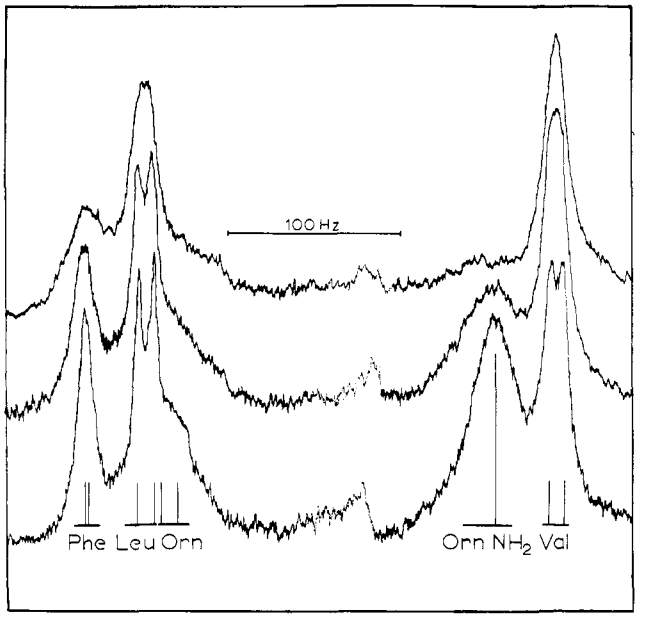

Figure 3. 220-MHz spectra of the N–H resonances of gramicidin S in methanol (10 wt %, 20°) showing the effect of additions of 0, 1, and 2% radical. The assignments shown are from the literature.[7,9] The dotted absorption appears to be an impurity in the sample used. We thank Dr. J. J. Katz, Argonne National Laboratory, for the use of the 220-MHz spectrometer.

nance of reference 2,2-dimethyl-2-silapentane-5-sulfonate ion.

We have examined the effect of radical on peptide spectra using the cyclic decapeptide gramicidin S, cyclo(L-Pro-L-Val-L-Orn-L-Leu-D-Phe)$_2$. Nmr and deuterium exchange studies of this peptide[7-10] agree in supporting a solution conformation of C_2 symmetry in which the N–H proton of each valine and leucine residue is directed inward, in the approximate plane of the peptide backbone, and transannularly hydrogen bonded to the carbonyl residue of an opposing leucine or valine residue. The N–H protons of these residues are thus shielded from interaction with other species. The effect of added radical on the N–H region of the spectrum of gramicidin S in methanol is shown in Figure 3. Although quantitative evaluation is difficult because of the overlap and multiplet structure of the resonances, it is clear from Figure 3 that only the protons assigned[7,9] to the valine and leucine residues escape extensive broadening. Measurements in dimethyl sulfoxide solutions also distinguish among the observed N–H proton resonances. The exposed ornithine (*ca.* 20 Hz/%) and phenylalanine (*ca.* 11 Hz/%) protons have greater line width increments than the shielded leucine proton (*ca.* 7 Hz/%). The valine proton is obscured by the aromatic proton absorption.

We think that this technique may be a useful adjunct to other methods, such as exchange rate and solvent or temperature dependence of chemical shift, in determining the environment of amide protons in studies of peptide conformation.

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On the Detection of the Helix–Coil Transition of Polypeptides by Ultrasonic Absorption Measurements in the Megahertz Range. Case of Poly-L-glutamic Acid

Sir:

The finding of ultrasonic absorption maxima on the plots of absorption *vs.* pH for aqueous solutions of poly-L-glutamic acid[1] (PLGA) and poly-L-ornithine[2] (PLO) has been recently reported. Moreover, Parker, *et al.*,[3] have shown that the excess ultrasonic absorption of poly-L-lysine (PLL) solutions increases with pH up to pH 10.2 where precipitation occurs. These three studies were performed at ultrasonic frequencies above 1 MHz and the results were interpreted as indicating a sensitivity of the ultrasonic absorption in the megahertz range to the equilibria between the helical and coiled conformations of the above polypeptides.

Following these reports similar absorption maxima on the plots of absorption *vs.* pH in protein solutions[4] have been found and have been interpreted as being due to proton transfer processes. Furthermore, the "site binding" of counterions by polyions in polyelectrolyte solutions has been shown to give rise to an excess ultrasonic absorption.[5,6] This effect, in conjunction with the variation of absorption associated with the conformational transition from coil to helix, has been interpreted[7] to be responsible for the ultrasonic absorption maximum appearing on the plots of absorption *vs.* concentration of added salt relative to PLL solutions.[7] The question remains, therefore, as to whether proton transfer and/or counterion site binding occur in solutions of synthetic polypeptides and, if so, as to their contribution to the observed absorption maxima in the cases of PLGA[1] and PLO[2] and to the excess absorption of PLL.[3] The purpose of this paper is to present methods by which a distinction can be made between the contributions of the three processes occurring (proton transfer, counterion site binding, helix–coil transition) and to show how these methods can be applied to ultrasonic studies of PLGA. Results from studies on PLL and PLO are reported elsewhere.[8]

Before discussing the experimental results it must be first pointed out that all of the studies of the effect of pH on the ultrasonic absorption of PLGA[1,9,10] have actually been performed on the sodium salt of PLGA

