tration increases. At 4 *M* $CaCl_2$ or above a very broad resonance remains in the trans region of the spectrum, indicating that at most only short sequences of form II structure remain at this and higher concentrations. This is in agreement with conclusions of Mattice and Mandelkern,[13] who followed changes in circular dichroism and intrinsic viscosity of poly(L-proline) as a function of $CaCl_2$ concentration.

In an effort to rationalize the stability of a poly(L-proline) chain containing a mixture of cis and trans peptide bonds in the salt solutions, the conformations of structures containing mixtures of cis and trans bonds were examined using CPK models. It was found that carbonyl oxygens in sequences such as trans–cis–trans or trans–cis–cis are favorably positioned to bind a cation. Evidence for Li^+ binding to poly(L-proline) in LiBr solutions has been reported by Kurtz and Harrington,[9] who concluded that *partial* rotation about the peptide bonds was due to the formation of a multiresidue Li^+ complex. Our picture of the complex indicates that it should contain at most 1 mol of bound salt for every 3 mol of peptide. Kurtz and Harrington found that the complex contained at least 1 mol of Li^+/5 mol of proline, which is consistent with the model proposed.

It has long been recognized that the rates for reactions of the type form I $\rightleftharpoons$ form II or form I $\rightleftharpoons$ [disordered state] are much smaller than the rate for the form II $\rightleftharpoons$ [disordered state] reaction. We believe that the results presented above firmly establish the presence of cis peptide bonds in salt solutions of poly(L-proline) and therefore conclude that all these reactions involve cis–trans isomerizations of the peptide bond. The activation enthalpy is about 20 kcal/mol for all these reactions. This implies that there is a large negative entropy of activation in reactions involving the isomerization of poly(L-proline) I but not of form II. Why this is so is not clear, but may be related to the extraordinarily compact structure of form I.

In summary, our data suggest that in D_2O, form I poly(L-proline) of low molecular weight isomerizes to form II in a stepwise fashion, starting at the carboxyl end of the chain. Addition of certain salts to aqueous solutions of poly(L-proline) in either form I or form II disrupts the organized polymer structure primarily by producing cis–trans isomerizations at the peptide bonds. At low to moderate salt concentrations the polymer contains ordered sequences of trans bonds and disordered sections containing a mixture of cis and trans bonds. At high salt concentrations the ordered regions are eliminated, leaving a polymer containing mixtures of cis and trans bonds along the entire length of the chain. It is suggested that the resulting disordered structure is stabilized by the formation of a multiresidue cation complex.

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Proton Nuclear Magnetic Resonance Study of Corticotropins

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ABSTRACT: This study outlines a proton nmr investigation of the hormone fragments of ACTH in neutral and acidic aqueous solution. Difference and addition proton nmr spectral studies of $ACTH^{1-10}$, $ACTH^{11-24}$, and $ACTH^{1-24}$ in the peptide NH and CH regions in acidic aqueous solution indicated the absence of interactions between sequences 1–10 and 11–24 in $ACTH^{1-24}$.

Much activity has been focused over the last few years on the isolation, purification, and synthesis of the adenohypophyseal hormone adrenocorticotropin (ACTH).[1]

$ACTH^{1-24}$

Ser-*Tyr*-Ser-*Met-Glu-His-Phe-Arg-Trp-Gly*-Lys-*Pro*-Val-Gly-
1 5 10
Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro
15 20

The 39 amino acid linear hormone ACTH is believed to stimulate steroidogenesis in the adrenal gland by the specific activation of enzymes. The amino acid residues at the C terminal and at positions 25–33 show species variation. The biologically active fragment $ACTH^{1-24}$ contains the basic amino acid sequence Lys-Lys-Arg-Arg at positions 15–18 which is currently believed to be the binding site for the receptor. The loss of adrenocorticotropic activity on protection of the N-terminal end and the results of amino acid modifications of the positions 1–10 of ACTH have led to the tentative identification of the functional site of the adrenocorticotropic activity with the N-terminal end.

