

Aqueous solubility and acidity constants of cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids

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Abstract Cholic acid, deoxycholic acid, chenodeoxycholic acid, and ursodeoxycholic acid were purified by a foam fractionation method. Using thermogravimetric analysis, the attached water molecule was found to be completely removed from solids of the latter three at 100°C, while cholic acid still had one water molecule of crystallization per two cholic acid molecules at that temperature. The acidity constants of the acids were accurately determined from aqueous solubility measurements at different pH values and at 15, 25, 35, and 45°C. The enthalpy change of dissolution from temperature dependence of solubility as undissociated acid monomer was much less than those of common ionic surfactants. This results from a smaller entropy increase on dissolution due to the hardly flexible hydrophobic structure of these bile acids.—Moroi, Y., M. Kitagawa, and H. Itoh. Aqueous solubility and acidity constants of cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids. *J. Lipid Res.* 1992. **33**: 49-53.

Supplementary key words bile acids • enthalpy change of dissolution

A number of papers have been published on physicochemical properties of bile acids since their physiological functions were made clear (1–15). Their vital functions are similar to ordinary surface-active agents in the sense that bile acids and their conjugates can solubilize and emulsify lipids for their transportation and absorption in vivo. They are also very sensitive to pH of living tissue, because their physiological functions are dependent on the degree of their ionization. Thus their dissociation constants should be determined as correctly as possible for insight into their physiological functions. Several papers have appeared on acidity constants of bile acids (16–19). However, these constants are not only inconsistent but also dependent on bile acid concentration, which requires determination of more reliable and more accurate acidity constants.

The acidity constant (K_a) of acids has been determined by titration, electrical conductivity, and spectrophotometric methods. These methods are, however, applicable only to acids whose aqueous

solubility is high enough for their concentration to be easily controlled. In a previous paper (20), an “isoextraction” method was introduced for pK_a determination for acids slightly soluble in water. This method is particularly valuable for studying the association equilibria in dilute solutions (21–23). A direct solubility method (24), which is a variation of the isoextraction method from a thermodynamical point of view, has an advantage over the isoextraction method in that there is no reaction of species in a coexisting excess phase of acid; only its dissolution into the aqueous phase takes place. This method is particularly useful for dibasic acids whose solubility in aqueous solution of low pH is too low to be determined precisely by normal analytical methods, for example bilirubin (25). Hence, the solubility method is very useful for studying the physicochemical solution properties of acids that cannot be investigated by other methods. This paper then aims to determine the solubility of bile acids at lower pH values and to obtain the correct acidity constants from their solubility data.

THEORY

Aqueous solubility of a chemical species depends on two intensive thermodynamic variables, on temperature at atmospheric pressure, due to two phases and two components (one chemical species and water). However, the total solubility of a slightly soluble organic acid is more sensitive to solution pH than to temperature. This is because undissociated monomeric acid is in equilibrium with the dissociated

Abbreviations: CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid.

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form, and the concentration ratio of dissociated to undissociated acids depends strongly on the solution pH. This pH-dependence is represented by the dissociation or acidity constant.

When an excess solid or liquid phase of a monobasic acid (AH) coexists with an aqueous phase, the total solubility (C_t), which is the sum of the concentration of dissociated acid (C_{A^-}) and the concentration of undissociated acid (C_{AH}), depends on the solution pH and can be expressed as

$$C_t = C_{AH} + C_{A^-} = C_{AH} + (C_{AH}K_a/\gamma_{A^-})/a_{H^+} \quad \text{Eq. 1}$$

and the acidity constant K_a is given by

$$K_a = C_{A^-}\gamma_{A^-}a_{H^+}/C_{AH} \quad \text{Eq. 2}$$

where the activity of the nonionic species is assumed to be equal to its concentration because it is extremely low. Because both C_{AH} and γ_{A^-} (activity coefficient of A^-) remain almost constant at constant temperature, pressure, and ionic strength, this equality predicts a linear relationship between C_t and $1/a_{H^+}$.