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(Na-PLG). In order to induce the helix–coil transition, the pH was decreased on adding HCl. The H^+ ions thus introduced into the solution form a covalent bond with the ionized carboxylic groups while the polyion-bound Na^+ ions are released. This brings about a disappearance of the absorption associated with the site binding of Na^+ by the poly-L-glutamate polyion (see below). However, when most of the carboxylic groups are protonated reaction 1 must begin to contribute to the absorption of the PLGA solution. This contribution which has been shown to exist for carboxylic acid[11] and for polycarboxylic acid[12] solutions

$$-CO_2H \rightleftharpoons -CO_2^- + H^+ \quad (1)$$

is known[4] to go through a maximum when the pH is changed. From the above, one should therefore expect proton transfer as well as site binding to contribute to the absorption of PLGA solutions.

Distinction between the absorption due to the counterion site binding process and that resulting from the helix–coil transition can be made by studying solutions of the tetramethylammonium (TMA) salt of PLGA. TMA^+ is such a large ion that it does not give rise to any excess absorption in becoming site bound by a polyion.[5,6] A tetramethylammonium poly-L-glutamate (TMA-PLG) was prepared by passing a Na-PLG solution through an ion-exchange resin which had been preallably neutralized by TMA-hydroxide. The ultrasonic absorption of the TMA-PLG solution was found to be lower than that of an equimolecular solution of Na-PLG. Since both Na-PLG and TMA-PLG have coiled conformations at neutral pH, as shown by optical rotation measurements, this decrease (of about 25×10^{-17} cm^{-1} sec^2 at 2.82 MHz) is concluded to be associated with the vanishing of the absorption due to the binding of Na^+. The effect of pH on the ultrasonic absorption of the TMA-PLG solution was then studied and ultrasonic absorption maxima were obtained at the same pH value (5.1) and with the same amplitude as with Na-PLG: 11 and 17 cm^{-1} sec^2 at 5.04 and 2.82 MHz, respectively. These results thus discard counterion site binding as a process responsible of the absorption maximum reported in ref 1.

In order to distinguish between the ultrasonic absorption due to the helix–coil transition and to proton transfer, comparative measurements were made on PLGA and poly-DL-glutamic acid (PDLGA). These two polypeptides are chemically identical but structurally different. (The sample of PDLGA studied in this work had a molecular weight of 15,000 and was of the type "A," *i.e.*, composed of long sequences of dextrorotatory and levorotatory monomeric units.[13]) Potentiometric and viscosimetric measurements performed on the PDLGA sample did not show any evidence of a helix–coil transition although these techniques are usually quite sensitive to conformational changes.[14] If the absorption maximum reported in ref 1 were due to reaction 1, an identical absorption *vs.* pH curve should be found for both PLGA and PDLGA. On the contrary, the absorption maximum should be strongly affected if it were due to the helix–coil transition. Our experiments support this last prediction since the absorption maximum observed with PLGA disappeared completely with PDLGA. However, the excess absorption measured at neutral pH, *i.e.*, outside the pH range in which the helix–coil equilibrium occurs, was about the same for PLGA and PDLGA. These results definitely show that the absorption maximum observed at pH 5.1[1] is indeed due to the helix–coil equilibrium.

In ref 1 an evaluation of the relaxation time τ characterizing the helix–coil transition, on the basis of our results at 5.04 and 26.2 MHz, led to a value about 10^{-8} sec. These calculations, however, were strongly dependent on the very small and thus inaccurate value of the amplitude A of the absorption maximum at 26.2 MHz. Moreover a much too large value of the nucleation parameter σ was used in these calculations. Using the value of A at 2.82 MHz and values of 5.1×10^{-3} and 1 cm^3/mol, respectively, for σ[15] and for the volume change[16] associated with the transition of PLGA, a value of $\tau \sim 10^{-6}$ sec is found by recalculation by means of eq 1–3 (ref 1). This result is in good agreement with that recently reported by Barksdale and Stuehr.[10]

The methods outlined above unequivocally show that the absorption maximum found in the megahertz range for aqueous solutions of PLGA is due to the helix–coil equilibrium. These methods are applicable to any polypeptide and should aid workers in avoiding misinterpretation of results as was done in the studies on PLO[2] and PLL.[3]

Acknowledgment. The author is pleased to acknowledge Mr. B. Michels for his technical assistance and Professor L. Saint Pierre for proofreading this article.

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Dependence of Sigmatropic Mechanisms on Excited State Multiplicity. Mechanistic and Exploratory Organic Photochemistry. LXVII[1]

Sir:

In studying the photochemistry of *trans*- and *cis*-5,6-diphenylbicyclo[3.1.0]-2-hexene (**1a** and **1b**) and of the 4,5-diphenylbicyclo[3.1.0]-2-hexenes (**2a** and **2b**) we have encountered some fascinating sigmatropic rearrangements. We report: (1) selectivity in the interconversion of these compounds, (2) a unique 1,1-antara–antarafacial rearrangement and a novel 1,3-antarafacial sigmatropic migration with migrating carbon inversion, (3) evidence for a concerted singlet electrocyclic interconversion, (4) an interesting multiplicity-dependent partitioning of sigmatropic pathways, (5) support for nonintersecting potential energy

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