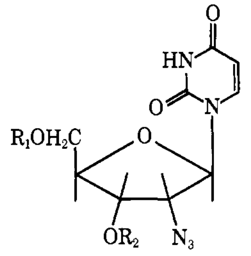
alkaline phosphatase, snake venom phosphodiesterase, and micrococcal nuclease.[11] As required by the absence of a 2′-OH function, poly U_z was completely resistant to degradation by pancreatic ribonuclease.[12]



I, $R_1 = R_2 = H$
II, $R_1 = (C_6H_5)_3C-; R_2 = H$
III, $R_1 = (C_6H_5)_3C-; R_2 = CH_3CO$
IV, $R_1 = H; R_2 = CH_3CO$
V, $R_1 = PO_3^{2-}; R_2 = H$
VI, $R_1 = P_2O_6^{3-}; R_2 = H$

The thermal stability of (presumably) single-stranded poly U_z is novel (Figure 1A), because with $T_m = 12°$, it surpasses $T_m = 6°$ for poly U under comparable conditions.[13] The shape and midpoint of the phase transition did not depend on the solvent (1.0 M NaCl, pH 7.5, or 0.01 M $MgCl_2$, pH 7.4), but became less cooperative and markedly elevated (~10°) in the presence of 0.1 M $MgCl_2$. Poly U_z formed a 1:1 complex with poly A as evidenced by the eutectic point at 50 mol %. This complex underwent a smooth cooperative transition with $T_m = 59°$ (Figure 1B), not markedly different from poly A·poly U.[14] All transitions were completely reversible.

Poly U_z is the first example of a stable secondary structure in a single-stranded polynucleotide without a 2′-oxygen function.[3] When the 2′-hydroxyl of poly U is replaced by chlorine, the single-stranded poly U_{Cl} is destabilized,[3] whereas substitution by the azido group has the opposite effect. Since both poly U_z and poly U_{Cl} form equally stable double-stranded complexes with poly A, the predominant stabilizing influence must differ in the single-stranded forms.

The availability of a model as unique as poly U_z should prove helpful in further studying the nature of the forces operative in stabilization, transcription,[15] and interferon stimulation[16] now in progress.

After this paper was submitted for publication, the syntheses and physical properties of poly (2′-fluoro-2′-deoxyuridylic acid)[17] (poly U_F) and poly(2′-amino-2′-deoxyuridylic acid)[18] (poly U_A) appeared. Surprisingly, both poly U_F and poly U_A are devoid of significant secondary structure at temperatures $> 2°$. The latter authors[18] were also able to prepare poly U_z but did not characterize it further. Thus, while 2′-fluoro, 2′-chloro, 2′-amino, and 2′-deoxy substituents decrease the observed secondary structure of polyuridylic acid, the 2′-methoxy and 2′-azido substituents *alone* give rise to an increase in secondary structure.

