Studies on Bile Salt Solutions. Part 2. Acid Catalyzed Hydrolysis of Trimethyl Orthobenzoate in Solutions of Glycine and Taurine Conjugated Bile Salts

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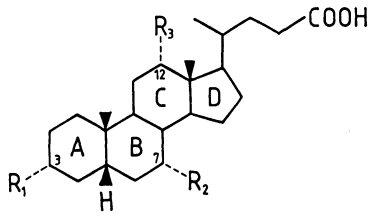
Synopsis. The reactivity of trimethyl orthobenzoate has been studied, at 310.5 K, in solutions of the glycine and taurine conjugates of the cholanic acids. The rate-concentration profiles are characterized by peaks and troughs which coincide with discontinuities in the slopes of the surface tension-concentration profiles.

Trimethyl orthobenzoate **1** is one of the few substrates whose acid catalyzed hydrolysis is subject to catalysis by anionic surfactants.[1-6] The kinetic effects are accentuated when the hydrophobic interactions between **1** and surfactants are accentuated. Increasing the hydrocarbon chain length of sodium alkyl sulfates causes a more dramatic increase in rate.[1]

Bile salts **2** are important surfactants in the biological system and they offer an attractive opportunity for



correlating chemical composition with micellar properties in a series of molecules differing only slightly in their chemical structure. It is therefore surprising that only five publications can be identified[7-11] which report kinetic investigations by these amphiphiles in aqueous solution in the absence of enzymes.

We now report on the reactivity of **1** in solutions of the taurine and glycine conjugates of trihydroxy- and dihydroxy cholates; taurocholate, TC, and glycocholate, GC, ($R_1=R_2=R_3=OH$); taurochenodeoxycholate, TCDC, and glycochenodeoxycholate, GCDC, ($R_1=R_2=OH$, $R_3=H$); and taurodeoxycholate, TDC, and glycodeoxycholate, GDC, ($R_1=R_3=OH$, $R_2=H$). In contrast to the literature studies[7-11] on reactivity in bile salt solutions which have concentrated, for the most part, on concentrations greater than CMC and in solutions of alkaline pH where all the bile salts are converted to the anionic form, we have used solutions of pH<7. Inevitably this has placed a relatively low limit on the maximum concentration able to be solubilized so that many of our data refer to concentrations well below CMC.

Experimental

Materials. The bile salts and bile acids were Sigma products. Their purity was monitored by TLC analysis using the solvent system of acetone: dibutyl ether in the ratio 3:7 and development in an iodine chamber. Acetic acid used for preparation of buffer solutions was freshly distilled. All water was triply distilled and the pH of each solution was measured, using as a standard BDH buffer for blood pH determination with pH 6.840 at 311 K, with a Radiometer PHM 4d pH meter and a dual walled sample holder held at 310.5 K by means of a Grant thermocirculator. Trimethyl orthobenzoate was prepared according to the method of McElvain and Venerable[14] (Bp 387—388 K at 25 mmHg).

Measurement of Rate Constants of Hydrolysis. The hydrolysis of **1** was followed by measuring the appearance of methyl benzoate at 228 nm. (Under acidic conditions methyl benzoate is converted slowly to benzoic acid but the extinction coefficients of the intermediate and final products of hydrolysis are identical at 228 nm.) The reaction was initiated by injecting 8—20 μl of a 5.8×10^{-4} mol dm^{-3} solution of **1** in absolute ethanol into the contents of the reaction cell which had previously been brought to equilibrium temperature (310.5 K) in the cell compartment of a Cary 219 recording Spectrophotometer. Pseudo first order rate constants were calculated from the absorbance data on a Burroughs B6700 or Commodore 3032 minicomputer. Rate data were duplicated and showed a reproducibility of $<+2\%$.

Results and Discussion

The pseudo first order rate constant, k_ψ, for hydrolysis of **1** in acid solution is linearly dependent on the hydronium ion concentration. At 310.5 K in 0.03 mol dm^{-3} sodium acetate buffer the values of k_ψ are 2.07×10^{-2} and 0.070×10^{-2} s^{-1} at pH values of 4.07 and 5.47 respectively. All rate measurements described in this investigation were made within this pH range and using this buffer medium and temperature.

Figures 1—3 show the values of k_ψ for hydrolysis of **1** in the bile salt solutions. Superimposed on all the rate concentration profiles are plots of the surface tension values of these same bile salt solutions measured under identical conditions of pH and temperature. Determination of these surface tension values has been reported separately.[12]

In contrast to the relatively simple multiphasic rate-concentration profile reported for hydrolysis of **1** in sodium alkyl sulfate solutions[1] the profiles presented here are quite complicated, being characterized by plateaux, peaks and troughs. Each change in slope in the rate-profile is matched by a discontinuity in the slope of the corresponding surface tension-profile.

We were able to explain the surface tension data by use of a multi-equilibrium model involving the step-wise aggregation of monomers or small oligomers. We believe that micelle formation of bile salts passes through several successive distinct stages, each one being characterized by constant properties of the solute. Our rate data support this postulate, for it seems that, at the pH values we have used, the association process in bile salts is one of multiple association rather than a monomer$\rightleftharpoons$*n-mer* equilibrium and that each of the small aggregates has its own characteristic binding constant leading to an individual kinetic identity, as well as an unique ability to modify the surface tension properties of the solvent.

