Table 1. Products of Reactions of 5-Hexenyl Chloride in Ethers with Alkali Metals[a]

metal	solvent	MCP	1-hex	2-hex	1 + 2
Na	DME	2	94	4	<1
Na	THF	3	85	3	9
Na	diethyl ether	6	94	<1	0
Na	dioxane	1	98	1	0
Cs	DME	44	10	12	34[b]
Cs	THF	18	7	18	57
Cs	diethyl ether	10	5	16	69
Cs	dioxane	4	67	30	<1

[a] Yields are mole percent of the products shown. No others were detected at significant levels. MCP = methylcyclopentane; 1-hex = 1-hexene; 2-hex = 2-hexene. Reactions were carried out at 25.0 ± 0.3 °C in sealed vessels under solvent vapor pressure. Solutions of 5-hexenyl chloride, initially 0.005–0.01 M, were agitated over metal mirrors for ~18 h and then neutralized with acetic acid. Analyses were by VPC using internal standards. The conditions used separated 1-hexene, *cis*- and *trans*-2-hexenes, *cis*- and *trans*-3-hexenes, methylcyclopentane, cyclohexane, hexane, and 1,5-hexadiene. **1** and **2** were collected by preparative VPC and characterized by their C,H analyses and off-resonance decoupled ^{13}C NMR spectra. [b] For Cs in DME the reaction time was 5 min. Alkene isomerizations were found to occur over a period of hours for products left in contact with a Cs mirror in DME.

3-Hexenes were absent and 1,5-hexadiene was formed in no more than trace quantities (distinctly <1%). Table I gives the product distributions for experiments with sodium and cesium, which represent the extremes observed for sodium, potassium, rubidium, and cesium, in THF, DME, diethyl ether, and dioxane.

The unexpected products imply 1-propylallyl intermediates: radicals, anions, or both. Isomerization of 5-hexenyl to 1-propylallyl radicals has not been found; instead, 5-hexenyl radicals cyclize as depicted in eq 1.[4] The possibility that surface-adsorbed radicals behave differently from solution radicals and are responsible for the isomerization observed is ruled out by the fact that the 1-propylallyl-derived products are also found from reactions of homogeneous solutions of alkali naphthalenes, potassium benzene, and alkali metals (i.e., solutions of M^- and e^-) with 5-hexenyl chloride.

Intermediate 1-propylallyl anions would protonate to 2-hexenes (as well as 1-hexene), and they would react with 5-hexenyl chloride to give **1** and **2**, thus rationalizing these products. Control experiments show that alkenes are not isomerized under the reaction conditions employed. Thus, a mixture of hexyl chloride and 1-hexene in THF reacts with K or Cs to give only hexane and 1-hexene. Further, when product isomerization is deliberately allowed to occur (by prolonged reaction of the Cs/5-hexenyl chloride/DME system), additional peaks appear in the C_{12} region of the VPC traces, but such peaks are absent for the product mixtures reported in Table I. This militates against intermolecular base- or metal-promoted reactions of 1-hexene as sources of 1-propylallyl anions, leaving the intramolecular process (eq 2) as the only reasonable possibility.

It is significant, we believe, that there exists a possible transition state for the 1,4 proton transfer of a 5-hexenylalkali in which the metal ion is simultaneously in good contact with both the original and the developing centers of negative charge in the anion (see eq 2).

Since isomerization of 5-hexenylsodium is a minor process, the conclusions of previous studies, in which the 5-hexenyl probe was applied to reductions of alkyl halides by sodium arenes and related species, are left unchanged.[1a-h]

Users of the 5-hexenyl probe should be aware that 5-hexenyl anions can isomerize prototropically. Products derived through 1-propylallyl anions must be included in the reckoning of the yield of 5-hexenyl anions.

Another caveat is appropriate here: cyclization of 5-hexenyl anions is not always negligible. It competes with the other reactions of 5-hexenyl anions, and since all of these may be affected by counterion, solvent, and temperature, the extent of anion cyclization can be expected to depend on these conditions.[5] We have found situations with the higher alkali metals and solvents containing crown ethers in which 5-hexenyl anions cyclize to an extent of ~6%.