EXPERIMENTAL

Materials and methods

Cholic acid (CA) and deoxycholic acid (DCA) were of guaranteed reagent grade (Nacalai Tesque, Inc.) and chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) were from Sigma Chemical Company. Each bile acid was purified by foam fractionation of the sodium salt solution of the acid until more than 15% of original solution was removed with foam, and the concentration was below the critical micellar concentration of the sodium salt of the bile acid (Fig. 1). This purification method is very effective in eliminating hydrophobic impurities from aqueous solution by adsorption of the impurities at the air/solution interface of bubbles. After foam fractionation, the retentate was acidified by HCl solution to separate the bile acids from the solution as a precipitate, and the precipitate was washed with a large amount of distilled water and dried over P_2O_5 in a desiccator under reduced pressure. The water content of solid bile acids was determined by thermogravimetric analysis. Attached water was found to be completely removed from the solids at 100°C except for CA, which has a water of crystallization as $CA \cdot 1/2H_2O$. Removal of the water started at 120°C and was complete at 180°C followed by decomposition of the bile acid at 210°C. The weight loss between 120° and 180°C was 1.9% of the original weight at 100°C, which indicates the water of crystallization mentioned above. Hence, the elemental analysis was performed after drying the acids at 100°C for 20 min. The purity was checked by elemental

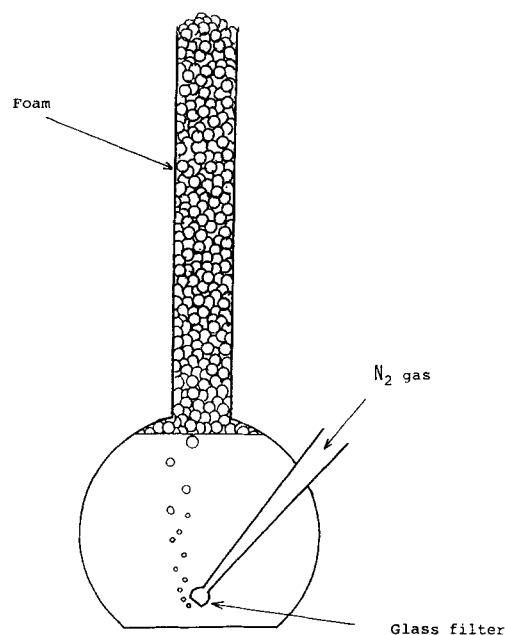


Fig. 1. Simple apparatus for foam fractionation.

analysis ($CA \cdot 1/2H_2O$: C 68.84(69.01), H 9.84(9.89); DCA: C 73.24(73.43), H 10.19(10.19); CDCA: C 73.29(73.43), H 10.27(10.19); UDCA: C 73.43(73.43), H 10.23(10.19)%), where the figures in parentheses are the theoretical values), acid-base titration, and thin-layer chromatography. A given amount of bile acid (ca. 5×10^{-4} mol) dissolved in 30 ml ethanol-water 6:4 (v/v) was titrated by NaOH solution. Purity was found to be more than 99.5, 99.3, 99.2, and 99.4% for CA, DCA, CDCA, and UDCA, respectively. Thin-layer chromatography showed only one spot for each bile acid and the R_f values were 0.110, 0.462, 0.411, and 0.493 for CA, DCA, CDCA, and UDCA, respectively. Silica-gel was used as a stationary phase and chloroform-acetone-acetic acid 7:2:1 (v/v/v) was the developing solvent.

