: The solutions were prepared containing 5.5 mM of cholinester and 17.4 mM of sodium lauryl sulfate (0.5%). After one end of the cellulose casing was tied with thread, 20 ml of the solution was pipetted into the casing, most of air was forced out, and the sac was closed with knots of thread. The sac was set surrounded by the dry powder of Sephadex G-25 and then pressure was supplied onto the sac and powder. About one hour later the sac was picked up and the remaining solution (about 80% of initial volume) was transferred into a measuring cylinder. After the determination of volume change, the concentration of cholinester was determined with Hestrin's method.[12]

Result and Discussion

When sodium lauryl sulfate (SLS) solution was mixed with cholinester solution, the formation of transient turbidity was observed but it disappeared promptly by the addition of excess mole of SLS. It seemed that the turbidity depended on the length of acyl chain of cholinesters. As both components, SLS and cholinesters, are strong electrolytes, they are assumed to be completely dissociated in aqueous solution and subject to metathesis. Therefore the precipitate should be cholinester–lauryl sulfate and the longer the acyl chain, the lower the solubility in water. The electroconductivity of the solutions containing octanoylcholine iodide, SLS, or octanoylcholine–lauryl sulfate was determined to verify this assumption. The results are given in Fig. 1 and 2. Since the solubility of dodecanoylcholine–lauryl sulfate in water was extremely low, so that it was impossible to measure the conductivities over a wide concentration range, but this substance was considered in complete dissociation like the other substances menitoned above.

The concentration of substances in aqueous and micellar phases (per unit volume of the solution) in 0.5% SLS solution was determined by the condensation method and shown in Table I.

There was no concentration change before and after the condensation when 5.5 mM of octanoylcholine iodide and 0.2 mM of SLS (below c.m.c.) were mixed and treated. It was concluded that there was no apparent interaction among the solute, surfactant below c.m.c., and the cellulose membrane.

9) M. Loury, *C. R. Acad. Sci. Paris*, **209**, 682 (1939).
10) All melting points are uncorrected.
11) O. Yoda, K. Meguro, T. Kondo, and K. Ino, *Nippon Kakagu Zasshi*, **75**, 1272 (1954).
12) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

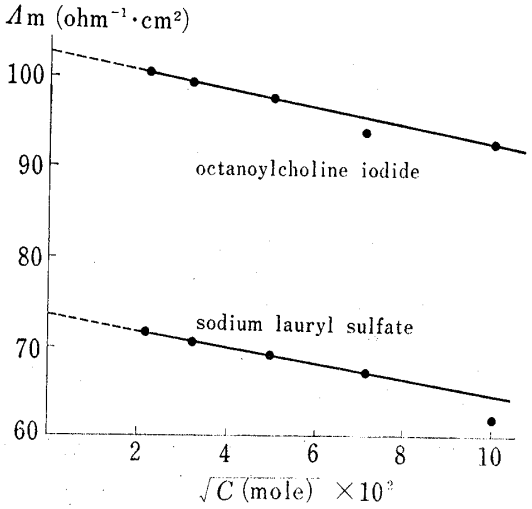

Fig. 1. Molar Conductance of Sodium Lauryl Sulfate and Octanoylcholine Iodide at 25°

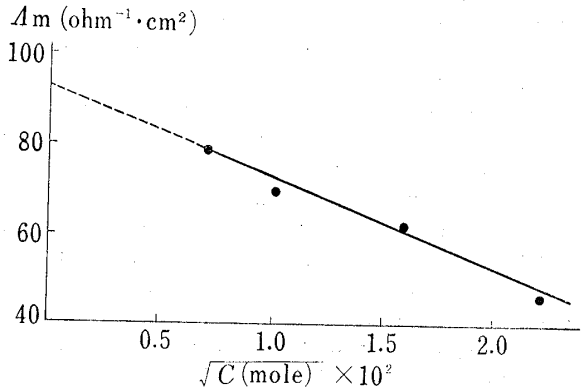

Fig. 2. Molar Conductance of Octanoylcholine-Lauryl Sulfate at 25°

Table I. Distribution of Cholinesters between Aqueous and Micellar Phases

	C_{aq}(mM)	C_m(mM)	C_m/C_{aq}
C_3 Propionylcholine iodide	1.05	4.45	4.2
C_4 Butyrylcholine iodide	0.94	4.56	4.8
C_6 Hexanoylcholine iodide	0.76	4.74	6.2
C_8 Octanoylcholine iodide	0.57	4.93	8.7
C_{12} Dodecanoylcholine iodide	0.03	5.47	18.3

C_{aq}: concentration of cholinester in aqueous phase (per unit volume of the solution)
C_m: concentration of cholinester in micellar phase (per unit volume of the solution)
The values given in the table were the mean of three measurements.

As the phosphate buffer solution was used in the kinetic study of hydrolysis of cholinesters in the previous report,[8] the apparent distributions of substrate between aqueous and micellar phases were determined under the equivalent gegen-ion concentration (NaCl 60 mM) as shown in Table II.

Table II. Effect of Sodium Chloride on Distribution of Cholinesters between Aqueous and Micellar Phases (NaCl 60 mM)

	C_{aq}(mM)	C_m(mM)	C_m/C_{aq}
C_4 Butyrylcholine iodide	0.63	4.87	7.7
C_8 Octanoylcholine iodide	0.37	5.13	14.0
C_{12} Dodecanoylcholine iodide	0.02	5.48	27.4

C_{aq}: concentration of cholinester in aqueous phase (per unit volume of the solution)
C_m: concentration of cholinester in micellar phase (per unit volume of the solution)
The values given in the table were the mean of three measurements.

