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SELECTIVE ACETYLATION OF STEROLS IN IMIDAZOLE-FUNCTIONALIZED SURFACTANT VESICLES

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Summary. In imidazole-functionalized surfactant coaggregates, acetyl groups are stereoselectively transferred from p-nitrophenyl acetate to 3β vs 3α-cholestanol, and regioselectively transferred to 3β vs 6β-cholestanol.

There have been studies of stereoselectivity attending the degradative esterolyses of chiral substrates in micellar or vesicular aggregates,[1,2] but there have been relatively few reports of *synthetic* selective reactions in these media. Apposite examples include peptide coupling reactions in the interior water pools of reverse micelles,[3] the oxymercuration of dienes in (aqueous) anionic micelles,[4a] the hydroboration of enones in cationic micelles,[4b] and the epoxidation or hydroxylation of sterols in steroidal porphyrin/phospholipid/substrate coaggregates.[5]

Recently, we found that coaggregates of cholesterol and the N,N-dihexadecylcholine ester of imidazole-4-carboxylic acid (**1**)[6] cleaved p-nitrophenyl acetate (PNPA) with the transient formation of N-acetylimidazole surfactant **1-Ac**, followed by transfer of the acetyl fragment to the cholesterol.[7] The efficiency of this intravesicular, imidazole-mediated transacylation reaction depended on the fluidity within the coaggregate, and was higher in the "liquid crystalline" coaggregate than in the more ordered "gel" phase.

Here we report on the stereo- and regioselectivity of related transacylations mediated by **1** coaggregated with various sterol substrates. We find strongly expressed, microviscosity-dependent stereoselection between 3β and 3α-cholestanol, and pronounced regioselectivity between 3β and 6β-cholestanol. These selective transacylations can be rationalized by a model in which the long axis of the sterol is aligned parallel to the long alkyl chains of the surfactant molecules within the coaggregates.

The sterols used were the "standard" substrate, 3β-cholestanol (**2a**), its 3α-OH epimer (**2b**), their 5β-H coprostanol stereoisomers, **2c** and **2d**, the position isomer, 5α-cholestan-6β-ol[8] (not illustrated), and cholesterol (3β-hydroxy-Δ^5-cholestene). Ternary coaggregates of **1**, **2a**, and a second sterol were generated by rapid injection[9] of a dilute ethanolic solution (160 μl, $\leq$ 0.05 M) of all 3 components into 4 ml of rapidly stirred 0.01 M tris buffer, μ = 0.01 (KCl) at 55°C. Additional stirring for 1 min at 50-55°C, followed by slow cooling to 25°C gave optically clear solutions.

Competitive acetylations were initiated by addition to these coaggregates of a 5-fold molar excess (relative to total sterol) of PNPA. Reaction solutions were maintained for 2 h at either 5° or 45°C, then quenched with 1 drop of 10% aq. HCl and lyophilized to dryness. Residues were extracted with 2 x 1 ml of hexane; steroids were recovered by evaporation,

dissolved in 20 μl of $CHCl_3$, and analyzed by gc.[10] The efficiencies of the acetylations were such that conversions to the product sterol acetates[11] were <15%, permitting simple computations of the sterol relative reactivities from the molar gc product ratio[10] and the initial molar sterol ratio. Appropriate controls demonstrated that the reaction and isolation procedures neither selectivity destroyed nor fractionated a typical product mixture. For comparisons to the aggregate reactions, competitive acylations were also carried out under homogeneous conditions, using Ac_2O/pyridine at 25°C. The data appear in Table I.

Ethanol-injected holovesicles of 1 are multilamellar, 2000-2300 Å aggregates, with a major phase transition (T_c) at 43.2°C.[6] Although the sharp phase transition is suppressed by sterols,[7] the ternary coaggregates of 1 are in a relatively fluid phase at 45° and in a much more ordered phase at 5°C. This is shown by the observed microviscosities (η)[12] of various blends of 1, 2a, and 2b (Table I, Cases 1-6), where η is always higher at 5° than at 45°C.