Solubility

A suspension of bile acid powder in buffer solution was stirred by a disk rotor in a 10-ml injector tube that was kept at constant temperature for more than 10 h until solubility equilibrium was reached. The pH buffer solutions were prepared from acetic acid and sodium hydroxide keeping the ionic strength at 0.05. The temperature was kept constant at 15, 25, 35, and 45°C and controlled within $\pm 0.1^\circ C$. The filtrate was withdrawn by applying pressure upon the injector through the filter of 0.2- μm pore size (Millipore; FGLP01300) and the pH at solubility equilibrium was measured by dipping a pH electrode directly into the suspension. The concentration of total bile acid (C_t) in the filtrate was determined by fluorometry: the

filtrate was diluted with 0.1 N NaOH solution; 1 ml of the diluted filtrate in a test tube was completely evaporated in a desiccator with P_2O_5 under reduced pressure; 3 ml of sulfuric acid (ultra-fine grade) was injected into the test tube and heated at 50°C for 6 h for fluorometric analysis (26, 27). The excitation and emission wavelengths were 500 and 535, 470 and 488, 500 and 521, and 440 and 487 nm for CA, DCA, CDCA, and UDCA, respectively.

RESULTS AND DISCUSSION

A calibration curve is indispensable for the determination of the concentration of bile acids. The calibration curves used for the present study are shown in Fig. 2; there was excellent linearity for each bile acid. Plots of the total solubility of each bile acid against $1/a_{H^+}$ are illustrated in Fig. 3, Fig. 4, Fig. 5, and Fig. 6 for CA, DCA, CDCA, and UDCA, respectively. There was good linearity for each bile acid at the different temperatures. In addition, the intercept values are very close to the solubility in 0.01 N HCl which can be regarded as that of the undissociated bile acid, as can be expected from Eq. 1. These results strongly support the reliability of the solubility measurements and the validity of the theory. The value of K_a/γ_A^- can be determined by dividing the slope by the interception value of the ordinate as mentioned in the Theory section. The temperature dependence of the activity coefficient is calculated using the following equation of 1-1 electrolyte (28):

$$\log \gamma_A^- = -A |z_1 z_2| \sqrt{I} / (1 + Ba\sqrt{I}) \quad \text{Eq. 3}$$

where 5 \AA is used for ionic radius, a ; the ionic strength I is 0.05; and the numerical values in Appendix 7.1 of the above reference (28) are used for A and B in Eq. 3. The values of activity coefficients then become 0.827,

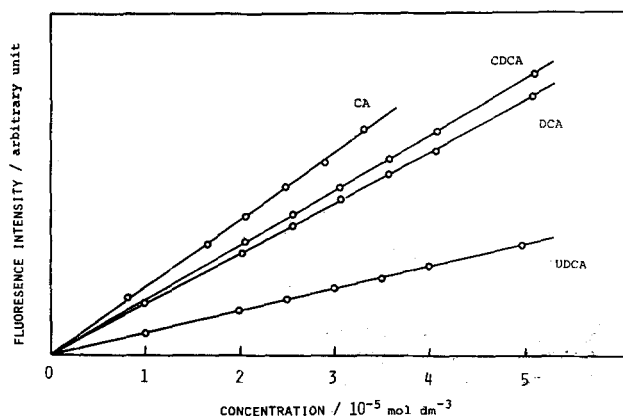


Fig. 2. Calibration curves to determine the bile acid concentration.

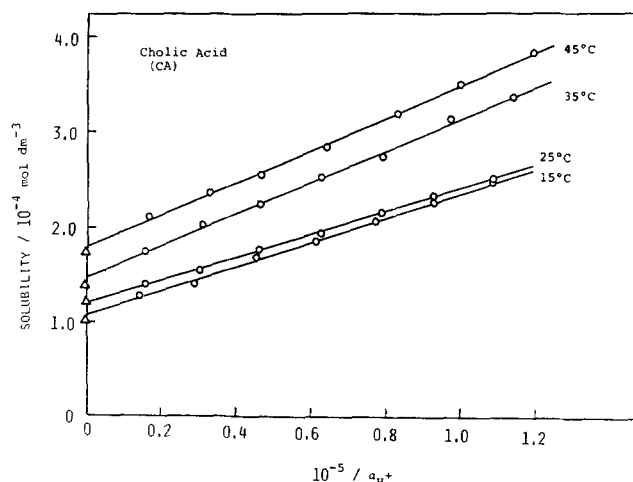


Fig. 3. Plots of total solubility against $1/a_{H^+}$ for cholic acid, where Δ is the solubility in 0.01 N HCl solution.

