peroxides. Furthermore, these results are consistent with previous mechanistic interpretations of endoperoxide decomposition.[6] An important implication of these results is the possibility of observation of magnetic isotope effects (^{17}O and ^{13}C) on the thermolyses of aromatic endoperoxides. At low magnetic fields a hyperfine mechanism may determine the rate of intersystem crossing in ^1D. The results of investigations examining this prediction will be reported shortly.

Acknowledgment. The authors thank the Air Force Office of Scientific Research and the Department of Energy for their generous support of this research. They also thank Dr. Y. Tanimoto for helpful discussions and comments, and Professor Steven Lippard for loan of a variable-field magnet.

References and Notes

(1) Reviews of magnetic field effects on chemical reactions: (a) Sagdeev, R. Z.; Salikhov, K. M.; Molin, Y. M. *Russ. Chem. Rev.* **1977**, *46*, 297. (b) Buchachenko, A. L. *ibid.* **1976**, *45*, 375.
(2) For a discussion of theoretical studies of the influence of an external magnetic field on chemical reactions, see: Hayashi, H.; Nagakura, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2862, and references therein.
(3) (a) Atkins, P. *Chem. Br.* **1976**, *12*, 214. (b) Buchachenko, A. L. *Russ. Chem. Rev.* **1976**, *45*, 761.
(4) (a) Kaptein, R. *Adv. Free-Radical Chem.* **1975**, *5*, 381. (b) Closs, G. In "Chemically Induced Magnetic Polarization", Lepley, A. R.; Closs, G. L.; Ed.; Wiley: New York, 1973; p 95.
(5) For a review of the magnetic isotope effects, see: Buchachenko, A. L. *Russ. J. Phys. Chem.* **1977**, *51*, 1445.
(6) Turro, N. J.; Chow, M.-F.; Rigaudy, J. *J. Am. Chem. Soc.* **1979**, *101*, 1300.
(7) Berndt, A.; Fischer, H.; Paul, H. "Magnetic Properties of Free Radicals", Landolt-Börnstein, New Series; Springer Verlag: New York, 1977; Vol. 9b.
(8) Magnetic field effects on the quenching of delayed fluorescence of aromatic hydrocarbons by molecular oxygen have been interpreted in terms of a triplet-triplet annihilation to produce singlet oxygen. In this case, the formation of singlet oxygen would be *enhanced* by an external magnetic field: Tachikawa, H.; Bard, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 1672.
(9) The yield of the "cage" product (phenyl benzoate) formed in the singlet-photosensitized decomposition of dibenzoyl peroxide decreased proportionally to the square root of the applied field: Tanimoto, Y.; Hayashi, H.; Nagakura, S.; Sakuragi, H.; Tokumaru, K. *Chem. Phys. Lett.* **1976**, *41*, 267.

Nicholas J. Turro,* Ming-Fea Chow
Chemistry Department, Columbia University
New York, New York 10027
Received March 19, 1979

A Synthetic Amphiphilic Helical Docosapeptide with the Surface Properties of Plasma Apolipoprotein A-I

Sir:

Plasma apolipoprotein A-I (apo A-I) contains six highly homologous 22 amino acid segments.[1,2] These domains have a high helix-forming potential[3] and in the α-helical conformation the hydrophobic and hydrophilic groups are segregated on opposite sides of the cylindrical segments.[4] If, indeed, the secondary structure of these segments is responsible for the binding of the whole apolipoprotein to lipid surfaces, then any amphiphilic helical oligopeptide should also display the unusual surface properties of apo A-I.[5,6] In order to test this hypothesis, we designed a docosapeptide (peptide I, see Figure 1) which a priori has a high potential to form amphiphilic helices (P_α = 1.28, P_β = 0.77).[3] The particular simple amino acid sequence was chosen to give an equal distribution of acidic and basic residues on the hydrophilic side and to be as different as possible from the repeating apo A-I sequence. The peptide has only 41% homology with the 198–219 fragment of human apo A-I[7] and considerably less with the rest of the A-I molecule.

