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The Dissolution State of a Triglyceride Molecule in Water and Its Orientation State at the Air-Water Interface

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The relations between the surface tension and the concentration of the aqueous solutions of triacetin, tripropionin, tributyrin, tricaproin, and tricaprylin were determined at 37°. The solubilities and the adsorbed amounts of triglycerides were obtained from these results. Since the solubilities of tricaproin and tricaprylin were extremely low, the corrections for adsorption at the interfaces were necessary to obtain their solubilities. The linear relationship between the logarithm of solubility and the total surface area of hydrophobic groups in a triglyceride molecule which could contact maximally with the water, was found to hold.

The smallness of the slope of this straight line in comparison with those of hydrocarbon and alcohol, was interpreted as showing that three hydrophobic chains in a triglyceride molecule may aggregate intramolecularly with one another in the water; three-fifths of the total surface area of hydrophobic group are in contact with water and the two-fifths are in contact with hydrophobic group. Moreover, from the molecular area occupied at the air-water interface, it was suggested that the hydrophobic chains in adsorbed monolayer of triacetin might correspond to the "tune-forming" conformation like the β-form crystal of triglyceride; the chains in 1- and 3-position were directed toward the air and the chain in 2-position was directed toward the water.

It has been said that pancreatic lipase hydrolyses selectively the triglyceride which is dispersed without dissolving in the water.[2,3] Therefore, the solubility of triglycerides in the water and an orientation state at the interface need to be studied. A few studies have already been reported on the solubility in the water[4,5] but its reliability is low in regard to the purity of materials and the method for the measurement.

Then, the authors determined the solubility and the adsorbed amount from the concentration dependence of the surface tension of the aqueous solutions of triglycerides and discussed the states of triglyceride molecule in the water and at the air-water interface.

Experimental

Materials——Triacetin and tributyrin used were of analytical grade from the Nakarai Chemicals (purity 99%) and tricaproin and tricaprylin used were obtained from Sigma Chemical Company (purity 99%). Tripropionin from the Tokyo Kasei Organic Chemicals (purity 90%) was distilled under reduced pressure (purity 96.6%). The purity of tripropionin was determined by means of gas chromatography. The water was twice distilled from all-glass apparatus and used within two days. *n*-Hexane of analytical grade was twice distilled.

Preparation of Aqueous Solutions of Triglycerides——A given amount of triglyceride was added into 100 ml of the water through the following ways:

Gravimetric method; a weighing vessel containing a weighed quantity of the sample was added and stirred. As a weighing vessel, glass disk (*ca.* 1 cm in diameter) was used in the case of a small amount such as tributyrin and a glass micro-beaker was used in the case of a relatively large amount such as triacetin.

Volumetric method; a given volume of the sample was dropped into the water through a Muromachi Kagaku micro-syringe ("Alfa" accuracy ±0.0002 ml).

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[1] Location: *Misasagi Yamashina, Higashiyama-ku, Kyoto, 607, Japan.*

[2] L. Sarda and P. Desnuelle, *Biochim. Biophys. Acta*, **30**, 513 (1953).

[3] G. Benzonana and P. Desnuelle, *Biochim. Biophys. Acta*, **105**, 121 (1965).

[4] H. Sobotka and D. Glick, *J. Biol. Chem.*, **105**, 199 (1934).

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Spread method; a given volume of n–hexane solution of the sample was spread on the water surface through the microsyringe and stirred after several minutes, when the solvent seemed to evaporate out.

The aqueous solutions of each triglyceride were prepared for triacetin by the gravimetric method, for tripropionin by the volumetric method, for tributyrin by the three methods, and for tricaproin and tricaprylin by the spread method because of the extremely low solubilities of the last two substances. The concentrations of n–hexane solutions of triglycerides were 1.785 mg/ml for tricaproin and 1.28 μg/ml for tricaprylin. The densities of tripropionin and tributyrin were put to be equal to 1.10 and 1.02,[6] respectively. The concentration was expressed in terms of the number of moles in 1 liter of the water.

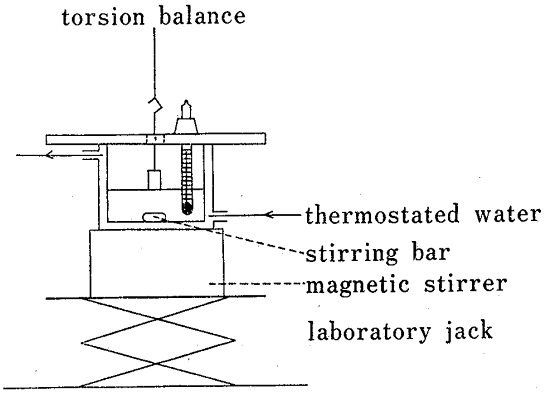

Fig. 1. Thermostated Apparatus for the Measurement of Surface Tension

Measurement of Surface Tension——Surface tension was measured by the hanging plate method as shown in Fig. 1. The buoyancy of a hanging plate was kept zero by regulating appropriately the position of the thermostated cell with the laboratory jack. The temperature of the aqueous solution was kept at 37 ± 0.2° by circulating the thermostated water through a glass cell. The aqueous solutions were stirred magnetically for 50 minutes after the addition of triglyceride and then, surface tension was measured. Glass cell, a glass plate (2 cm in perimeter), and a teflon stirring bar were washed with cromic-sulfuric acids mixture.