Paralleling the effect of temperature on coaggregte microviscosity is the enhancement of stereoselectivity favoring the vesicular acetylation of 2a over 2b. In each of the matched pairs of runs (Cases 1-6), the 2a/2b relative reactivity is enhanced at 5°C. Indeed, there is a good linear correlation ($\underline{r}$ = 0.97, significant at the 99% confidence level) between $RT\ln\underline{k}_{rel}$ and η for cases 1-6, suggesting that the magnitude of $\Delta\Delta G^{\ddagger}$ for acetyl transfer from vesicular surfactant 1-Ac to either 2a or 2b depends directly on the microviscosity (or order) within the coaggregates' bilayers. Interestingly, the vesicular 2a/2b stereoselectivity straddles that of the homogeneous Ac_2O/pyridine acetylation ($\underline{k}_{rel}$ = 0.26) and can be either higher or lower, depending on η. Case 1 represents an 8-fold greater stereoselectivity for the vesicular vs. the solution acetylation.[13]

A/B-*trans* (5α) sterols, such as 2a or 2b are relatively flat molecules (*cf.*, 3), and fit well into the vesicular bilayers, with the steroidal long axis parallel to the surfactant alkyl chains, and the 3-OH groups at the aqueous/surfactant head group interface.[14] With 3β-cholestanol (2a = 3a), the OH group is directed along the sterol axis; it will point toward the acetylimidazole head group of a neighboring 1-Ac surfactant in an ordered aggregate, and should readily participate in acetyl transfer. In contrast, the 3α-OH group of 2b(3b) is normal to this sterol's long axis and it should be less accessible to acetylation by 1-Ac in a "rigid" bilayer matrix. This model also accounts for the comparable reactivity of cholesterol and 2a (Table I, Cases 15 and 16). Like 2a, cholesterol is a "flat" sterol with a 3β-OH; it should enjoy a similar competitive advantage in vesicular transacetylation.[15]

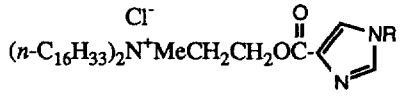
The enhanced regioselectivity exhibited in the vesicular acetylation of 2a vs. 5α-cholestan-6β-ol (Cases 13 and 14) is also rationalized. The intrinsically greater reactivity of 2a (~8-fold with Ac_2O) is augmented more than 10 times in the coaggregates with 1. If the sterols must align their long axes parallel to the hydrocarbon chains in the bilayers, then a 6β-OH substituent will be kept away from the surfactant head groups, and strongly disadvantaged (relative to a 3β-OH) in competitive acetylation by 1-Ac.

The relative reactivities of the A/B-cis (5β) coprostanols 4 (2c and 2d) are less readily understood. Compared to the homogeneous Ac_2O acetylation ($\underline{k}_{rel}$ = 0.27), sterol 2c gains in reactivity under the vesicular conditions (Cases 7 and 8), although it remains less reactive

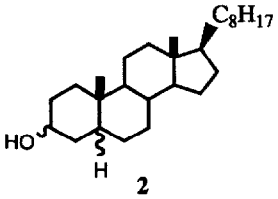
Table I. Reactivities of Sterols in Acetylation, Relative to 3β-Cholestanol[a]

Case	Sterol	[sterol] / [2a]	[1][b] / [tot. sterols]	Temp, °C	η, cP[c]	k_{rel}[d] Vesicle	k_{rel}[d] Ac_2O/pyr, 25°C
1	**2b**	1:1	4:1	5	184	0.033	0.26
2	**2b**	1:1	4:1	45	63	0.23	
3	**2b**	4:1	4:1	5	122	0.053	
4	**2b**	4:1	4:1	45	16	0.48	
5	**2b**	4:1	9:1	5	91	0.19	
6	**2b**	4:1	9:1	45	14	0.58[e]	
7	**2c**	1:1	4:1	5		0.66	0.27
8	**2c**	1:1	4:1	45		0.51	
9	**2d**	1:1	4:1	5		0.78	0.80
10	**2d**	1:1	4:1	45		0.91	
11	**2d**	4:1	4:1	5		2.8[f]	
12	**2d**	4:1	4:1	45		2.5	
13	6β-chol[g]	1:1	4:1	5		<0.01	0.12
14	6β-chol	1:1	4:1	45		<0.01	
15	cholesterol	1:1	4:1	5		1.3	1.0
16	cholesterol	1:1	4:1	45		1.3	