The peptide was synthesized using the Merrifield solid-phase method[8] employing the blocking groups and cleavage method described previously.[9] Purification of the deblocked peptide was carried out by gel permeation chromatography (Sephadex G-25, 0.2 M acetic acid), followed by lyophilization and ion-exchange chromatography on CM Sephadex C-25 (0.02 M sodium phosphate buffer, pH 6.5, with a sodium chloride gradient from 0 to 0.5 M). After lyophilization and desalting, peptide I was purified further by ion-exchange chromatography on CM Sephadex C-25 (0.02 M sodium phosphate buffer, pH 6.6, with a stepwise sodium chloride gradient 0, 0.2, 0.3 M; the peptide eluted at 0.3 M). The yield of pure peptide I was 1.5% based on the starting amount of Boc-Ala-resin. No impurities were detected in the final product by automated Edman degradation, amino acid analysis, high voltage paper electrophoresis, and thin layer chromatography on cellulose and silica. Based on the limits of detection inherent in each of these methods, the purity of the peptide was estimated to be at least 99%.

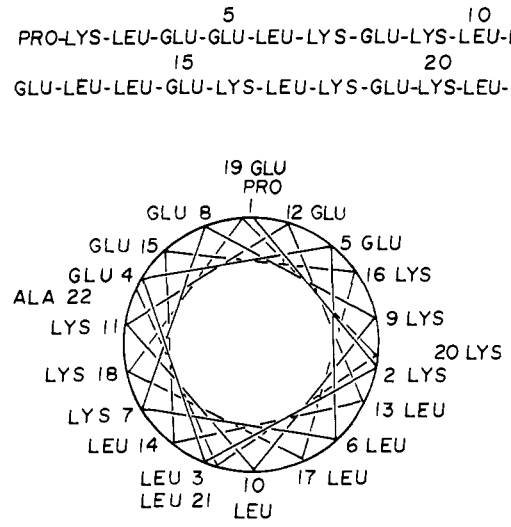


Figure 1. Amino acid sequence of peptide I and the axial projection of the α-helical peptide showing the relative location of the side chains with the segregation of the hydrophobic and hydrophilic residues.

Helical content was estimated from the molar ellipticity of I measured at several concentrations. From the concentration dependency of molar ellipticity, the peptide was found to form tetramers cooperatively with a $K_{dissoc} = 9.7 \times 10^{-16}$ M^3 (at 222 nm $\theta_{monomer} = -4.6 \times 10^3$ deg cm^2/dmol and $\theta_{tetramer} = -6.8 \times 10^4$ deg cm^2/dmol). The tetramerization of I was also demonstrated by gel permeation chromatography on Sephadex G-50. We found 61% helicity in 50% trifluoroethanol; in 0.1 M phosphate buffer, pH 7.0, at 1.7×10^{-4} M peptide, the helicity was 50%, whereas at 3×10^{-6} M it was 30%.

The binding of I to pure egg lecithin single bilayer vesicles[11] was quantitated by rapid ultrafiltration sampling.[12] The 2.0-mL incubation mixtures contained 0.025 M buffer (morpholinopropanesulfonic acid, MOPS, pH 7.0) and 0.16 M KCl. After 3-h incubation two 100-μL aliquots were removed, and the remaining solution was subjected to ultrafiltration (100-Å pore Amicon XM 100 membrane), the first 250 μL of ultrafiltrate being discarded and the next ~250 μL being collected, followed by the removal of two 100-μL aliquots from it for analysis. To each aliquot was added sequentially 250 μL of 2-propanol, 250 μL of 0.4 M borate buffer (pH 9.7), and 250 μL of 6.56×10^{-3} M *o*-phthalaldehyde in the same borate buffer. Fluorescence intensity (λ_{ex} 340 nm, λ_{em} 465 nm) was proportional to the peptide concentration over the entire concentration range employed. The adsorption of the peptide to the outer surface of the vesicle obeys a simple Langmuir isotherm. The data were analyzed in the same fashion as those described earlier for A-I fragments,[12] indicating that formally the binding sites are equivalent and independent.[13] The surface of a single vesicle was found to accommodate 48 peptides with a dissociation constant of $K_D = 1.9 \times 10^{-6}$ M. The binding of I to single bilayer vesicles was shown to be independent of pH