Anion cyclization cannot account for the large yield of methylcyclopentane found in the reaction of 5-hexenyl chloride with Cs in DME (Table I) unless such cyclization is dependent on the heterogeneity of the system or some other detail of reaction conditions. When 5-hexenylcesium is produced in DME by the reduction of 5-hexenyl chloride with cesium naphthalene, <3% of the reduction products is methylcyclopentane.[6]

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(3) Many previous experiments have shown that no normal Wurtz coupling results when simple primary alkylsodiums, e.g., hexyl- and 5-hexenylsodium, are generated from corresponding alkyl chlorides.[1a] Clearly, such alkylsodiums are protonated by the solvent rapidly compared with their reactions with their parent alkyl chlorides.

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(5) In fact, cyclization of 5-hexenylmagnesium halides to cyclopentylmethylmagnesium halides is well known and intensely studied. See H. G. Richey, Jr., and H. S. Veale, *J. Am. Chem. Soc.*, **96**, 2641 (1974). See also, for additional background and related material, E. A. Hill, *Adv. Organomet. Chem.*, **16**, pp 131–165 (1977), and E. A. Hill, *J. Organomet. Chem.*, **91**, 123 (1975).

(6) For example, 10^{-1} M cesium naphthalene in DME reacts with 3.5×10^{-2} M 5-hexenyl chloride to give reduction products consisting of <3% methylcyclopentane: F. E. Barton, II, Dissertation, University of Georgia, 1969.

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Mechanism of Lipid–Protein Interaction in Lipoproteins. A Synthetic Peptide–Lecithin Vesicle Model

Sir:

We are engaged in an investigation of the relationship between structure and function in lipoproteins, exploring the mechanism by which small fragments of a protein molecule may exhibit some of the fundamental properties of the native protein itself. The tertiary structure of an enzyme is known to bring into close proximity the functional groups necessary for catalysis but far apart in the linear sequence of amino acids. Therefore, making the assumption that the catalytic activity of an enzyme could be imitated by a small synthetic portion of it surrounding one active site residue would be naive, in general. In contrast, the functional units of structural proteins

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A. His-Val-Asp-Ala-Leu-Arg-Thr-His-Leu-Ala-Pro

B. Leu-Gly-Glu-Glu-Met-Arg-Asp-Arg-Ala-Arg-Ala-
His-Val-Asp-Ala-Leu-Arg-Thr-His-Leu-Ala-Pro

C. $CH_3\overset{O}{\overset{\|}{C}}$-Glu-Met-Glu-Leu-Tyr-Arg-Gln-Lys-Val-Glu-
Pro-Leu-Arg-Ala-Glu-Leu-Gln-Glu-Gly-Ala

D. Gly-Lys-Asp-Leu-Met-Glu-Lys-Val-Lys-Ser

Figure 1. Peptides synthesized. Peptide A corresponds to residues 158-168 of apolipoprotein A-I, B to residues 147-168, and C to residues 114-133 acetylated at the N terminal (H. N. Baker, A. M. Gotto, Jr., and R. L. Jackson, *J. Biol. Chem.*, **250**, 2725 (1975)). Peptide D corresponds to residues 22-31 of apolipoprotein A-II (H. B. Brewer, Jr., S. E. Lux, R. Ronan, and K. M. John, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1304 (1972)).