Results

Solubility by the Measurement of the Surface Tension

Since the solubilities of triglycerides in the water are extremely low in general, the preparation of the aqueous solutions of triglycerides need to be devised. Fig. 2 shows the relation between the surface tension, γ and the concentration, C_t of the aqueous solution of tributyrin prepared by the three methods described above. Since the concentration, C_t is the number of moles divided by the volume of the water, it is the apparant one above the solubility. Therefore, the solubility, C_s is the concentration of the kink point above which the surface tension is unvaried. In the case of tributyrin, all the three methods give almost identical results as shown in Fig. 2.

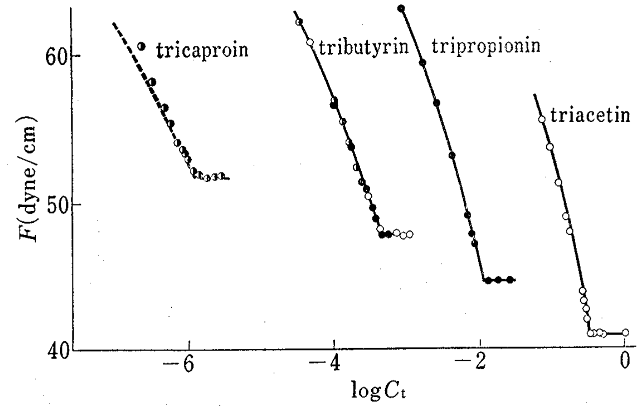

Fig. 2. The Relation between Surface Tension and the Logarithm of the Concentration of Triglyceride Solution

○ : gravimetric method, ● : volumetric method, ◑ : spread method

———, -------- calculated from the Eq. (1)

The results for other triglycerides are also shown in Fig. 2. The relation between γ and log C_t of tricaproin is convex downward below the solubility, being different from the others. This is attributed to the decrease in the concentration of the solution due to the adsorption of tricaproin at the air-water interface.

In order to correct this error, we assume the Szyszkowski's equation for the relationship between the surface tension and the true concentration, C:

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6) Beilsteins Handbuch, 2E-2, Springer-Verlag, Berlin 1942, p. 222, 249.

$$\gamma_w - \gamma = \Gamma_0 RT \ln\left(1+\frac{C}{a}\right), \tag{1}$$

where γ_w is the surface tension of the pure water, Γ_0 is the adsorbed amount of saturation, and a is a constant. Substituting the Eq. (1) into the Gibbs adsorption equation:

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln C}, \tag{2}$$

we obtain the Langmuir's equation:

$$\Gamma = \frac{\Gamma_0 \dfrac{C}{a}}{1+\dfrac{C}{a}}, \tag{3}$$

where Γ is the adsorbed amount. When S is the area of the air-water interface and V is the volume of the water examined, the total amount of tricaproin, $C_t V$ is the sum of the amount adsorbed at the air-water interface and the dissolved amount:

$$C_t V = \Gamma S + CV. \tag{4}$$

Substituting the Eqs. (1) and (3) into the Eq. (4), we obtain the Eq. (5):

$$a\left\{\exp\left(\frac{F}{\Gamma_0 RT}\right)-1\right\} = C_t - \frac{\Gamma_0 S}{V}\left\{1-\exp\left(-\frac{F}{\Gamma_0 RT}\right)\right\}. \tag{5}$$

Substituting V=100 ml and S=341.9 cm^2 in the present experiment, and the results of Fig. 2 into the Eq. (5), we can determine the appropriate values of Γ_0 and a by the trial and error method. Thus, we obtain $\Gamma_0 = 1.7\times10^{-10}$ mole/cm^2 and $a = 1.9\times10^{-8}$ mole/liter. Dotted line in Fig. 2 and the solubility of tricaproin were calculated from the Eq. (1) with the values of Γ_0 and a thus obtained and γ_s=51.9 dyne/cm, the surface tension of the saturated solution of tricaproin.

The solubility of tricaprylin was calculated similarly to that of tricaproin. Since the solubilities of triacetin, tripropionin, and tributyrin are relatively high, the correction due to the adsorption was not necessary. Solid lines in Fig. 2 for these three triglycerides were calculated from the Szyszkowski's equation (1) with the appropriate values of Γ_0 and a.