0002-7863/79/1501-3703$01.00/0

over the range pH 5 to 8.2, using MOPS, citrate, and phosphate buffers where appropriate. The binding of pure apo A-I to the egg lecithin single bilayer vesicles was measured under the same experimental conditions as the binding of I, but the free and bound protein were separated by rapid gel permeation chromatography using Sepharose 6B. A simple Langmuir isotherm is again sufficient to interpret the data, and it yields $K_D = (9.4 \pm 1.7) \times 10^{-7}$ M with a maximum of six apo A-I molecules per vesicle. Rapid gel permeation chromatography using Sepharose CL-4B showed that both the vesicle–peptide and vesicle–apo A-I complexes have the same hydrodynamic properties ($d = 250$ Å) as the pure vesicle, indicating the absence of any major reorganization of the lipid structure such as disk formation or fusion into large liposomes.

At the air–water interface insoluble monolayers of I form spontaneously by adsorption from dilute solutions or by spreading of concentrated solutions. The monolayers are stable for at least several hours at a variety of surface pressures, even after repeated compression and expansion. Analysis of the force area curve shows that the surface behavior of the monolayers can be described by the same equation as the one observed for apo HDL and apo A-I monolayers:[5,6,14] $\pi[A - A_{00}(1 - K\pi)] = C$, where K is the surface compressibility and A_{00} is the limiting molecular area extrapolated to zero surface pressure. The compressibility seen for peptide I is $K = 2.1 \times 10^{-2}$ cm/dyn, the same as found for apo HDL, $K = 1.8 \times 10^{-2}$ cm/dyn. Most importantly, the collapse pressure ($\pi = 22$ dyn/cm) of the peptide and protein monolayers appear to be indistinguishable. We find that the limiting area of the peptide extrapolated to zero surface pressure is equal to 23 Å^2 per amino acid residue, whereas $A_{00} = 16.3$ Å^2/amino acid for apo HDL.[6] These values suggest that the stable form of the peptide at the air–water interface is a relatively compact folded conformation, presumably a helix. Peptide I is also able to penetrate phospholipid monolayers at the air–water interface to the same extent as apo A-I does; addition of peptide I to the subphase of a $\pi = 14$ dyn/cm egg lecithin monolayer results in an increase of the surface pressure to 24 dyn/cm.

Our results show that a docosapeptide of high amphiphilic helix potential does behave in solution, at phospholipid–water interfaces, and at the air–water interface in a manner similar to that of apo A-I. Since peptide I epitomizes apo A-I in its tendency to form an amphiphilic helix and yet reproduces the essential surface properties of the intact apolipoprotein, we conclude that the structural role of apolipoprotein A-I is fulfilled by its helical segments, and it is not necessary to invoke a highly organized tertiary structure thereof. The synthesis of amphiphilic helical oligopeptides of simple sequence and well-defined surface properties opens the way to the systematic study of the structure–function relationship of lipid-associated proteins and to the construction of water-soluble lipid–peptide complexes of desirable and useful physical and physiological properties.

Acknowledgments. The work presented in this paper was supported by USPHS Program Project HL-18577, USPHS Medical Scientist Traineeship 5TGM-07281, and USPHS HL-15062 (SRC). The authors thank Dr. R. L. Heinrikson for the sequence analysis of the peptide and Dr. A. M. Scanu for providing a sample of apo A-I.