Several approaches to the study of secondary structures of these hormones by physical techniques have recently been reported. Eisinger[2] has studied the efficiency of singlet energy transfer from tyrosine residues at positions 2 and 23 to the tryptophan at position 9 in $ACTH^{1-24}$. The measured intramolecular distances (Tyr-2–Trp 9 = 10 Å, Trp-9–Tyr 23 $\geq$ 19 Å) obtained from an analysis of emission data using Forster's theory suggest some form of loop or helical segment between residues 2 and 9. Edelhoch and Lippoldt[3] have studied the effect of pH, temperature, and guanidine on both the tyrosyl and tryptophan emissions in $ACTH^{1-25}$. They concluded that none of the structural parameters revealed struc-

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TABLE I
THE NUMBER OF EXCHANGEABLE PEPTIDE NH, LYSINE AND ARGININE SIDE CHAIN NH, AND AROMATIC PROTONS IN ACTH FRAGMENTS IN AQUEOUS SOLUTION AT 23°

Sample in 5% CH_3CO_2H–H_2O	7.9–8.8 ppm			7.3–7.9 ppm		6.9–7.3 ppm		6.5–6.9 ppm	
	Pep. NH	Arom	Other	Lys	Arom	Arom	Arg	Arom	Arg
$ACTH^{1-10}$	9	1	0	0	2	11	1	2	3–4
$ACTH^{11-24}$	10	0	1	9–10	0	2	2	2	6–7
$ACTH^{1-24}$	20	1	0	8–11	2	13	2–3	4	9–10

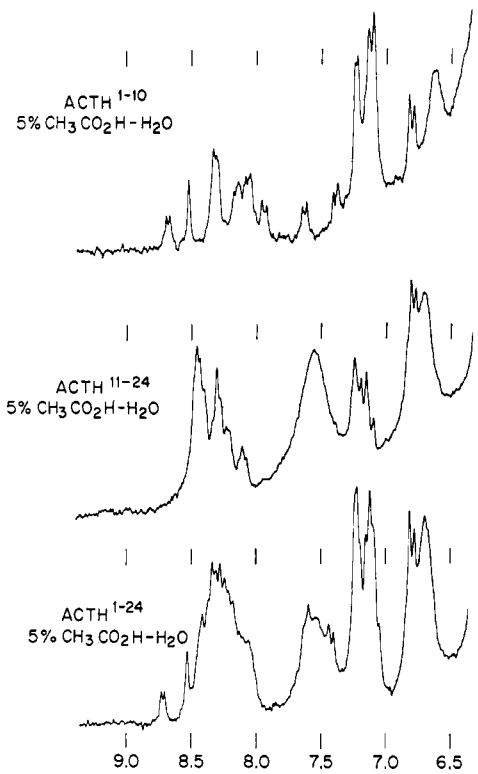

Figure 1. Proton nmr spectra (220 MHz) of exchangeable hydrogens of ACTH fragments in 5% CH_3CO_2H–H_2O between 6.5 and 9.0 ppm.

tural modifications. Circular dichroism studies by both these groups indicate that the α-helical content, if any, is small.

The availability fragments of ACTH permitted us to undertake a proton nmr study to try to elucidate the nature of the secondary structure of these hormones. From our related studies of glucagon[4] and secretin[5] we were aware that the peptide NH and CH protons were sensitive probes for polypeptide conformation and consequently our main effort was directed toward these regions of the spectra. The availability of the fragments $ACTH^{1-10}$, $ACTH^{11-24}$, and $ACTH^{1-24}$ permitted a difference and addition spectral analysis[6] of the peptide NH and CH region to elucidate the interactions, or lack of them, between the residues 1–10 and 11–24.[7]

Experimental Section

The ACTH fragments used in this study were prepared at Ciba, Basel. They are unprotected at both N- and C-terminal ends with all side chains free. The fragments were in the form of lyophilisates containing some hydrate water and acetic acid to neutralize the basic side chains. The samples were dissolved in 5% acetic acid–water (protonated or deuterated) and their proton spectra studied on a 220-MHz Varian nmr spectrometer equipped with a variable-temperature unit. Spectra were also run at neutral pH. All spectra were time averaged to improve signal to noise on a Fabri-Tek computer of average transients with 1024 channels. Addition and difference nmr spectra[6] were obtained by adding to or subtracting from previously stored information in the computer.

