terized spectroscopically.
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(23) The fluorine resonance of the neutral compound 5-fluoro-6-methoxy-5,6-dihydrouracil in Me_2SO-d_6[21] occurs 5 ppm to lowfield of that for II. The influence of solvent is minimal as we have observed that the resonance for III shifts only 2 ppm on going from phosphate buffer to Me_2SO-d_6.
(24) Recipient of a Faculty Research Award (FRA-144) from the American Cancer Society.

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Catalysis of *p*-Nitrotrifluoroacetanilide Hydrolysis by an Imidazole Derivative of Polyethylenimine "Ghosts"

Sir:

We have recently prepared a cross-linked derivative of polyethylenimine (PEI) which could be useful in such areas as solid-phase organic synthesis, solid-phase sequencing of biopolymers, catalyst immobilization, and affinity chromatography. PEI ghosts are made in a three-step process (Figure 1). First, PEI is adsorbed to porous alumina beads. The PEI layer is then cross-linked. In the final step the inorganic core is removed by treatment with acid or base resulting in the formation of hollow polymer "ghosts". These structures have great chemical and mechanical stability. Other advantages include compatibility with a wide range of solvents, high capacity (1 mequiv of primary amine/g), and ease of preparation.

Soluble polymeric catalysts with enzyme-like characteristics have been reported by a number of workers.[1-5] We believe that the use of insoluble polymers in enzyme modeling studies is attractive for at least three reasons: the synthetic procedures are simplified, catalytic groups such as thiols or metal ions can not deactivate by forming disulfides or binuclear compounds, and the application of the catalyst in a heterogeneous system would be possible.

We report here the preparation of an effective catalyst of amide hydrolysis at pH 8.2. PEI ghosts containing lauroyl and histidyl groups catalyze the hydrolysis of *p*-nitrotrifluoroacetanilide more than 200 times faster than the rate brought about by imidazole alone.

PEI-600 (Dow, 400 mL of an 8.3% solution in methanol) was mixed with 100 mL of porous alumina beads.[6] After removal of trapped air, the mixture was gently agitated for 30 min. The beads were washed with five portions of methanol (200 mL each) and then dried in vacuo at room temperature. The beads (25 g) were reacted with 250 mL of 0.4% aqueous glutaraldehyde. Trapped air was removed and the reaction was continued for 30 min. The glutaraldehyde solution was then replaced with 100 mL of an 8.3% solution of PEI in methanol. After 15 min of gentle agitation, the PEI solution was replaced with 100 mL of methanol. A total of 2 g of $NaBH_4$ was added in small increments over a period of 30 min. The beads were washed and dried as described in the previous step. PEI ghosts were produced by treatment of PEI-alumina (10 g) with 100 mL of 1 N HCl. After ~15 min, the change in density is complete. The acid was decanted and replaced with a second 100 mL-portion of 1 N HCl. After 30 min, the PEI ghosts were washed with water and methanol and then dried in vacuo at room temperature.

Histidyl residues were introduced by reaction of PEI Ghosts with Boc-His(^{Im}DNP)-ONp. After treatment with 0.2% ethanolic KOH, the PEI ghosts (4-mL settled volume) were mixed with 100 mg of nitrophenyl ester and 2 equiv of triethylamine in 5 mL of dry dioxane. This mixture was rotated for

