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IMIDAZOLE MEDIATED ACYLATION OF CHOLESTEROL IN FUNCTIONAL VESICLES: A SIMPLE ANALOGUE OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE

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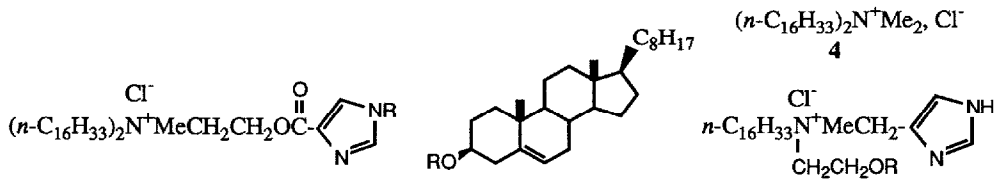
Summary. Imidazole-functionalized surfactants transfer acetyl groups from p-nitrophenyl acetate to cholesterol in vesicular coaggregates.

Human plasma lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) catalyzes acyl transfer from the sn-2 position of lecithins to cholesterol. It is responsible for the esterification of cholesterol and for transformations of high density lipoproteins (HDL) in plasma.[1] Mechanistically, LCAT appears to operate by an initial histidine (imidazole) potentiated serine hydroxyl scission of the lecithin's 2-acyl group. The resulting Ser-O-acyl moiety is then transferred, within the LCAT, to a cysteine-SH. It is the cysteine thioester that ultimately esterifies the cholesterol.[2,3] *In vivo* LCAT functions in covesicular (or comicellar) HDL aggregates that contain LCAT, lecithin, cholesterol, and apolipoprotein A-I; the latter activates the enzyme.[1,4]

Some years ago, it was found that imidazole-functionalized micellar surfactants cleaved reactive esters with the formation of acylimidazole intermediates that subsequently transferred the acyl fragments to comicellized alcohols.[5] Imidazole-functionalized *vesicular* surfactants also cleave reactive esters,[6,7] so that a simple (if inexact) LCAT analogue might be constructed from an imidazole-functionalized surfactant/cholesterol covesicle.

Here, we report that vesicles of the N,N-dihexadecylcholine ester of imidazole-4-carboxylic acid (**1**),[7] doped with cholesterol (**2**), afford 3β-cholesteryl acetate (**3**) upon reaction with p-nitrophenyl acetate (PNPA). These reactions are found to proceed via acylimidazole intermediates, and their efficiency improves above the vesicular T_c (*i.e.*, the transition temperature above which the ordered vesicular "gel" phase goes over to the more fluid "liquid crystalline" phase).

Covesicles of **1** and **2** were generated by rapid injection[8] of 0.01-0.02 M ethanolic solutions into rapidly stirred 0.01 M, pH 8 tris buffer, μ = 0.01 (KCl), at 50°C. Additional stirring for 1 min at 50-55°C was followed by slow cooling to ambient temperature. *Holovesicles* of **1** created in this way were characterized[7] as multilamellar (electron microscopy), 2000-2300 Å (dynamic light scattering) aggregates, with a major phase transition (T_c) at 43.2°C (differential scanning calorimetry). At 23°C, the size of 1/2 *coaggregates* varied from ~2500 Å at 20 mol-% **2** to ~1750 Å (polydisperse) at 50 mol-% **2**. The coaggregates' T_c (by fluorescence polarization of 1,6-diphenylhexatriene[9]) was 42.5°C for **1** alone, but the



1, R = H; **1-Ac**, R = $COCH_3$ **2**, R = H; **3**, R = CH_3CO **5**, R = H; **6**, R = $COCH_3$

sharp phase transition was suppressed[10] at 20 or 33 mol-% cholesterol, although the observed decrease in fluorescence polarization with increasing temperature was similar to that observed with 1 alone. Apparent microviscosities (η) of the various aggregates were estimated from the measured excited state lifetimes and fluorescent polarizations of the included diphenylhexatriene probe.[11] At 22°C, η was 34.2, 106, or 239 cP, respectively, for 1, 4:1 1/2, or 2:1 1/2. At 45°C, the microviscosities of the 4:1 and 2:1 coaggregates decreased to ~35 and 53 cP.

The kinetics of PNPA cleavage by vesicular 1 were followed by monitoring the release of p-nitrophenoxide ion at 400 nm in pH 8.0, 0.01 M aqueous tris buffer, μ = 0.01 (KCl), at 22°C. Holovesicular 1 (1 x 10^{-4} M) cleaved 1.0 x 10^{-5} M PNPA with pseudo-first-order kinetics, k_ψ = 1.6 x 10^{-2} s^{-1}. Coaggregates of 1 and 2 were less reactive, with k_ψ = 6.3 x 10^{-3}, 1.8 x 10^{-3}, and 5.7 x 10^{-4} s^{-1} at 4:1, 2:1, and 1:1 molar ratios of 1/2, respectively. Acetylimidazole intermediates could be detected at 255 nm[5c] during these reactions. From the time course of their decay, we estimate[5c] that $k_{deacyl} \sim k_{acyl}$ for vesicular 1.