Results and Discussion

The amino acids arginine and lysine contain exchangeable NH hydrogens in their side chains. Under conditions of slow exchange with water these NH resonances would be observable in the aromatic and peptide NH regions. The amino acids Arg and Lys, protected at N- and C-terminal ends, were investigated over a pH range in aqueous solution. In 5% CH_3CO_2H–H_2O, the arginine and lysine peptide NH doublets appear at 8.4 ppm. Upfield from these resonances are the single secondary guanidinium proton of Arg at 7.22 ppm and four primary guanidinium protons of Arg at 6.70 ppm and the three amino protons of Lys at 7.54 ppm. Since the Arg and Lys exchangeable side chain NH protons have different chemical shifts, they can be differentiated. The Arg and Lys exchangeable side chain NH protons show pH-independent chemical shifts but are broadened by rapid exchange with water around pH 7 for arginine and the lower pH 5.5 for lysine. This pH dependence can also be used to differentiate between Lys and Arg exchangeable side chain hydrogens. Internal hydrogen bonding of these positively charged exchangeable hydrogens of Lys and Arg with negatively charged (carboxylate) and uncharged (carbonyl) groups can result in large downfield shifts of up to 4–5 ppm.[8,9]

Figure 1 outlines the 6.5–9.0 ppm region of $ACTH^{1-10}$, $ACTH^{11-24}$, and $ACTH^{1-24}$ in 5% CH_3CO_2H–H_2O at 23°. All three ACTH fragments are free at N- and C-terminal ends. In $ACTH^{1-10}$ and $ACTH^{1-24}$ the doublet due to the Tyr-2 peptide NH proton at 8.7 ppm was used as a calibrant to measure areas under remaining peaks. The areas for $ACTH^{11-24}$ and $ACTH^{1-24}$ were calibrated using the upfield 18-proton valine region around 1 ppm. Table I summarizes the data for these three ACTH fragments. $ACTH^{1-10}$ contains one each of the aromatic amino acids and one arginine. The four exchangeable side chain protons of arginine appeared at 6.6 ppm. They are thus unchanged from their position in the free amino acid. $ACTH^{11-24}$ contains a single tyrosine, two arginines, and four lysines. There is a strong peak at 7.6 ppm of 9–10 hydrogens corresponding to the four lysines in $ACTH^{11-24}$. Using the upfield 18-proton valine resonance as a calibrant, two and six to seven exchangeable hydrogens were found in the 6.9–7.3-ppm and 6.5–6.9-ppm ranges over and above the aromatic and peptide NH protons. The ex-

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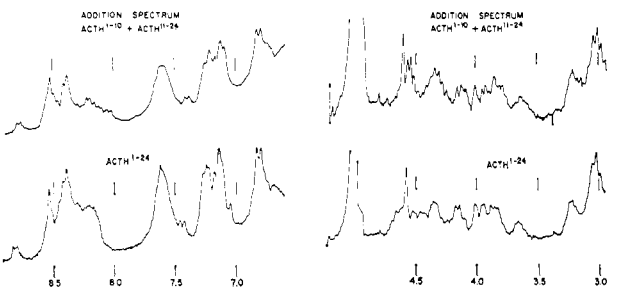

Figure 2. Comparison of the 220-MHz spectrum of $ACTH^{1-24}$ with the addition spectrum ($ACTH^{1-10}$ + $ACTH^{11-24}$) in 5% CH_3CO_2H–H_2O in the peptide NH and in 5% CD_3CO_2D–D_2O in the peptide CH region.

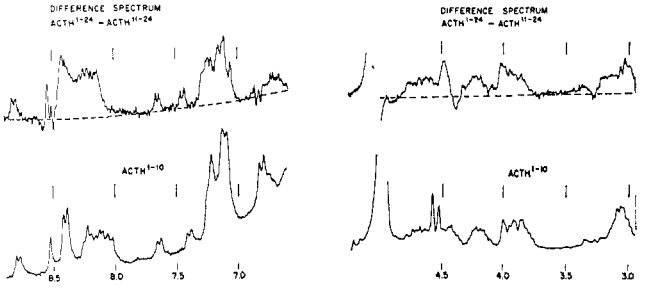

Figure 3. Comparison of the 220-MHz spectrum of $ACTH^{1-10}$ with the difference spectrum ($ACTH^{1-24}$ – $ACTH^{11-24}$) in 5% CH_3CO_2H–H_2O in the peptide NH and in 5% CD_3CO_2D–D_2O in the peptide CH region.