However, the changes we have observed are small. The maximum percentage change observed was

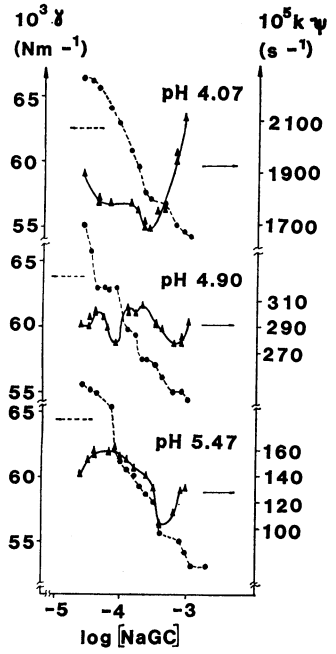


Fig. 1.

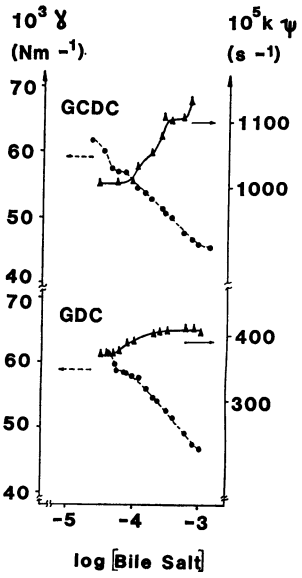


Fig. 2.

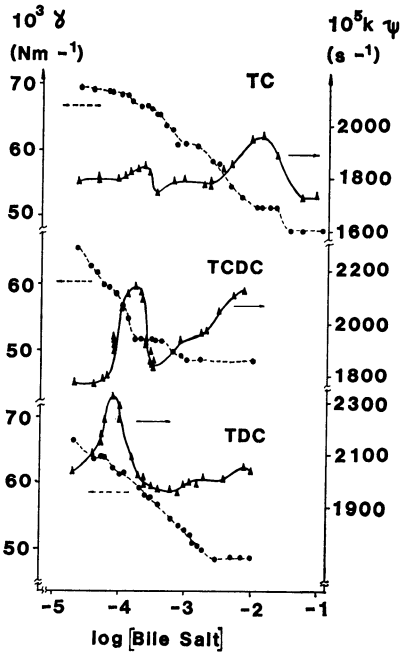


Fig. 3.

Fig. 1—3. The pseudo first order rate constant of hydrolysis, k_ψ, of trimethyl orthobenzoate (▲ and solid line) and the equilibrium surface tension values, γ, (● and broken line) in solutions of bile salts in 0.03 mol dm^{-3} acetate buffer at 310.5 K. Fig. 1, sodium glycochoate at pH values of 4.07, 4.90, and 5.47 (43%, 10%, and 3% protonation respectively); Fig. 2, sodium glycochenodeoxycholate at pH 4.34 and sodium glycodeoxycholate at pH 4.77 (both 43% protonated); Fig. 3, sodium taurocholate, sodium taurochenodeoxycholate and sodium taurodeoxycholate at pH 4.09.

+100% for GC at pH 5.47 and TDC was the only other salt to show a significant increase in rate (+22%) at its maximum value. At the minimum values of k_ψ all the salts except TDC were slightly inhibitory (the maximum inhibition of −16% was observed for GC at pH 4.07).

The pK_a values for GC, GDC and GCDC range from 3.8—4.1, 4.7—4.8, and 4.2—4.3 respectively[13] while those for the taurine conjugates have pK_a values less than 2. This means that a substantial portion of the glycine conjugates is present in the undissociated form at the pH values we have used (43%, 10%, and 3% protonation at pH values of 4.07, 4.90, and 5.47 respectively) while the taurine conjugates will be 100% deprotonated. Increasing the pH from 4.07 to 4.47 increases the value of k_2 (min) for GC (where $k_2=k_\psi/[H_3O^+]$ is the second order rate constant) from 198 to 301 dm^3 mol^{-1} s^{-1} and the value of k_2 (max) from 248 to 472 dm^3 mol^{-1} s^{-1}. Thus the presence of the acid form of the bile salt inhibits the rate of hydrolysis. The bile acids are sparingly soluble in water and cannot themselves form micelles, but they are solubilized as "mixed" micelles of anion and acid. Solubilization of free acid in the mixed micelles will lessen the hydrophobic environment and thereby inhibit the catalytic efficiency for hydrolysis of **1**.

The hydrophobicity of the bile salts increases in order TC<TCDC<TDC and this is also the order of increasing values of k_2 (max), *i.e.* the more hydrophobic the surfactant, the more of **1** is in the micellar phase and the greater is the catalysis. However in these micellar systems the aggregation number, the pK_a value and the binding constant of substrate to micelle will depend very delicately upon the reaction conditions and these will also affect the rate of hydrolysis. In the light of these factors further discussion of the small rate enhancements does not seem warranted.

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