Interaction of fatty acid sodium salts with sodium deoxycholate

G. V. Shilnikov, A. P. Sarvazyan, M. Okon, J. Zakrzewska,* J. Hranisavljevic,* and D. Vucelic*

Institute of Biological Physics, USSR Academy of Science, Pushchino, Moscow Region, USSR, and Institute of General and Physical Chemistry, Belgrade University Institute of Physical Chemistry,* Faculty of Science, Belgrade University, Belgrade, Yugoslavia

Abstract Interaction of homologous fatty acids (C_3–C_{18}) with sodium deoxycholate was investigated. From NMR and ultrasonic results it was found that short chain homologues (up to C_9) do not participate in the formation of mixed micelles with sodium deoxycholate. Fatty acid homologues with longer chains (starting with C_9) form mixed micelles by "burying" hydrophobic chains in hydrophobic environment of a sodium deoxycholate micelle.—**Shilnikov, G. V., A. P. Sarvazyan, M. Okon, J. Zakrzewska, J. Hranisavljevic, and D. Vucelic.** Interaction of fatty acid sodium salts with sodium deoxycholate. *J. Lipid Res.* 1987. **28:** 1259–1262.

Supplementary key words NMR • ultrasound velocity • micelle

During fat digestion and absorption the intestinal content consists of two phases: micellar and emulsified oil phase. Absorption of dietary lipids takes place mainly from the micellar solution of bile salts containing lipolytic products of fat digestion, i.e., fatty acids and monoglycerides (1, 2). Investigations proved very early that fatty acids with chains exceeding 10 carbons are absorbed and transported through the lymphatic system while short chain fatty acids are transported via portal blood to the liver (3–5). The latter transport mechanism is especially important for newborns because of the high content of short chain fatty acids in colostrum and milk.

At present it still remains unknown whether these phenomena are due to different interactions between bile salts and fatty acids or to some other effect.

Published data on the interaction of homologous fatty acids with bile acids are quite obscure. Verkade and Meerburg (6) investigated solubility of homologous fatty acids (C_6–C_{15}) in aqueous sodium-glycocholate solution. Solubility constantly decreased with increasing chain length, with large fluctuation with C_{10} and C_{12} acids. Also, solubility rather depended on the molar ratio of bile acid to fatty acid component than on the concentration.

Phase equilibria for a three-component system: sodium taurodeoxycholate–fatty acid (sodium salt)–water, at pH 12, has been investigated (7). For the three fatty acids (C_{18}, C_{16}, C_{14}) at 20°C, critical micellar equilibria were below molar partial ratios: 0.7:0.3; 0.6:0.4; and 0.4:0.6, respectively. Interpretations of solubilities for long chain fatty acids in bile salt solutions based on these data must be made with caution. Kinetic effects are different due to slow equilibration with long chain fatty acids and may lead to erroneous solubilities.

From phase equilibria of the sodium cholate–sodium oleate–water system, Small (8) has found that mixed micelles are built over a wide range of molar ratios. In the work of Benzonana (9) the oleate–deoxycholate system was examined. According to this author, mixed micelles are characterized by lower CMC values (2.5–3.0 mM) while fatty acid seems to be "buried" in deoxycholate micelles.

MATERIALS AND METHODS

Sodium deoxycholate (NaDC) and n-alkyl carboxylates (Sigma) were used without further purification. To the 36 mM deoxycholate solution, fatty acid salts were added up to corresponding concentrations with intensive stirring. NaOH was added up to pH 11. A new, fresh sample was prepared for each experimental point.

At pH 11, long chain fatty acids dissolved readily at room temperature and neutral soaps were formed in the whole range of the fatty acids that were studied.

Densities were measured on a Paar DMA 60 differential microdensimeter with a DMA 602 measurement cell and a DMA 602 HT resistance cell. Under special conditions, to prevent evaporation, samples were thermostated at 25°C with an absolute precision of ±0.005°C. Precision of density measurements was 1.5×10^{-6} g/cm^3.

Abbreviations: CMC, critical micellar composition, NaDC, sodium deoxycholate.

Velocity of ultrasonic waves was measured on a home-made interferometer with constant length, described elsewhere (10). Differential operation mode enabled measurements with an error of 10^{-4}%.