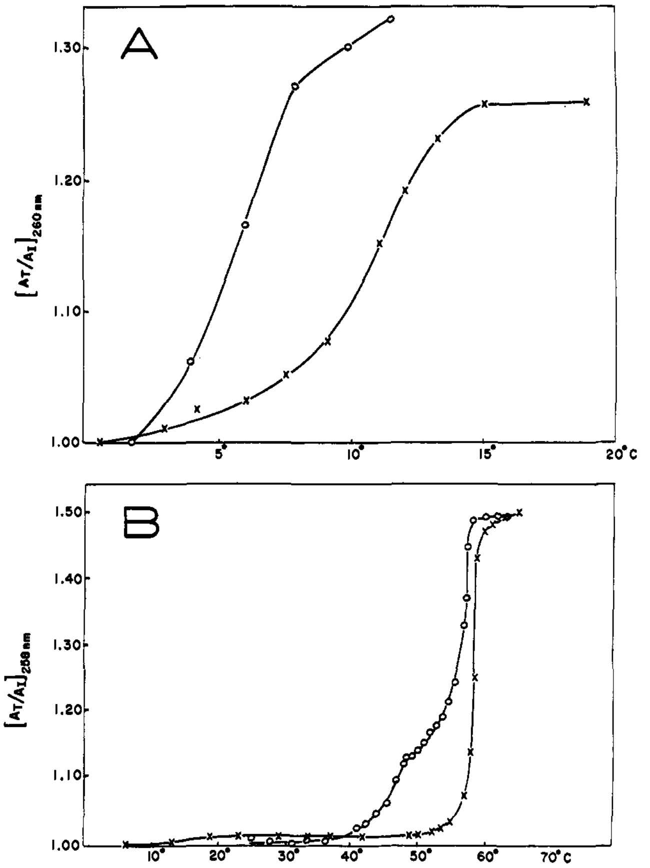


Figure 1. (A) Uv absorption–temperature profile of poly U (O) and poly U_z (X) in 0.01 M $MgCl_2$, pH 7.6. A_T/A_I is the ratio of absorbance at temperature T over the absorbance at the initial temperature. (B) Uv absorption–temperature profile of the complex poly A·2 poly U (O) and poly A·poly U_z (X) in 0.1 M NaCl, 0.01 M NaH_2PO_4, pH 7.5. The inflection at 45° represents melting of the triple-stranded poly A·2 poly U complex while the transition at 57° represents melting of the double-stranded poly A·poly U complex (also see ref 14).

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[19] National Institutes of Health Staff Fellow, July 1969–present.

Helix–Coil Transition of a Synthetic Polypeptide Monitored by Fourier Transform Carbon-13 Nuclear Magnetic Resonance

Sir:

We wish to report the preliminary results of the first[1] application of ^{13}C nmr to the study of the helix–coil transition of a synthetic homopolypeptide. We

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[1] (a) Dr. P. A. Temussi of the CNR laboratories in Naples, Italy, has obtained some ^{13}C nmr spectra of poly(γ-benzyl-L-glutamate) with results which seem to be similar to our findings (communicated to the 6th Conference of the Italian Association of Physical Chemistry, Siena, Italy, Dec 1971). (b) NOTE ADDED IN PROOF. These results now appear in: L. Paolillo, T. Tancredi, P. A. Temussi, E. Trivellone, E. M. Bradbury, and C. Crane-Robinson, *J. Chem. Soc., Chem. Commun.*, 335 (1972).

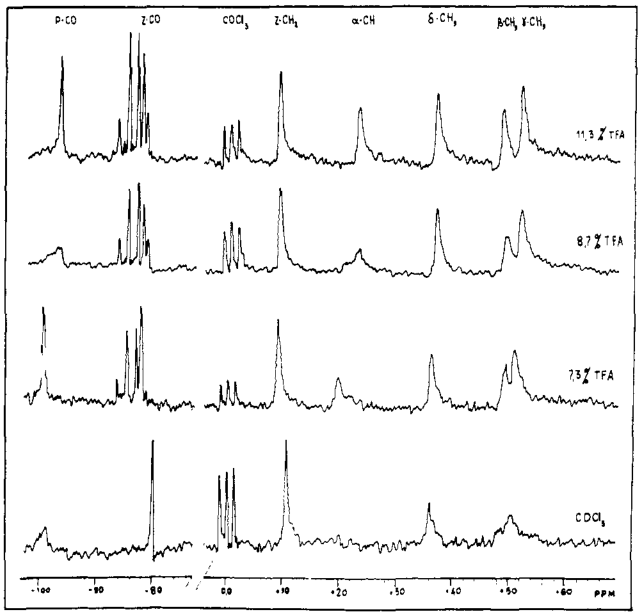

Figure 1. Fourier transform ^{13}C nmr spectra of 7% w/v solutions of poly(*N*-δ-carbobenzoxy-L-ornithine) in different $CDCl_3$–TFA mixtures. Solvent compositions are expressed as volume fractions. The assignments are marked at the top with reference to the formula in Figure 2. The unmarked quartet in the Z–CO region is due to the TFA carbonyl. The number of transients accumulated per spectrum is 20,000.

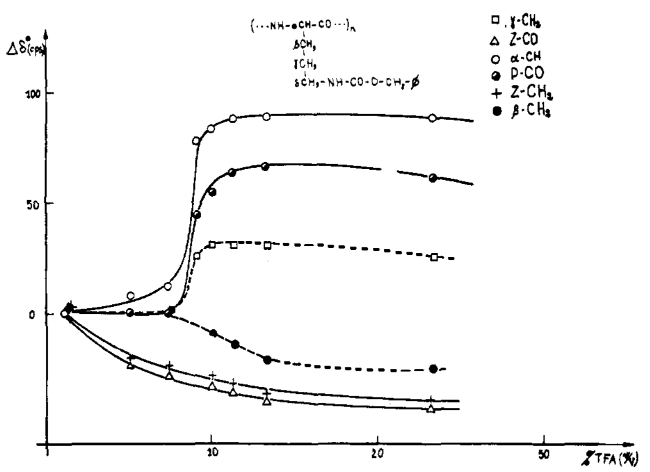

Figure 2. Relative chemical shifts of the various carbon atoms of poly(*N*-δ-carbobenzoxy-L-ornithine) in different mixtures of $CDCl_3$–TFA. The shifts are all referred to their value in a mixture containing 0.01 volume fraction of TFA.

have chosen poly(*N*-δ-carbobenzoxy-L-ornithine) (PCBO), a polymer on which optical, calorimetric, and proton magnetic resonance studies[2] have been recently performed in these laboratories.