changeable side chain NH protons of Arg-17 and Arg-18 can contribute two secondary and eight primary protons. Thus the lysine and arginine protons are accounted for and their chemical shifts correspond to those of the free amino acid. In $ACTH^{1-24}$ one has three arginines and four lysines. The strong peak at 7.6 ppm has an area of 9–12 protons (varied in samples) relative to the peak at 8.7 ppm of one proton. These are assigned to the 8–11 side chain exchangeable protons of four lysines and 1 proton of tryptophan indole doublet. Thus the lysines are approximately accounted for and occur at the same chemical shift. This suggests the absence of secondary structure or hydrogen bonding for the lysines in $ACTH^{1-24}$ in acidic medium. The three arginines in $ACTH^{1-24}$ have their exchangeable side chain NH resonances at 7.2 ppm (2–3 protons) and 6.6 ppm (9–10 protons) corresponding to their secondary and primary nature. In $ACTH^{1-24}$ we also observe that the arginine and lysine side chain NH resonances appear at their characteristic amino acid positions.

The peptide NH and CH proton resonances which are directly attached to the polypeptide backbone should be a sensitive function of the secondary structure of the polypeptide. The interaction, or lack of it, between the ACTH sequences 1–10 and 11–24 in $ACTH^{1-24}$ was approached by investigating addition and difference spectra in peptide NH and CH region of $ACTH^{1-10}$, $ACTH^{11-24}$, and $ACTH^{1-24}$ in acidic aqueous solution. The N- and C-terminal ends of these polypeptide fragments were free.

Our current knowledge indicates that peptide NH resonances in a helix are downfield while those in a pleated sheet are upfield relative to their positions in a coil in polar nonaqueous solvents. Consider end effects in a comparison of ($ACTH^{1-10}$ + $ACTH^{11-24}$) with $ACTH^{1-24}$. In $ACTH^{1-10}$, amino acid 10 will have an unprotected C-terminal side (relative to $ACTH^{1-24}$) resulting in shielding by the CO_2^- group of the C-terminal Gly-10 peptide NH resonance. In $ACTH^{11-24}$, Lys-11 will have an unprotected N-terminal side (compared to $ACTH^{1-24}$) resulting in deshielding by the NH_3^+ group of the NH peptide resonances from the N-terminal side.

Figure 2 outlines a comparison of the peptide NH, exchangeable side chain NH, and aromatic regions of $ACTH^{1-24}$ with those of the addition spectrum ($ACTH^{1-10}$ + $ACTH^{11-24}$). The peptide NH region encompasses the 8.0–8.7-ppm region. The Tyr-2 peptide NH doublet at 8.7 ppm corresponds to a single proton in both spectra. (The lower spectrum is attenuated ~50% over the top addition spectrum.) Comparison of the spectra indicate resonances at 8.5 and 8.05 ppm in the addition spectrum which are absent in this region in $ACTH^{1-24}$. These differences are readily accounted for by end effects originating in the N-terminal side of $ACTH^{11-24}$ and the C-terminal side of $ACTH^{1-10}$, respectively, discussed above. Beyond these changes there are no observable differences in the peptide NH, side chain NH, and aromatic regions on comparison of the addition spectrum with $ACTH^{1-24}$. Comparison of $ACTH^{1-10}$ with the difference spectrum ($ACTH^{1-24}$ – $ACTH^{11-24}$) must take into account end effects (Figure 3). Position 10 is free at the C-terminal end in $ACTH^{1-10}$ but a peptide bond in $ACTH^{1-24}$ in the difference spectrum. The C-terminal CO_2^- in $ACTH^{1-10}$ shifts the Gly-10 peptide NH resonance to 8.05 ppm for this fragment. Thus an end effect accounts for the resonance at 8.05 ppm in $ACTH^{1-10}$ which is absent in the difference spectrum. Subtraction of $ACTH^{11-24}$ from $ACTH^{1-24}$ must consider the effect of the free N-terminal position at Lys-11 in the former fragment when compared with a peptide bond at this position in the latter fragment. The observed upfield shift of negative resonances at 8.55 ppm to the positive region at 8.3 ppm in the difference spectrum reflect the deshielding effect of the free peptide NH_3^+ group at Lys-11 in $ACTH^{11-24}$ on the peptide NH resonances of amino acids 13 and 14 (there is a proline at position 12). Thus the variations between the peptide NH regions of $ACTH^{1-10}$ and the difference spectrum ($ACTH^{1-24}$ – $ACTH^{11-24}$) can be accounted for by end effects. Figure 1 also indicates that the exchangeable 7.6-ppm side chain Lys NH resonances for this amino acid at positions 11, 15, 16, and 21 in $ACTH^{1-24}$ exactly cancel out their corresponding lysine resonances in $ACTH^{11-24}$. This indicates that amino acids extending from positions 1 to 10 have no effect on the chemical shifts of the four lysines in the fragment 11–24. Figure 4 outlines a comparison of the 6.5–9.0-ppm region of $ACTH^{11-24}$ with the difference spectrum ($ACTH^{1-24}$ – $ACTH^{1-10}$). The downfield shift of the negative resonance at 8.05 ppm to the positive region at 8.2 ppm in the difference spectrum is due to a C-terminal CO_2^- at Gly-10 in