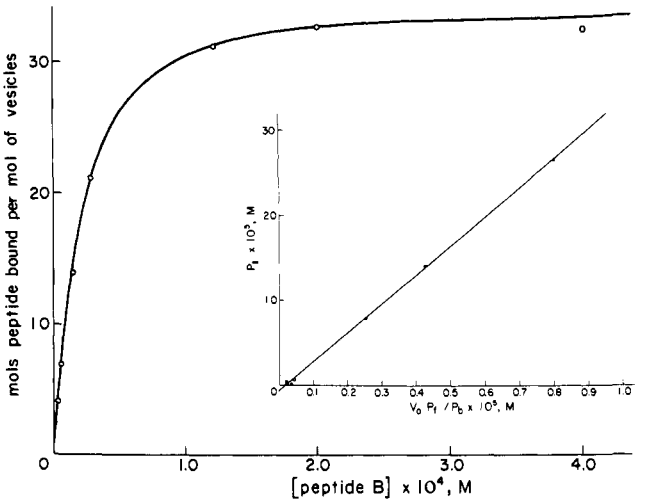

Figure 2. Binding of peptide B to vesicles as a function of peptide concentration. The insert shows the data plotted according to the equation $P_f = (nV_0P_f/P_b) - K$.

might be confined to smaller, repeating domains on a single polypeptide chain. For example, a substantial part of the apolipoprotein A-I molecule has been shown to contain a region of repeating structural units of 22 amino acids, possibly divided into two similar 11 residue domains.[1] The ability of the apolipoproteins to bind lipids may be related to their potential for forming "amphiphilic" helical structures.[2] We thought that preparation of a small apolipoprotein fragment might enable us to test the hypothesis that the functional role of the apolipoproteins is carried by the oligopeptide segments of the repeating amino acid sequence.

To test this proposal, peptides A-D, shown in Figure 1, homologous to repeating units of apolipoproteins A-I and A-II, were synthesized by the Merrifield solid-phase method.[3] The thermodynamics and stoichiometry of binding of these peptides to highly purified egg lecithin[4] single bilayer vesicles were studied as a model for lipid-protein interactions in the lipoproteins. These vesicles were chosen for their remarkable stability[5] and for their similarity to lipoproteins both with respect to the size and composition of their aliphatic chains. Vesicles (diameter, 230 Å) were prepared by injecting ethanolic solutions of the pure lipid into aqueous solutions, separated from multilamellar liposomes by chromatography on Sepharose CL-4B, and concentrated by ultrafiltration.[6]

In a typical binding experiment 500 μL of the vesicle solution (10^{-2} M lecithin) was incubated for 2 h at room temperature with varying concentrations of peptide. The mixture was then chromatographed on a 1.5 × 43 cm column of Sepharose 4B, using 0.16 M KCl as the eluent and monitoring the effluent spectroscopically. Peptide content of the fractions was determined after reaction with *o*-phthalaldehyde to produce a fluorescent product (λ_{ex} 340, λ_{em} 455).[7] Alternatively, mixtures of vesicles and peptides were subjected to ultrafiltration through 100-Å-pore cellulose acetate membranes (XM 100 A, Amicon, Inc., Lexington, Mass.), 2-propanol (20% by volume) was added to destroy the vesicle structure, and the filtrate was analyzed for peptide concentration.

Both methods showed the formation of a complex between the vesicles and peptide B. Preincubation of peptide B with the vesicles for varying times demonstrated that binding equilibrium is reached in <2 h. Vesicle-peptide complexes cochromatographed with single bilayer vesicles and no more than 70% of the phospholipid in the complexes is subject to hydrolysis by *C. atrox* venom phospholipase A_2.[8]

At pH 6.0, 21 °C, 0.16 M KCl, the binding of peptide B displayed saturation behavior as shown in Figure 2. Analysis of the binding data was performed by assuming independent binding sites governed by a single binding constant, according to the equation $P_f = (nV_0P_f/P_b) - K$, where P_f is the molarity of free peptide, P_b is the molarity of bound peptide, V_0 is the initial vesicle molarity (3200 phospholipids per vesicle), n is the maximum number of peptide molecules that can be bound per vesicle, and K is the dissociation constant for the peptide-lipid complex. The experimental data indeed yielded a straight line (see insert of Figure 2), and we calculated $n = 34$ and $K = 6.9 \times 10^{-6}$ M.