Table I summarizes the values of the solubility C_s, the constant a, the surface tension of the saturated solution γ_s, the molecular cross-sectional area of the adsorbed film in equilibrium with the saturated solution, A_s, and the area A_o which is calculated from the Eq. (6):

$$A = \frac{10^{16}}{N\Gamma} \tag{6}$$

with the value of $\Gamma = \Gamma_0$.

TABLE I. The Values of C_s, A_o, a, γ_s, and A_s of Triglycerides

Substance	C_s (mole/liter)		A_o (Å^2/molec.)	a (mole/liter)	γ_s (dyne/cm)	A_s (Å^2/molec.)
Triacetin	0.343	(0.328) 5)	39	2.67×10^{-3}	41.3	42
Tripropionin	0.0118	(0.0205) 5)	45	8.45×10^{-4}	44.9	48
Tributyrin	4.41×1^{-4}	(6.6×10^{-4}) 5) (3.88×10^{-4}) 4)	67	1.47×10^{-5}	48.1	69
Tricaproin	1.17×10^{-6}	(1.1×10^{-5}) 5)	97	1.9×10^{-8}	51.9	99
Tricaprylin	8.5×10^{-7}	(8.5×10^{-7}) 5)	127	5.1×10^{-9}	52.8	—

The relations between log C_s or log a and the total numbers of carbon atom, m in acyl groups of a triglyceride molecule are shown by the closed circles and the open circles in Fig. 3. Half-closed circles in Fig. 3 are the literature values 4,5) of the solubility. It is found that the following relationships hold in Fig. 3 except tricaprylin:

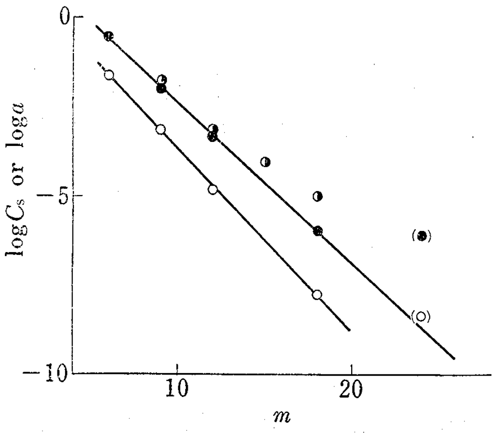

Fig. 3. The Relations between log C_s or log a and m

● : log C_s *vs* m (this work), ◑, ◓ : log C_s *vs* m (literature value),[4,5] ○ : log a *vs* m (this work)

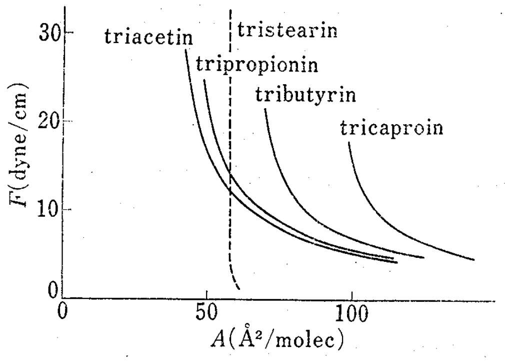

Fig. 4. The Pressure-Area Curves of Various Triglycerides

$$\log C_s = -0.482m + 2.43 \qquad (7)$$

and

$$\log a = -0.521m + 1.50. \qquad (8)$$

Pressure-Area Curve

The following equation is obtained from the Eqs. (1), (3), and (6):

$$F = \frac{10^{16}kT}{A_0} \ln\left(\frac{A}{A-A_0}\right). \qquad (9)$$

The solid lines in Fig. 4 show the surface pressure-area curves calculated from the Eq. (9) with the values of A_0 in Table I. The pressure-area curve of tristearin at 25° with technique of insoluble monolayer[7] is also shown by dashed lines for comparison.

Discussion

Solubility

The solubilities of triglycerides in the water have been measured[4,5] on the basis of Sobotka's method[8]; triglyceride is dropped into aqueous solution of Sudan IV till a liquid dropplet of triglyceride begins to appear in the aqueous solution. This method is simple but it has the disadvantages that Sudan IV may affect the dissolution equilibrium and that the adsorption at the interface is neglected. Actually, the literature values deviate from the linear relationship between log C_s and m with m increasing. The solubility of tricaprylin in this work seems to be too high, suggesting that tricaprylin is adsorbed considerably on the glass cell as well as at the air-water interface compared with the dissolved amount. The lower limit of the spread method for the solubility measurement seems to be *ca.* 10^{-6} mole/liter.