0.825, 0.822, and 0.819 at 15, 25, 35, and 45°C , respectively. The acidity constants thus obtained are tabulated in Table 1.

The acidity constants are quite reasonable judging from the chemical structure of the bile acids and, at the same time, quite similar to the acidity constant of *n*-valeric acid, 1.56×10^{-5} at 25°C . Solubilities of bile acids at low pH have also been reported, but they do not agree with one another [Igimi and Carey (17) and Roda and Fini (19)]. Our results on the solubilities of undissociated forms of the bile acids are closer to those by Roda and Fini (19). The fact that the acidity constants from the aqueous solubilities are quite reasonable strongly indicates that the solubility values are correct, not only at very low pH but also at higher pH.

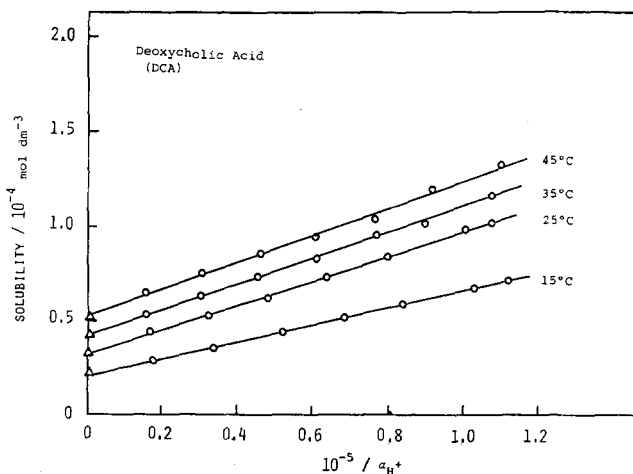


Fig. 4. Plots of total solubility against $1/a_{H^+}$ for deoxycholic acid, where Δ is the solubility in 0.01 N HCl solution.

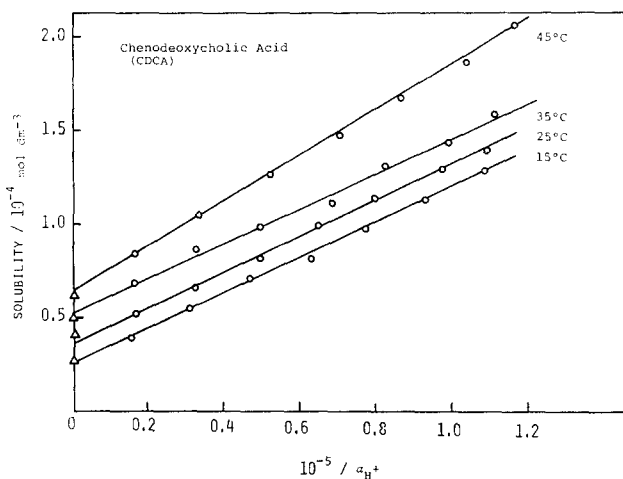


Fig. 5. Plots of total solubility against $1/a_{H^+}$ for chenodeoxycholic acid, where Δ is the solubility in 0.01 N HCl solution.