Apparent molal adiabatic compressibilities and apparent molal volumes were calculated from well known equations.

$$\phi_k = B_o \left(\frac{M}{\varrho_o} - 2 \times 10^3 \left(\frac{v - v_o}{v_o c} + \frac{\varrho - \varrho_o}{\varrho_o c}\right)\right) \quad \textit{Eq. 1)}$$

$$\phi_v = \frac{M}{\varrho} - 10^3 \frac{\varrho - \varrho_o}{\varrho c} \quad \textit{Eq. 2)}$$

where: B_o is the solvent adiabatic compressibility; M is the molecular mass of dissolved substance; ϱ and ϱ_o, respectively, are solution and solvent densities; v and v_o are ultrasonic velocities in the solution and solvent, respectively; and c is the molal concentration.

Proton spectra were obtained on a Bruker WH-400 spectrometer with an Aspect 2000 computer. The experimental conditions were: number of scans 32–1048, aquisition time 2.3 sec, delay time 10 sec, pulse width 5 sec (60°), digital resolution 0.5 Hz/point, no exponential multiplication. In NMR experiments, fatty acid carboxylate and deoxycholate concentrations were kept at constant values of 3 mg/ml and 15 mg/ml (36 mM).

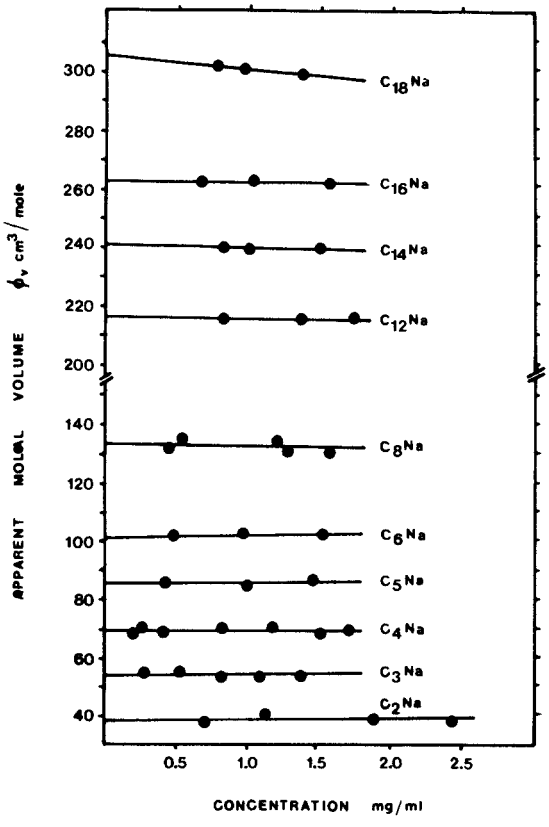

Fig. 1. Concentration dependence of apparent molal volumes of fatty acids in micellar solution of sodium deoxycholate.

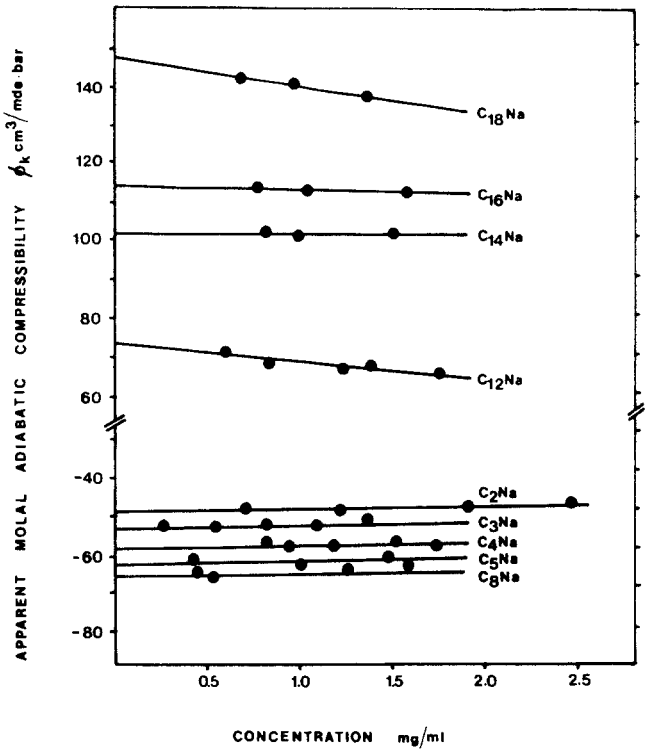

Fig. 2. Concentration dependence of apparent molal compressibilities of fatty acids in micellar solution of sodium deoxycholate.