PCBO in the solvent system trifluoroacetic acid (TFA)–deuteriochloroform shows a sharp helix–coil transition at room temperature when the TFA volume fraction is ~0.10.[2b] Figure 1 shows the spectra of 7% w/v solutions of PCBO in different TFA–$DCCl_3$ mixtures.

The sample of PCBO used was prepared by polymerization of the corresponding *N*-carboxyanhydride in dimethylformamide, using isopropylamine as initiator and a monomer–initiator molar ratio of ~20.

Natural abundance ^{13}C spectra were obtained on a Varian XL-100-15 spectrometer operating at 25.2 MHz and having a field–frequency control system based on the deuterium resonance signal derived from the $CDCl_3$ contained in the solvent mixture. All the proton lines were decoupled by broad-band (~3000 Hz) irradiation from an incoherent 100-MHz source. A constant flow of room temperature air through the probe maintained the sample temperature near 35°. The sample was contained in 12-mm o.d. tubes and was not degassed. Radiofrequency pulses were generated by a Varian VFT-100 accessory associated with a 620i computer.

The assignment of the different carbon signals is marked on Figure 1 and was made by comparison with known spectra of ornithine and of carbobenzoxylate amino acids.[3] The first thing to be noted is a marked line-width effect on the peptide backbone signals. Both the carbonyl and the α-C carbons are broad in pure $CDCl_3$ (α-C is not even detectable in this condition), they get narrow (especially the carbonyl signal) at low (<0.07) and high (>0.11) TFA volume fractions, and they are broad again within the range of TFA concentrations corresponding to the conformational transition. Broadening in pure $CDCl_3$ is a common phenomenon also in proton nmr and it is almost certainly due to association among helices to form very *rigid* structures. Very small amounts of TFA are known to be capable of breaking these tertiary structures and yielding solutions containing isolated molecules, hence, for low molecular weight polymers, narrow lines. It is more difficult to interpret the broadening appearing in the neighborhood of the transition point. The phenomenon may well have the same origin as that which produces the appearance of two α-CH peaks in the transition region as found in some of the proton spectra of low molecular weight polypeptides.[4] A careful study of the line shape under conditions of enhanced resolution and sensitivity is necessary, however, before one can reach any significant conclusion.

The chemical shifts of the various carbon atoms (which were measured with respect to the $CDCl_3$ carbon signal) relative to their value in a solvent containing ~0.01 vol fraction of TFA are plotted in Figure 2. The values for the phenyl and δ carbons have not been plotted being practically insensitive to solvent variation.

The other carbon atoms can be divided into three classes according to the behavior of their chemical shift: (i) the backbone carbons, *p*-CO and α-C, which show a sharp shift of approximately 80 Hz upfield in the transition region, which appears to be centered, under the present experimental conditions, at ~0.08 TFA volume fraction; (ii) the carbon atoms of the urethane group and its immediate neighborhood, Z–CO and Z–CH_2, which show a gradual exponential downfield shift to a limit of about 50–60 Hz with respect to their value in the 1% solution; (iii) the β and γ carbon atoms, which remain insensitive to solvent variation from 1 to

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7% volume TFA and show opposite shifts to limits of about ±25 Hz only after the transition has started.

The superiority of the information contained in the chemical-shift behavior in ^{13}C nmr with respect to ^1H nmr for these systems is clearly indicated by these results. In the proton spectra only the α-CH and, when observable, the NH signals show significant shifts limited to the transition region. In the carbon spectra the chemical shifts seem to map in a very detailed way both the conformation and the solvation state of the polymer.

It is hopeful that the utilization of this information, qualitatively and quantitatively improved by experiments at higher resolution, coupled with the careful study of solvation effects in model systems may bring about a detailed understanding of the peptide-solvent interaction.