The acetylation of 2 by PNPA in 1/2 coaggregates was examined with 2 x 10^{-3} M vesicular 1, containing varying amounts of 2.[12] Cholesteryl acetate (3), and unreacted 2, were identified by TLC and by analytical GC, which also quantitated the 2→3 conversion.[13] Data appear in Table I.

At 22°C, with an 8-fold excess of PNPA, conversion of 2 to 3 proceeds ~30% in vesicles of 1 containing 20 mol-% of 2 (run 1). Comparisons with runs 3 and 6 show that increased 2 within the coaggregte improves conversion to 3, even though the molar excess of PNPA to cholesterol is thereby decreased. The reaction of PNPA with covesicular 1/2 initially forms the acetylimidazole surfactant 1-Ac, so that the efficiency of subsequent acyl transfer to 2 depends on the quantity of vesicular 2. In runs with less 2, more of the 1-Ac is competitively cleaved by water.

Similarly, comparison of run 3 with run 5 (2-fold dilution of 1 with the nonfunctional surfactant 4) shows that decreasing the access of 1-Ac to substrate 2 decreases the conversion to 3. Control run 12 demonstrates that coaggregates of 4 and 2 lead to only traces of 3; without an imidazole unit to transfer the acetyl fragment, 2 is not esterified, and the PNPA simply hydrolyzes.

More striking is the dependence of the conversion on temperature; cf., runs 1 vs. 2, 3 vs. 4, and 6 vs. 7. Acetyl transfer is more efficient at 45°C than at 22°C, particularly with the 20 mol-% cholesterol coaggregates (run 1 vs. 2). The coaggregates at 45°C are above the T_c of vesicular 1; they are more fluid than the vesicles at 22°C, and exhibit much lower

Table I. Acetylation of Cholesterol Coaggregated with 1 by PNPA[a]

Run	Surf.[b]	[Cholesterol], M	[PNPA]/[Chol.]	Temp., °C	% Conv.[c]
1	1	5×10^{-4}	8	22	30
2	1	5×10^{-4}	8	45	67
3	1	1×10^{-3}	4	22	35
4	1	1×10^{-3}	4	45	46
5[d]	1	1×10^{-3}	4	22	15
6	1	2×10^{-3}	2	22	38
7	1	2×10^{-3}	2	45	49
8[e]	1	2×10^{-3}	2	22	10
9[e]	1	2×10^{-3}	2	45	35
10	5	2×10^{-3}	2	25	24
11	6	2×10^{-3}	2	25	40
12	4	5×10^{-4}	8	25	~0.1

[a]See text for the experimental conditions and analytical procedure; [PNPA] = 4×10^{-3} M throughout. [b][1] = 2×10^{-3} M throughout. [c]Per cent conversion is defined as 100 x molar ratio of 3 formed/initial 2. Reproducibilities were ±0.5% when indentical coaggregate preparations were used, and ±3% with different preparations. [d]2 equivalents of surfactant 4 were included in the covesicle. [e]3β-cholestanol was substituted for cholesterol.

microviscosities (see above). We suggest that 1-Ac more efficiently acetylates 2 in the fluid 45°C coaggregates because it can there more readily attain the appropriate acyl transfer geometry than it can in the stiffer, more ordered gel phase vesicles at 22°C , and also because the mutual lateral diffusion rate and collision frequency of 1-Ac and 2 are greater in the 45°C coaggregate.[14] There are reports of similar increases in the efficiency of LCAT esterification of cholesterol with increasing vesicular fluidity, but Jonas has not observed such effects with carefully defined HDL discoidal coaggregates of phosphatidylcholines, cholesterol, and A-I.[16]

3β-Cholestanol is also acetylated by PNPA in coaggregates with imidazole surfactant 1 (runs 8 and 9). The conversion at 22°C is lower than with cholesterol (run 6), but it is much improved at 45°C (run 9). Finally, micellar imidazole-functionalized, single chain surfactants 5 or 6[5c] mediate transacetylation from PNPA to comicellized 2 (runs 10 and 11) with efficiencies similar to that of vesicular 1 (run 6). We are now studying the regio- and stereoselectivities of the vesicular acetylation reaction with various substrate sterols.

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(12) Thus, 0.4 ml of a 0.1 M solution of PNPA in CH_3CN was added to 10 ml of the vesicle solution in the tris buffer solution at 22° or 45°C. The final [PNPA] was ~4 x 10^{-3} M, or twice [1]. After 2 hrs of stirring (sufficient for completion of all of the reactions), the reaction was quenched by acidification to pH 3 (HCl), followed by partial lyophilization and ethereal extraction to obtain the products.

(13) GC analysis employed a Varian model 3700 flame ionization instrument fitted with a 2 m, 2 mm (i.d.), 3% OV-17 on 80/100 Gas-Chrom QII glass column. On-column injection was used with the following operating parameters: injector, 300°; column, 260°; detector, 350°; N_2 carrier gas, 31 ml/min. Retention times (min) were cholestane (internal standard), 6.5; 2, 13.0; 3, 16.0. Corresponding relative molar detector sensitivities were 1.00, 0.84, and 0.91.

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