RESULTS AND DISCUSSION

Fig. 1 and **Fig. 2** show concentration dependencies of apparent molal volumes and compressibilities of fatty acid sodium salts in 36 mM sodium deoxycholate solution. Linear dependence over a sufficiently wide range of concentrations enables extrapolation at zero concentration. The obtained values are presented in **Table 1** (columns 2 and 6), and in **Fig. 3** for compressibilities. Also, the literature results for homologous fatty acid sodium salts in aqueous solution (11, 12) and in solubilized states in Na decanoate solution (13) are displayed in Table 1. Apparent molal volumes of homologues in aqueous solutions, without and with micelle formation, are given in columns 3 and 4, respectively. Column 5 presents apparent molal volumes in Na decanoate. Corresponding apparent molal compressibilities are given in columns 7,8, and 9. Results for C_{16} and C_{18} fatty acids are critical due to their low solubility. Calculated values are shown in parentheses. (Calculation is based on the logarithmic approximation from the homologous series.)

TABLE 1. Apparent molal volumes and compressibilities of sodium n-alkylcarboxylates at 25°C

Homologues	ϕ_v° cm³/mol				ϕ_k° cm³/mol · bar			
	In NaDC, pH 11, 15 mg/ml	In H_2O	In H_2O (Micelle)	In $C_{10}Na$ 1.0 M	In NaDC, pH 11, 15 mg/ml	In H_2O	In H_2O (Micelle)	In $C_{10}Na$ 1.0 M
C_2Na	38.9	39.3[a]			− 48.6	− 60.0		
C_3Na	54.1	53.7[a]			− 56.8	− 67.5		
C_4Na	69.8	69.3[a]	69.2[b]	75.8[b]	− 58.8	− 71.2[b]		− 29.0[b]
C_5Na	86.0	85.1[a]	85.2[b]	91.7[b]	− 61.6	− 73.2[b]		− 26.4[b]
C_6Na	102.2	100.6[a]	101.1[b]	107.6[b]	− 63.5	− 75.2[b]		− 16.0[b]
C_7Na	115.6	115.8[a]	116.8[b]	126.9[c] 124.3[b]	− 68.3	− 71.0[b]	39[c]	1.4[b]
C_8Na	132.8		132.4[b]	142.4[c] 141.4[b]	− 66.1	− 74.0[b]	39[c]	19.5[b]
C_9Na	151.7		148.2[b]	158.7[c] 158.2[b]	− 52.8	− 75.0[b]	45[c]	37.2[b]
$C_{10}Na$	173.3		164.2[b]	175.0[c] 176.3[b]	− 19.9	− 78.0[b]	51[c]	55.6[b]
$C_{11}Na$	186.6		179.9[b]	190.9[c] 193.3[b]	+ 57.0	− 77.0[b]	59[c]	69.5[b]
$C_{12}Na$	216.7		195.5[b]	207.3[c] 211.5[b]	+ 87.9	− 81.0[b]	70[c]	85.0[b]
$C_{14}Na$	240.5		226.4[b]	240.8[c]	+ 103.0	− 82.0[b]	89[c]	
$C_{16}Na$	263.0(260)				115.0(134)			
$C_{18}Na$	305.5(276)				150.0(170)			
Column	2	3	4	5	6	7	8	9
Estimated standard deviation	± 0.4	± 0.2	± 0.2	± 0.2		± 2		± 2

[a]Reference 11.
[b]Reference 13.
[c]Reference 12.

It is evident that low molecular homologues in micellar solution of deoxycholate have volumes close to those obtained for aqueous solutions without micelle formation (columns 2 and 3). This also indicates that low molecular homologues do not form mixed micelles with NaDC within the range of fatty acid concentrations investigated. With increasing length of the hydrophobic chain, starting with C_9, apparent molal volume also increases, reaching in the case of C_{10} and C_{11} the values obtained for volumes in aqueous micellar solution (columns 2 and 4). Considering that in all cases, except for C_{16} and C_{18}, fatty acid concentrations were significantly lower than their own CMCs (11, 12), this increase might be ascribed only to incorporation of fatty acid molecules into deoxycholate micelles.