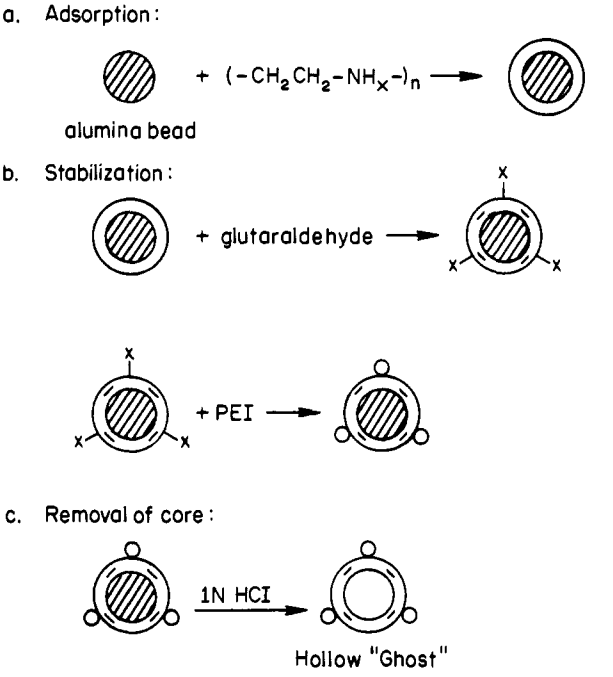

Figure 1. Preparation of PEI ghosts

12 h at 60 °C. The Boc group was removed with 30% TFA in $CHCl_3$ (5 mL, 12 h, at 25 °C). The DNP group was removed by thiolysis (0.1 M mercaptoethanol, pH 9, 12 h, 25 °C). After hydrolysis (6 N HCl, 100 °C, 24 h), amino acid analysis was done. The result was 0.4 mequiv of His/g. Lauroyl groups were then introduced by treatment with nitrophenyl laurate (500 mg in 25 mL of dioxane, containing 0.2 mL of triethylamine) for 72 h at 70 °C. Estimates based on loss of ninhydrin reactivity suggest incorporation of 0.2 mequiv of lauroyl groups/g. A sample which contained no histidyl residues was lauroylated in the same way and used as a control.

The hydrolysis of *p*-nitrotrifluoroacetanilide was followed at 410 nm (0.01 M *N*-ethylmorpholine–HCl buffer, pH 8.2; 40 mL of substrate, 10^{-4} M; 25 °C; 7 mg of catalyst). At timed intervals samples were removed for analysis by means of a syringe fitted with a tube covered by nylon net. The corrected second-order rate constants for hydrolysis catalyzed by imidazole and lauroylhisdidyl-PEI ghosts were estimated to be 0.0036 M^{-1} s^{-1} and 0.816 M^{-1} s^{-1}, respectively.[7] The rate constants were calculated on the basis of total imidazole content in both cases. PEI ghosts substituted only with lauroyl groups do not produce a rate enhancement over background. Therefore, the sizable rate enhancement of 230 times for the lauroylhistidyl preparation is probably the result of the lauroyl groups binding substrate in close proximity to the imidazole ring of histidine. Constant activity after repeated use with *p*-nitrotrifluoroacetanilide and after acylation with large excesses of nitrophenyl esters would suggest that the catalyst is regenerated.

Current investigations with the lauroylhistidyl catalyst are directed at the dependence of reaction rate on pH, substrate concentration, and temperature.

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Proton Nuclear Magnetic Resonance Demonstration of Conformationally Nonequivalent Phospholipid Fatty Acid Chains in Mixed Micelles

Sir:

In the last few years, NMR techniques have been used extensively to examine the structure and packing of phospholipids in multibilayers and sonicated vesicles as membrane models.[1] We have employed these techniques to study phospholipids in mixed micelles[2] which serve as an ideal substrate for lipolytic enzymes such as phospholipase A_2.[3] We have now discovered that, with ^1H NMR, one can detect subtle differences in the environment of the two α-CH_2 groups in the fatty acyl chains of phospholipids, such as dipalmitoylphosphatidylcholine[4] (**1**), in mixed micelles formed with nonionic surfactants such as Triton X-100.[2] Such differences have not been reported for vesicles or multibilayers;[1a-d] mixed micelles with greater motional freedom and narrower line widths[2] provide an ideal membrane-like interface for exploring conformational questions. The differences in the signals from the two chains are not sensitive to changes in chain length or unsaturation of the fatty acids of the phospholipid, but are sensitive to changes in the polar group. Phosphatidylcholine and phosphatidylserine show similar chemical shift differences between the α-CH_2 protons in the *sn*-1 and *sn*-2 fatty acyl chains, whereas, for phosphatidylethanolamine, only a single unresolved peak is observed. This difference in behavior shows that the conformational details or environment of phosphatidylethanolamine is not identical with that of the other two phospholipids.