The stability increase of cholinesters by the addition of SLS were compiled in Table III and the relationship between apparent distribution and stabilization effect was illustrated in Fig. 3.

The linear relation was observed between apparent distribution and logarithm of stabilization effect with exception of dodecanoylcholine iodide. As the apparent distribution is in

the function of two factors, *i.e.* solubilities in aqueous and micellar phases, it can not be interpreted simply by the effect of alkyl chain length of acyl chains, but the following explanations may hold.

Table III. Half Lives of Cholinesters in Sodium Lauryl Sulfate Solution at 70° (pH 7.7)[a)]

Choilinester (5.5 mM)	Concn. of sodium lauryl sulfate 0 % (A)	0.5 % (B)	(B)/(A)
C_2 Acetylcholine chloride	48 min	80 min	1.7
C_3 Propionylcholine iodide	60	120	2.0
C_4 Butyrylcholine iodide	104	305	2.9
C_6 Hexanoylcholine iodide	100	894	8.9
C_8 Octanoylcholine iodide	100	5040	50.4
C_{12} Dodecanoylcholine iodide	110	29400	267

a) ref. 8

a) The solubilities of acylcholinesters in aqueous phase decrease as the increase of alkyl chain length in acyl moiety. The solubility in 0.5% SLS solution decreases to ½ (C_3 to C_8) and to 1/30 (C_3 to C_{12}). The distribution is the result of two effect as mentioned and a good linear relation between the two parameters might be observed for C_3 to C_8 except C_{12}.

b) The addition of 60 mM of sodium chloride reduced the solute concentration in aqueous phase approximately 2/3 for C_4 and C_8 but no significant change on the solubilizate concentration in micellar phase. These might result the shift to right direction in Fig. 3. It is frequently observed that c.m.c. reduced by the addition of salts.[13)] This would probably decrease the concentration of lauryl sulfate ion and the esters in aqueous phase.

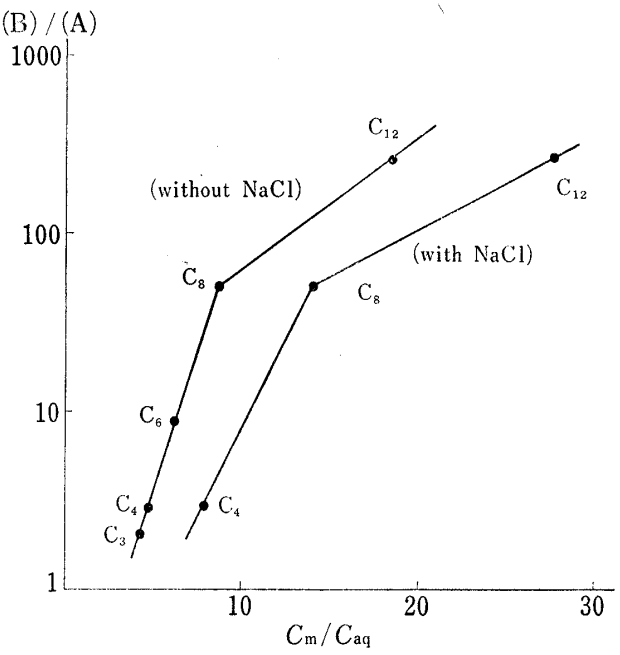

Fig. 3. The Relationship between Apparent Distribution and Stabilization Effect

c) The distribution was measured by condensation as a static factor of the system and the values of B/A were obtained from kinetic study as a dynamic factor. Therefore, it might be said that there was a good correlation as shown in Fig. 3 between the two different parameters in a solubilized system. It may be possible, however, that the deviation of dodecanoylcholine iodide from the linear relationship due to other mechanisms, *e.g.* the difference of solubilization site or expansion of micellar dimention due to the larger volume of the solubilizate. Further investigation is necessary to draw a conclusion about this point.

13) *a*) T. Tachibana (ed.), "Kaimenkagaku," Maruzen, Tokyo, 1956, p. 119; *b*) I. Nishi, I. Imai, and M. Kasai (eds.), "Kaimenkasseizai Binran," Sangyotosho, Tokyo, 1960, p. 137.

Dyer[14] measured the pK_a values of weak acids and bases by solubility studies using different pH buffer solutions containing surfactant. His experiments can be interpreted that the dissociated form exists only in aqueous phase and undissociated form does in micellar phase, and the complex which can be solubilized into organic solvent will not be formed in this case. On the other hand, from hue change c.m.c. of anionic surfactants were determined with cationic dye, *e.g.* pinacyanol chloride, and those of cationic surfactants with anionic dye, *e.g.* Sky Blue FF.[15] These show abrupt change of environments of dye at c.m.c. Considering following results that the hydrolytic reaction was suppressed in the combination of SLS and methantheline bromide as well as SLS and cholinesters (the latter case) far greater than in the combination of cetylethyldimethylammonium bromide and acetylsalicylic acid (the former case), it may be understood that the mechanism of stabilization of methantheline bromide or cholinesters by SLS is due to the formation of ion pair which is soluble in micellar phase by metathesis and the protective action of the micellar phase against hydrolysis.

Acknowledgement The authors express their thanks to Nikko Co., for the supply of sodium lauryl sulfate and alkyl acid chlorides.

14) D.L. Dyer, *J. Colloid Sci.*, **14**, 640 (1959).
15) ref. 14 (a) p. 140.