Compressibility results shown in Table 1 and Fig. 3 are in full agreement with the results for apparent molal volumes. As can be seen in Fig. 3, fatty acid homologues (up to C_8) in deoxycholate have negative compressibilities, very close to the values for nonmicellar homologues in aqueous solutions. In micellar aqueous solution, all the homologues, including C_7 and C_8, have positive values (Fig. 3, upper curve). Starting with C_9, compressibility in deoxycholate solution increases, so that C_{11} and C_{12} have positive values identical with C_{11} and C_{12} compressibilities in aqueous micellar solutions. Molal compressibility of C_{14} exceeds micellar values and approaches those obtained in sodium decanoate. The same is true for C_{16} and C_{18} (especially for corrected values). Very close compressibilities obtained in $C_{10}Na$ and in NaDC positively indicate that fatty acid molecules are "buried" in a hydrophobic environment of deoxycholate micelles.

Corresponding proton spectra of some fatty acid homologues in 36 mM deoxycholate under the same conditions

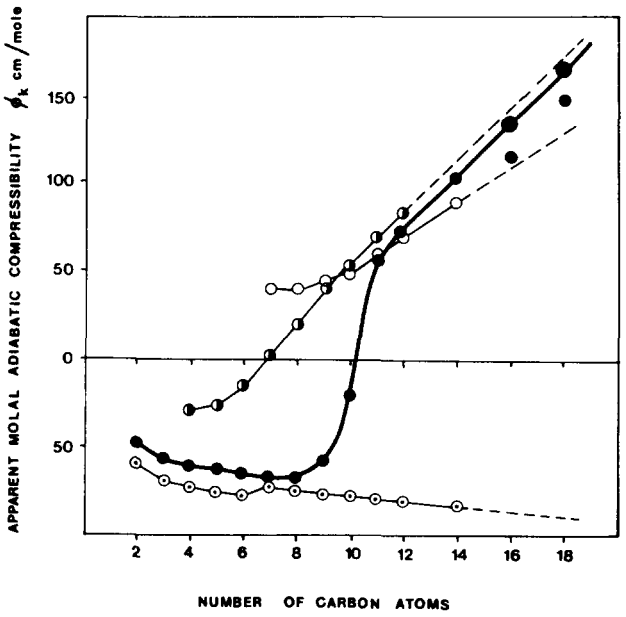

Fig. 3. Apparent adiabatic compressibility of homologous fatty acids in: (●) micellar sodium deoxycholate solution (double ring points, values in brackets from Table I); (○) micellar aqueous solutions (ref. 12); (⊙) nonmicellar aqueous solutions (ref. 13); (◑) micellar sodium decanoate solution (ref. 13).

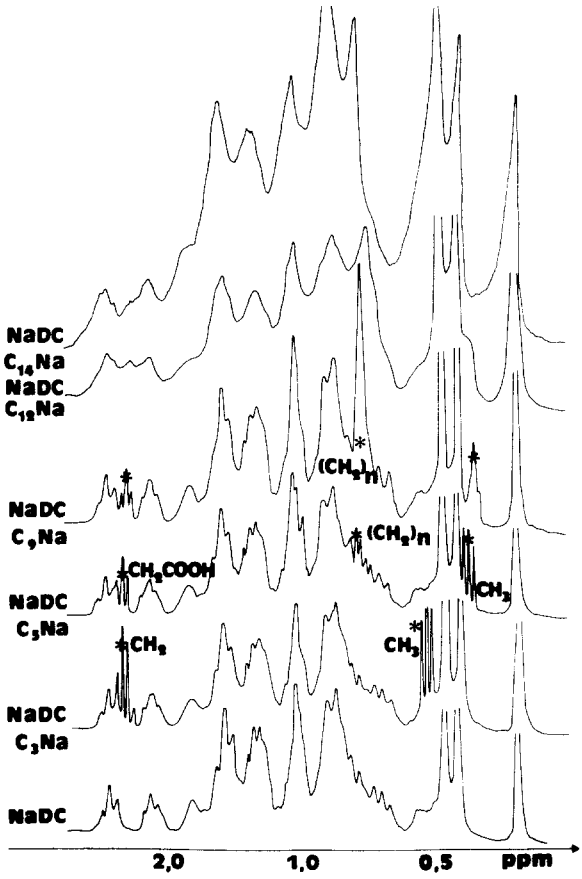

Fig. 4. ^{1}H NMR spectra of some fatty acids in micellar sodium deoxycholate solution.