Under the above conditions, peptides A, C, and D showed no detectable binding. However, the binding of the peptides to vesicles was found to be dependent on pH. Using 3.2×10^{-4} M peptide and 3.12×10^{-6} M vesicles, maximum binding of peptide B (30% of the available peptide) was observed at pH 5 and 6. At pH 7 the amount bound was reduced to 22%, and at pH 8 less than 5% of the peptide complexed with the vesicles. At pH 6, 7, and 8 no measurable amount of peptide C was bound, but at pH 5 the vesicles retained 20% of the peptide. Peptides A and D did not form complexes in the pH range of 5-8.

The hydrolysis of phosphatidylcholine in the peptide B-vesicle complex by *C. atrox* venom phospholipase A_2 was followed using a pH stat.[9] Reaction mixtures were isotonic to the vesicles (0.16 M KCl, 5 mM $CaCl_2$) and contained enough peptide B and vesicles to ensure 75% occupancy of the binding sites. Values of k_{cat}/K_m, estimated from the initial rates of the reactions, were 1×10^5 M^{-1} s^{-1} for vesicles alone, 6×10^5 M^{-1} s^{-1} for vesicles with peptide B, and 20×10^5 M^{-1} s^{-1} for vesicles with 1% albumin.

These experiments demonstrate that even relatively small fragments of apo A-I have the intrinsic ability of forming mixed peptide-phospholipid surfaces. The size, composition, and accessibility of the phospholipid to the enzyme prove that the structure of the complex is vesicular. In contrast to our results, it is doubtful whether the structural integrity of 1,2-dimyristoyl-*sn*-3-phophatidylcholine vesicles is preserved when they bind apolipoproteins or their fragments.[10] The binding must occur in the outer leaflet of the phospholipid bilayer since no structural reorganization of the vesicle is observed upon complex formation. Although the binding formally shows the presence of a discrete number of "binding sites", calculations indicate that, in fact, the stoichiometry is a result of the geometry of vesicles. The curvature of the outer surface creates between the phospholipid head groups a free area of 1.8×10^4 Å^2 per vesicle which would be equivalent approximately to 24 Å^2 per amino acid bound. This value is in excellent agreement with the area found at the air-water interface for denatured protein molecules retaining their secondary structure.[11] The

noncooperative nature of the binding also indicates a random, nonspecific interaction between the peptide and the phospholipid surface, without the necessity to invoke the presence of discrete binding sites. The free energy of complex formation is 0.6 kcal/amino acid, a reasonable value if the binding energy is the result of the transfer of a potential amphiphilic helix from water into an amphiphilic surface.[12]

Circular dichroism spectra show that peptides A and D are in a random, rather than in a helical conformation, even in 50% trifluoroethanol. Peptides B and C contain an estimated 10–15% helical structure[13] in aqueous buffers over a wide pH range, the amount of helicity increasing to 40% for peptide B and 33% for C in 50% trifluoroethanol. Thus, the differences in the abilities of the synthetic peptides to bind to lipid vesicles seem to correlate with helix-forming potential. The pH dependency of the binding shows the importance of the state of ionization of the peptides: the isoelectric point is 7.1 for peptide B and 4.6 for peptide C, whereas the vesicles have a negative ζ potential, as indicated by their electrophoretic mobility. Thus, the electrostatic repulsion of negatively charged peptides must reduce their affinity for the vesicles, although the major binding force has to be of a lyophilic nature.

The enzymatic digestion shows that the presence of bound peptide increases the rate of hydrolysis of the phospholipids by a factor of 6, thereby yielding a hydrolytic rate comparable with that observed for human plasma LDL.[14] Thus, the egg lecithin vesicle-peptide complex is a suitable model for the lipoprotein surface by the criterion of this sensitive enzymatic probe. Previous phospholipid-protein binding studies using apoproteins or their fragments and dimyristoyl lecithin vesicles failed to yield a quantitatively analyzable system, mostly because of the major structural reorganization of the vesicles that occurred upon binding.[5] We feel that the present study demonstrates that the synthetic peptide–egg lecithin single bilayer vesicle system provides an excellent model for the study of lipoprotein structure. We are currently pursuing this line of investigations.