The coefficient of m in the Eq. (7) is −0.64 for *n*-aliphatic hydrocarbons, −0.60 for *n*-aliphatic fatty acids, and −0.58 for *n*-aliphatic alcohol.[9] However, it is recently said that the molecular surface area is correlated to the solubility of hydrophobic compounds in the water[10–13] more closely than the number of carbon atom or the molecular volume.[14]

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[14] C. MacAuliffe, *J. Phys. Chem.*, **70**, 1267 (1967).

Figure 5 shows the relation between log C_s and the total surface area, S_{hc}, of hydrocarbon chain in contact with the water. Open circles are data for aliphatic hydrocarbons[10] including normal, branched, and cyclic compounds, half-closed circles are those for n-aliphatic alcohols, and closed circles are those for triglycerides in this work. The values of S_{hc} for triglycerides were calculated, assuming that the water-contact area of three chains in a triglyceride molecule is simply three times as large as that of one alkyl chain (calculated from the Amidon's data).[12] But, these calculated values for triglycerides should be corrected as shown below.

The following relation is found in Fig. 5 for each homolog:

$$kT \ln C_s = -\alpha S_{hc} + \beta, \qquad (10)$$

where K is the Boltzmann constant and T is the absolute temperature. The quantity α represents the free energy per unit area of the water-hydrocarbon interface for transferring a hydrophobic group from the pure liquid to an aqueous solution[10] and seems to be independent of the kind of the hydrophilic group. The value of α is obtained to be equal to 25.5 cale/mole·Å^2 for hydrocarbons and alcohols and to 16.2 cale/mole·Å^2 for triglycerides from Fig. 5. This small value of α for triglycerides may be interpreted on the basis of intramolecular aggregation of three hydrophobic chains of a triglyceride molecule in the aqueous solution; only a fraction, 16.2/25.5, of the total surface area S_{hc} of hydrophobic chains is in contact with the water and the other is with the hydrophobic chains.

Orientation State at the Air-Water Interface

It is worth noting in Fig. 4 and Table I that A_s of triacetin is 42 Å^2/molec. This molecular area can not be explained as three hydrophobic chains exist all in the air as shown in Figs. 6b and 6c. In view of the value of A_s=42 Å^2/molec, energetical stability, and the conformation of triglyceride molecule in the crystal (described below), it is suggested that a triacetin molecule is in the orientation state as shown in Fig. 6a where the two of three chains exist in the air and the other does in the water. Bold line and fine line in Fig. 6 represent glyceryl group and acyl group, respectively. Tributyrin and tricaproin seem to be in a state of Fig. 6b and tristearin seems to be in a state of solid film as shown in Fig. 6c.

In relation to the "tune-forming" structure such as Fig. 6a, Lasson[15] and Jensen and Marbis[16] have been made clear from the throughout analysis of the crystal structures of tricaprin and trilaurin of β-form, the most stable crystal form, that the acyl chains in a triglyceride molecule correspond to the "tune-forming" structure. Moreover, Lasson has suggested that this conformation also exists in the collapsed material of a triglyceride monolayer.[17]

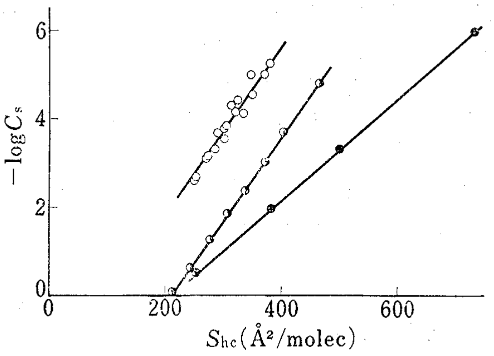

Fig. 5. The Relation between the Logarithm of the Solubility in the Water and the Total Surface Area of Hydrophobic Chain

● : triglyceride, ◑ : n-aliphatic alcohol, ○ : aliphatic hydrocarbon

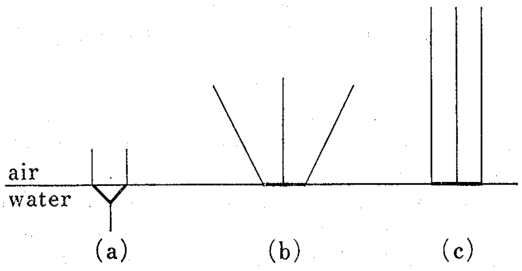

Fig. 6. Schematic Representation of Orientation State of Triglycerides at the Air-Water Interface

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[16] L.H. Jensen and A.J. Marbis, *Acta Cryst.*, **21**, 770 (1966).

[17] K. Lasson, M. Lundquist, S. Ställberg-Stenhagen, and E. Stenhagen, *J. Colloid Interface Sci.*, **29**, 268 (1969).