[a]All reactivities are relative to 2a and were determined in direct competition experiments. The reactivities are adjusted to a competitive sterol mole ratio of 1.0. [b]Total sterols were 0.5 mM in 2.0 ml of buffer solution with [1] = 2.0 - 4.5 mM as indicated. [c]Microviscosity as determined from the fluorescence of coaggregated 1,5-diphenylhexatriene; see ref. 12. [d]Reproducibilities were generally ≤ ±10%, unless otherwise noted. [e]Reproducibility was ±22%. [f]Reproducibility was ±14%. [g]5α-Cholestan-6β-ol.

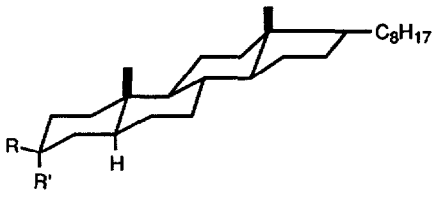


1, R = H; **1-Ac**, R = $COCH_3$



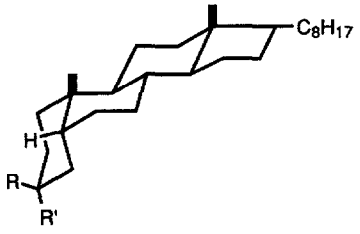
2

2a; 3β-OH, 5α-H
2b; 3α-OH, 5α-H
2c; 3β-OH, 5β-H
2d; 3α-OH, 5β-H



3a (=**2a**); R=OH, R'=H

3b (=**2b**); R=H, R'=OH



4a (=**2c**); R=OH, R'=H

4b (=**2d**); R=H, R'=OH

than sterol **2a**. The 3α-coprostanol (**4b** = **2d**) is comparably reactive under either homogeneous or vesicular conditions (Cases 9 and 10), but its reactivity is augmented (and even exceeds that of **2a**) in ternary coaggregates that are rich in **2d**; cf., Cases 11 and 12. The k_{rel} values thus depend on the precise composition of the ternary coaggregates, reflecting cooperative interactions that are presently ill-defined.

Coprostanols **4a** and **4b** are not flat; they do not pack as readily into the bilayers and may be less stringently oriented than cholestanols **3a** and **3b**.[14] Additionally, the coprostanols may be expected to exert different effects on the vesicular phase transitions and microviscosities than the cholestanols.[14] Accordingly, the relative reactivities of the coprostanols do not fit the simple model advanced for the cholestanols. We plan additional studies of chemoselectivity in sterol-surfactant coaggregates.

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References and Notes

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(9) S. Batzri and E. D. Korn, Biochim. Biophys. Acta, **298**, 1015 (1973).
(10) We used a Varian model 3700 instrument fitted with a 12 m, 0.2 mm diameter, 0.25 μm-layer BP-10 capillary column, operating at 260°C, with 0.30 ml/min of N_2 carrier gas. The flame ionization detector was calibrated with authentic sterol acetates.
(11) Authentic sterol acetates were either available commercially or were prepared by Ac_2O/pyridine acetylation of the sterols. The purities of all sterols and sterol acetates were ascertained by gc.[10]
(12) η was estimated from the measured excited state lifetimes and fluorescent polarization of an included 1,6-diphenylhexatriene probe (e.g., for Cases 1-4, [**1**] = 5 x 10^{-5} M, [sterols] = 1.25 x 10^{-5} M, [probe] = 5 x 10^{-7} M; see R. A. Moss and S. Swarup, J. Org. Chem., **53**, 5860 (1988), and references therein. Note that the data suggest augmentation of η with an increase in [**2a**], not [**2b**]. η for holovesicular **1** is only 15 cP at 45°C, similar to η for ternary coaggregates rich in **2b**; see Cases 4 or 6 vs 2.
(13) The *micellar* reagent related to **1** and **1-Ac** [in which one hexadecyl group is replaced by methyl] is not as supportive of stereoselectivity as the vesicular coaggregates: k_{rel} (5°C) = 0.57 for 20:1 surfactant/(4:1 **2b**/**2a**). Note that an excess of micellar reagent was required for complete sterol solubilization.
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