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Effect of Phenyl Substituents on the Hydrolyses of Trifluoroacetanilides Catalyzed by Imidazolyl Cation and Hydroxide Ion

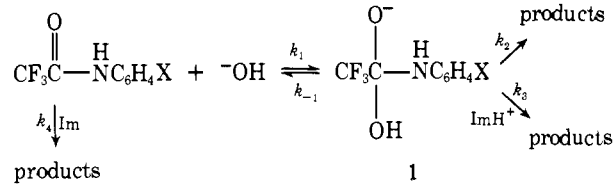
Sir:

We report a result of a simple model study which strongly supports acid catalysis by the imidazolyl cation in serine protease catalyzed hydrolyses of amide compounds, especially anilides. Acid catalysis by the imidazolyl cation of His-57 has been proposed in the α-chymotrypsin-catalyzed hydrolyses of anilides, on the basis of the finding that electron-donating substituents on the phenyl rings of anilides largely facilitated reaction;[1] for example, in the chymotrypsin-catalyzed hydrolyses of *N*-acetyl-L-tyrosine anilides $\rho = -1.8$ at 35 °C.[1e] The imidazolyl cation allegedly catalyzes the breakdown of the tetrahedral intermediate between Ser-195 and the anilide. However, nonenzymatic hydrolyses of trifluoroacetanilides, in which the breakdown of the tetrahedral intermediate between hydroxide ion and the anilide is catalyzed by water (as acid catalyst), showed a *positive* ρ value (+0.69) with respect to phenyl substituents.[2] Thus, there has been a big discrepancy between enzymatic hydrolyses of anilides and nonenzymatic ones previously studied. Here, the alkaline hydrolyses of five trifluoroacetanilides (H, *m*-Cl, *p*-Cl, *m*-NO_2, and *p*-NO_2) catalyzed by imidazolyl cation are reported, showing a large *negative* ρ value consistent with enzymatic reactions.

The alkaline hydrolyses of trifluoroacetanilides in the presence of imidazolyl cation (ImH^+) and imidazole (Im) proceeds as shown in Scheme I.[3,4] The tetrahedral intermediate (**1**) collapses to products via acid catalysis by the imidazolyl cation (k_3) and by water (k_2).[5] Furthermore, neutral imidazole directly catalyzes the hydrolyses (k_4).

The parameters, k_3/k_{-1}, k_1, and k_4, were evaluated from the plot of the observed rate constant of hydrolyses, determined spectrophotometrically, vs. the concentration of imidazole at pH 7.2, 70 °C. As described by Eriksson et al.,[4] this plot gives $k_3/(k_{-1} - k_2)$, k_1, and k_4. Since k_{-1} is much larger than

Scheme I



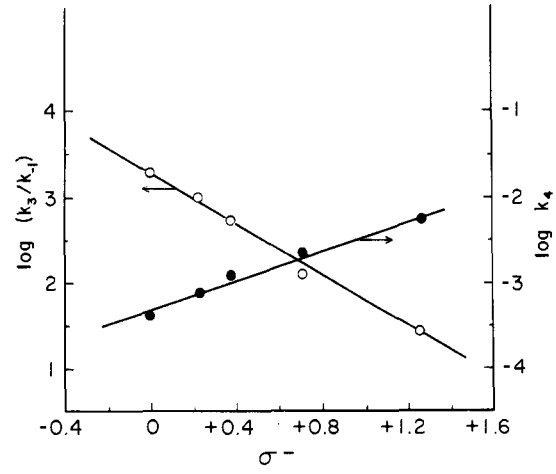


Figure 1. Plots of log (k_3/k_{-1}) and log (k_4) vs. σ^- in the hydrolyses of trifluoroacetanilides at 70 °C; see Scheme I concerning the notation of rate constants.

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