as for ultrasonic measurements are given in **Fig. 4**. No changes occur in the spectra up to C_9. Sharp lines originate from fatty acid, and the whole spectrum is simply a sum of independent fatty acid and deoxycholate spectra. However, fatty acid lines begin to broaden from C_9 so that C_{12} and C_{14} have already lost their structure and the basic deoxycholate spectrum becomes significantly broader. These results correlate with ultrasound data, proving, for short chain homologues, absence of any significant interaction that might lead to mixed micelle formation. Formation of mixed micelles is not followed by equal broadening of all groups in fatty acids. Broadening of the lines belonging to CH_3- and $(CH_2\text{-}CH_2)_n$ is more significant than that of the CH_2- group next to carboxyl. This is the consequence of gradual incorporation of a hydrophobic fatty acid chain in hydrophobic environment of sodium deoxycholate micelles.

The results enable elucidation of different fatty acid transport routes. Long chain acids (exceeding C_{10}) are transported from the intestinal lumen into epithelial cells as mixed micelles with bile acids, while short chain acid (below C_9) transport is nearly independent of the presence of bile acid micelles. At present it is not clear whether such differences can influence later intracellular events including different transport routes via lymph or blood.

Manuscript received 18 September 1986 and in revised form 23 March 1987.

REFERENCES

1. Hofmann, A. F., and B. Borgström. 1962. Physico-chemical state of lipids in intestinal content during their digestion and absorption. *Fed. Proc.* **21:** 43–50.
2. Hofmann, A. F., and B. Borgström. 1964. The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. *J. Clin. Invest.* **43:** 247–257.
3. Bloom, B., I. L. Chaikoff, and W. O. Reinhardt. 1951. Intestinal lymph as pathway for transport of absorbed fatty acids of different chain length. *Am. J. Physiol.* **166:** 451–455.
4. Kiyasu, J. Y., B. Bloom, and I. L. Chaikoff. 1952. The portal transport of absorbed fatty acids. *J. Biol. Chem.* **199:** 415–419.
5. Bollman, J. L., E. V. Flock, J. C. Cain, and J. H. Grindly. 1950. Lipides of lymph following feeding of fat: an experimental study. *Am. J. Physiol.* **163:** 41–47.
6. Verkade, P. E., and W. Meerburg. 1955. The solubility of some normal saturated fatty acids in an aqueous sodium glycocholate solution. *Recl. Trav. Chim. Pays-Bas.* **74:** 263–270.
7. Hofmann, A. F., and H. S. Mekhjian. 1973. Bile acids and the intestinal absorption of fat and electrolytes in health and disease. *In* The Bile Acids, Chemistry, Physiology and Metabolism. P. P. Nair and D. Kritchevsky, editors. Plenum Press, New York. 103–149.
8. Small, D. M. 1968. A classification of biological lipids based upon their interaction in aqueous systems. *J. Am. Oil Chem. Soc.* **45:** 108–119.
9. Benzonana, G. 1969. Study of bile salt micelles: properties of mixed oleate-deoxycholate solutions at pH 9.0. *Biochim. Biophys. Acta.* **176:** 836–848.
10. Sarvazyan, A. P. 1982. Development of methods of precise ultrasonic measurements in small volumes of liquids. *Ultrasonics.* **20:** 151–154.
11. Sakurai, M. 1973. Apparent molal volumes of some organic electrolytes in a dilute aqueous solution at 5,25 and 45°C. *Bull. Chem. Soc. Japan.* **46:** 1596–1602.
12. Vikingstad, E., A. Skauge, and H. Høiland. 1978. Partial molal volumes and compressibilities of the homologous series of sodium alkylcarboxylates, R_6COONa-$R_{13}COONa$, in aqueous solution. *J. Colloid Interface Sci.* **66:** 240–246.
13. Vikingstad, E. 1980. Partial molal volumes and compressibilities of sodium-alkylcarboxylates, disodium-alkylcarboxylates, and n-diols in micellar solutions of sodium-alkylcarboxylates at 25°C. *J. Colloid Interface Sci.* **73:** 500–507.