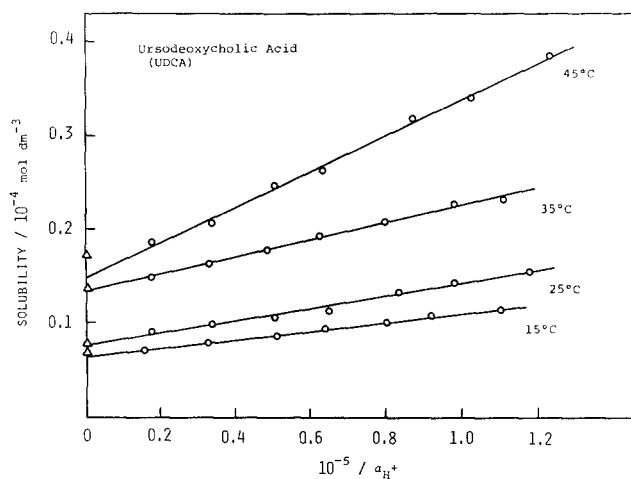


Fig. 6. Plots of total solubility against $1/a_{H^+}$ for ursodeoxycholic acid, where Δ is the solubility in 0.01 N HCl solution.

The intercept of the ordinate is the solubility of undissociated bile acids. The temperature dependence of the solubility makes it possible to evaluate the enthalpy change (Δh) of dissolution:

$$\Delta h = -R \left\{ \frac{\partial \ln S}{\partial (1/T)} \right\}_p \quad \text{Eq. 4}$$

where S is the solubility, R is the gas constant, and T is the absolute temperature. The values of enthalpy change are 16, 17, 23, and 22 kJ mol⁻¹ for CA, DCA, CDCA, and UDCA, respectively. They are very small

compared with those of conventional ionic surfactants with a flexible alkyl chain, e.g., about 50 kJ mol⁻¹ for ionic surfactants with only twelve carbons (29). This means a smaller contribution of entropy effect to dissolution, which is quite reasonable judging from the hardly flexible hydrophobic structure of the bile acids. It is also quite interesting that aqueous solubility of UDCA is very small compared with the others. The β position of the 7-OH makes a large contribution to the stability of UDCA in the crystalline state, while the

TABLE 1. Aqueous solubility and acidity constants

Bile Acids	Temp.	Aqueous Solubilities ^a	Aqueous Solubilities ^b	Acidity Constants	Igimi and Carey (Ref. 17)		Roda and Fini (Ref. 19)		Ekwall et al (Ref. 16)
					Aqueous Solubilities	Acidity Constants	Aqueous Solubilities	Acidity Constants	Acidity Constants
	°C	$10^{-4} \text{ mol} \cdot \text{dm}^{-3}$	10^{-5}	10^{-5}	$10^{-4} \text{ mol} \cdot \text{dm}^{-3}$	10^{-5}	$10^{-4} \text{ mol} \cdot \text{dm}^{-3}$	10^{-5}	10^{-5}
CA · 1/2H ₂ O	15	1.09	1.02	1.01	4.20 (20°C)		2.35 (25°C)	0.832	1.05 (20°C)
	25	1.20	1.22	0.846					
	35	1.48	1.39	0.921	4.60 (37°C)				
	45	1.80	1.73	0.772					
DCA	15	0.219	0.235	1.73	1.11 (20°C)				0.676 (20°C)
	25	0.326	0.340	1.65			0.28 (25°C)	0.955	
	35	0.436	0.435	1.29	1.14 (37°C)				
	45	0.533	0.530	1.10					
CDCA	15	0.256	0.280	3.01	2.29 (20°C)				
	25	0.355	0.410	2.24			0.27 (25°C)	0.851	
	35	0.526	0.500	1.43	2.56 (37°C)	0.0417 (37°C)			
	45	0.637	0.615	1.54					
UDCA	15	0.0657	0.0680	0.573	0.51 (20°C)				
	25	0.0780	0.0800	0.712			0.09 (25°C)	0.832	
	35	0.137	0.140	0.547	0.53 (37°C)	0.148 (37°C)			
	45	0.150	0.176	1.04					

^aBy extrapolation of solubility.

^bIn 0.01 N HCl solution.

greatest solubility of CA with three -OH groups is very much expected.

The aqueous solubility and the acidity constants of bile acids have been a matter of interest for a long time. The correct values in this paper, therefore, should contribute to further study of bile acids. Moreover, the solubility method will be useful for determination of the acidity constants of acids whose aqueous solubility is very low. ■

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