References and Notes

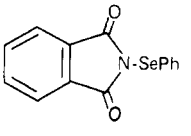
(1) Fitch, W. M. *Genetics* **1977**, *86*, 623–644.
(2) McLachlan, A. D. *Nature (London)* **1977**, *267*, 465–466.
(3) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 211–222.
(4) (a) Segrest, J. P.; Jackson, R. L.; Morrisett, J. D.; Gotto, A. M., Jr. *FEBS Lett.* **1974**, *38*, 247–253. (b) Morrisett, J. D.; Jackson, R. L.; Gotto, A. M., Jr. *Biochim. Biophys. Acta* **1977**, *472*, 93–133.
(5) Edelstein, C.; Kézdy, F. J.; Scanu, A. M.; Shen, B. W. *J. Lipid Res.* **1979**, *20*, 143.
(6) Camejo, G. *Biochim. Biophys. Acta* **1969**, *175*, 290–300.
(7) Osborne, J. C., Jr.; Brewer, H. B., Jr. *Adv. Protein Chem.* **1977**, *31*, 253–337.
(8) Erickson, B. W.; Merrifield, R. B. In "The Proteins", Neurath, H., Hill, R. L., Eds., Academic Press: New York, 1976; Vol. II, 3rd ed., pp 253–527.
(9) Kroon, D. J.; Kaiser, E. T. *J. Org. Chem.* **1978**, *43*, 2107–2113.
(10) Adler, A. J.; Greenfield, N. J.; Fasman, G. D. *Methods Enzymol.* **1973**, *27*, 675–735.
(11) Batzri, S.; Korn, E. D. *Biochim. Biophys. Acta* **1973**, *298*, 1015–1019.
(12) Kroon, D. J.; Kupferberg, J. P.; Kaiser, E. T.; Kézdy, F. J. *J. Am. Chem. Soc.* **1978**, *100*, 5975–5977.
(13) Analysis of the data according to either of the equations[12] $P_f = (nV_0P_f/P_b) - K$ or $P_b/P_fV_0 = nK - (P_b/V_0)K$ yielded the same results.
(14) Shen, B. W.; Scanu, A. M.; Kézdy, F. J. *Circulation* **1973**, *48*, Suppl. IV, 218.

Daikichi Fukushima, Josh P. Kupferberg
Shinji Yokoyama, Daniel J. Kroon
E. T. Kaiser,* F. J. Kézdy*
Departments of Chemistry and Biochemistry
University of Chicago, Chicago, Illinois 60637
Received February 8, 1979

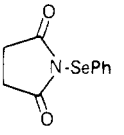
N-Phenylselenophthalimide (N-PSP) and *N*-Phenylselenosuccinimide (N-PSS). Two Versatile Carriers of the Phenylseleno Group. Oxyselenation of Olefins and a Selenium-Based Macrolide Synthesis

Sir:

In recent years the phenylseleno group became a very powerful tool in organic synthesis owing to its fertile and easily manipulated nature.[1-3] The most useful transformations of this group are its oxidative and reductive removal to introduce unsaturation and saturation respectively. In these organoselenium-based operations, the first and most crucial stratagem is the introduction of the phenylseleno (PhSe) group into the organic molecule. In this communication we report on the reactions of *N*-phenylselenophthalimide (N-PSP) and *N*-phenylselenosuccinimide (N-PSS), two new, versatile and useful carriers of the PhSe group. These readily available and



N-PSP



N-PSS

relatively stable reagents are highly effective for introducing the PhSe group into unsaturated substrates. Of particular importance is their utility in the oxyselenenation of olefins and cyclization reactions including the formation of cyclopropanes and macrolides. This new method of macrolide formation from long, open-chain, unsaturated carboxylic acids constitutes a new concept for the synthesis of these biologically important compounds.[4-6]

N-PSP[7] is readily prepared from potassium phthalimide and phenylselenenyl chloride and is a perfectly stable colorless crystalline solid. N-PSS is conveniently obtained from allylphenylselenide and *N*-chlorosuccinimide by the method of Sharpless and Hori[8] as a white crystalline solid. This substance is stable at −20 °C under argon for long periods of time although in the air and at 25 °C it slowly decomposes turning increasingly yellow. The following reactions of N-PSP and N-PSS illustrate the versatility and effectiveness of these newly introduced organoselenium reagents in organic synthesis.

I. Oxyselenation of Olefins. *N*-Phenylselenophthalimide and *N*-phenylselenosuccinimide react readily with olefins at 25 °C in methylene chloride in the presence of 2–3 equiv of water and acid catalyst (0.05–0.1 equiv, e.g., *p*-toluenesulfonic, pyridinium *p*-toluenesulfonate, camphorsulfonic) to afford *hy-*

0002-7863/79/1501-3704$01.00/0