These findings are of particular interest because the biological significance of these differences can be accessed by the susceptibility of these phospholipids to phospholipase A_2 action. This enzyme specifically catalyzes the hydrolysis of phospholipids by reaction at the carbonyl carbon adjacent to the α-CH_2 group of the *sn*-2 fatty acyl chain.[3b] We[5] have now found that phosphatidylcholine serves as an excellent substrate for phospholipase A_2 in the mixed micelle system, whereas phosphatidylethanolamine is hydrolyzed much more slowly. Phosphatidylserine appears to be a good substrate, but special metal effects complicate the quantitation of its activity.[5] Thus, the activity differences between phosphatidylcholine and phosphatidylethanolamine can be correlated directly with the structural conclusions reported here.

^1H NMR spectra of Triton-phospholipid mixed micelles were obtained at 220 MHz with a Varian HR-220/Nicolet TT-100 pulse Fourier transform system operating at 40 °C. Samples were prepared by adding solutions of Triton X-100 (Rohm and Haas) in D_2O to dry phospholipid; mixing was achieved by a few strokes with a Potter-Elvejhem homogenizer. Dipalmitoylphosphatidylcholine (**1**) and palmitic acid were obtained from Calbiochem. Egg phosphatidylethanolamine, prepared by transesterification of egg phosphatidylcholine, was obtained from Avanti Chemical. Egg phosphatidylcholine was prepared by the method of Singleton et al.,[6] and bovine brain phosphatidylserine was prepared as described elsewhere.[7] Lysophosphatidylcholine was prepared by phospholipase A_2 treatment of egg phosphatidylcholine and purification on alumina chromatography.[3,6] 1-Palmitoyl-2-[2′-$^2H_2$]palmitoylphosphatidylcholine was prepared by the acylation of 1-palmitoyllysophosphatidylcholine with [2′-$^2H_2$]palmitoylimidazolide by a modification of the procedure of Boss et al.[8a] The 1-palmitoyllysophosphatidylcholine was prepared by phospholipase A_2 treatment of dipalmitoylphosphatidylcholine and purification by ether precipitation at pH 3. The [2′-$^2H_2$]palmitoylimidazolide was prepared from [2′-$^2H_2$] palmitic acid (Merck Sharp & Dohme) and carbonyldiimidazole (Aldrich) as described elsewhere.[8b] This acylation procedure results in a small amount of acyl migration between the 1 and 2 positions.[8c] Line widths ($\delta\nu_{1/2}$) were measured as the full width at half-height maximum intensity on expanded spectra; field inhomogeneity was taken to be the line width of the HOD peak and this was subtracted from the reported values.

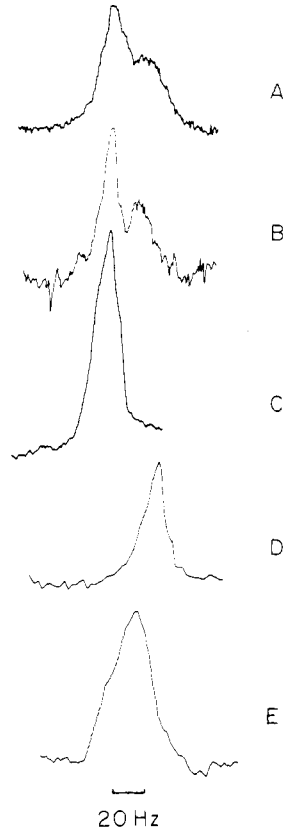
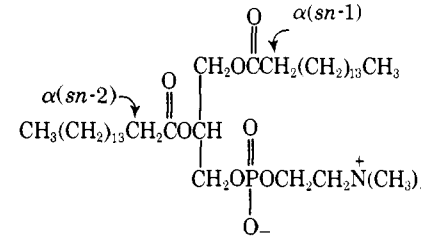


Figure 1. Typical 220-MHz NMR spectra of the α-CH_2 region of various lipids in mixed micelles with Triton X-100 at pH 8.0 and Triton/lipid molar ratios of 4:1: (A) dipalmitoylphosphatidylcholine, (B) same spectrum decoupled by irradiation of the β-CH_2 protons, (C) lysophosphatidylcholine, (D) palmitic acid (molar ratio 8:1), and (E) 1-palmitoyl-2-[2′-$^2H_2$]palmitoylphosphatidylcholine.



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The α-CH_2 proton signals of phospholipids in mixed micelles are well separated from other resonances in the 220-MHz spectrum.[2a,c] In mixed micelles of Triton and **1**, the α-CH_2 region is composed of two broad overlapping peaks as shown