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Isotopic Exchange of Molecular Hydrogen in Dimethyl Sulfoxide-Water Mixtures under Base Catalysis[1]

Sir:

The interaction between molecular hydrogen (or deuterium) and hydroxide ion in aqueous solution, leading to ortho-para conversion or isotopic exchange,[2] is conceptually one of the simplest of reactions, involving molecular species of only moderate complexity. It is of considerable interest theoretically as a possible model for proton-transfer processes. Also, the reaction could have utility in the production of D_2O,[3] although the rate of isotopic exchange in aqueous base is far too slow. Theory[4] predicts, however, that molecular hydrogen does *not* react with OH^- by rate-determining proton transfer, with formation of hydride ion, as might have been expected.[2] Instead, it is proposed[4] that formation of an addition complex $[HODD]^-$ is the rate-determining step in the overall exchange process.

We wish to report results on the isotopic exchange process which are pertinent to the theoretical and practical aspects referred to above. Our approach involves the use of dipolar media[5,6] and correlations with acidity functions applicable to reaction rates in strongly basic solutions.[7,8]

Isotopic exchange experiments were performed with Me_4NOH as base in a closed vessel containing the vigorously stirred DMSO-water solution saturated with D_2 at a partial pressure of 1 atm. The solubility of D_2 was estimated from measurements made with H_2 in these media; the H_2 solubility data have been reported.[9] Aliquots of gas were withdrawn periodically and analyzed mass spectrometrically for D_2, HD, and H_2. First-order rate constants were obtained from plots of log (mole per cent D_2) *vs.* time. The resulting vapor-phase data were converted to in-solution rate constants through multiplying by the factor (total moles of D_2 in the system)/(moles of D_2 dissolved in the liquid phase). Present results refer to 65°; data obtained at other temperatures, leading to activation parameters as a function of medium composition, will be reported subsequently.

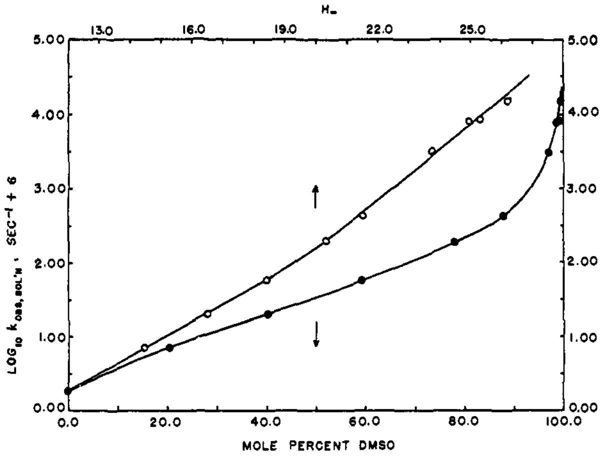

Figure 1. Dependence of the logarithm of the first-order, in-solution rate constants for D_2 exchange on medium composition (solid circles) and H_- (open circles) at 65°. The rate data have been extrapolated to a common base concentration, 0.011 *M* Me_4NOH. The value for pure water was calculated from the data of ref 2.

Our results on the rate of isotopic exchange as a function of medium composition are shown in Figure 1. It is seen that the rate of exchange increases by a factor of 10,000 as the medium composition is changed from purely aqueous to 99.5% DMSO. This increase in rate, although not particularly large for such a change in medium, is in accord with the generally observed enhanced reactivity of hydroxide ion in DMSO-water mixtures and is ascribed largely to progressive desolvation of hydroxide ion as the hydroxylic component is replaced by the poorly hydrogen-bonding DMSO.[5,6]

The plot of log (exchange rate) *vs.* H_-, the acidity function for the DMSO-water system, is somewhat curved, with an initial slope of 0.23 in the aqueous region increasing to 0.36 at the DMSO rich end. These slope values appear to be the smallest yet reported for an overall proton-transfer process. The slopes of log (rate)-H_- plots have recently been interpreted as signifying the degree of transfer of a proton from substrate to base.[10,11] Taking hydrogen isotope exchange in the CH_3SOCH_3-OH^--H_2O system, for example,[10] the observed slope of 0.93 was interpreted as indicating that the proton from CH_3SOCH_3 is practically completely transferred to hydroxide ion in the transition state. Thus the observed slope of 0.23-0.35 in the D_2-OH^- reaction is indicative of a very small degree of proton transfer from substrate to base in the rate-limiting tran-

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