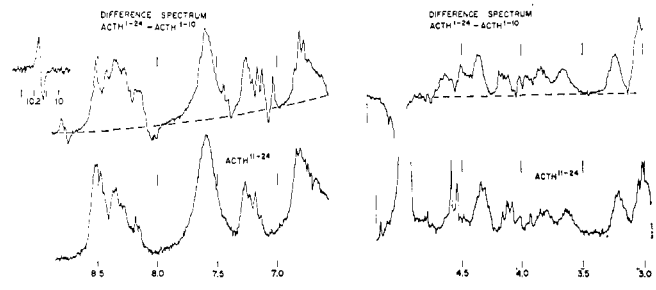

Figure 4. Comparison of the 220-MHz spectrum of $ACTH^{11-24}$ with the difference spectrum ($ACTH^{1-24}$ – $ACTH^{1-10}$) in 5% CH_3CO_2H–H_2O in the peptide NH and in 5% CD_2CO_2D–D_2O in the peptide CH region.

$ACTH^{1-10}$ but to a peptide bond at this position in $ACTH^{1-24}$. Comparison of $ACTH^{11-24}$ with the difference spectrum indicates variations between 8.4 and 8.5 ppm. End effects can account for this difference since in $ACTH^{11-24}$ peptide NH resonances near the protonated free N-terminal end are deshielded when compared with the same resonances in $ACTH^{1-24}$ where there is a peptide bond. Thus end effects can account for what apparently look like large differences in the peptide NH region. The Trp-9 indole NH resonance at 10.15 ppm, the Tyr-2 peptide NH proton at 8.75 ppm, and the His-6 C-4 resonance at 7.05 ppm show small chemical shift variations between $ACTH^{1-10}$ and $ACTH^{1-24}$ since they do not exactly cancel out in the difference spectrum. The doublets at 6.75 and 7.25 ppm in the difference spectrum correspond to the aromatic protons of Tyr-23. These resonances were not well resolved in $ACTH^{11-24}$ from the superimposable side chain arginine NH resonances. In summary, the addition and difference spectral studies in the 6.5–9.0-ppm region indicate no interaction between sequences 1–10 and 11–24 in $ACTH^{1-24}$ in acidic solution.

Addition and difference spectral analysis of the peptide CH resonances extending over 3.5–5.0 ppm are outlined in Figures 2–4. Peptide CH resonances in a helix are known to come upfield from their relative positions in a coil in polar nonaqueous solvents. End effects discussed earlier for the peptide NH region apply here also although their magnitude and direction are known with less certainty. There is a sharp side band ~100 Hz upfield from the HOD resonance.

Figure 2 outlines a comparison of the 3.5–5.0-ppm peptide CH region of $ACTH^{1-24}$ with the addition spectrum ($ACTH^{1-10}$ + $ACTH^{11-24}$). The spectra are very similar. The evaluation of end effects in the peptide CH region is not known. Figure 3 outlines the spectrum of $ACTH^{1-10}$ in the peptide CH region along with the difference spectrum ($ACTH^{1-24}$ – $ACTH^{11-24}$). Other than a shift from 4.4 ppm in $ACTH^{11-24}$ to 4.5 ppm in $ACTH^{1-24}$, the spectra lack differences. Comparison of the difference spectrum ($ACTH^{1-24}$ – $ACTH^{1-10}$ with $ACTH^{11-24}$ in the 3.5–5.0-ppm region is outlined in Figure 4. The patterns in the two spectra are similar. The data indicate that the addition and difference spectra in the peptide CH region do not provide evidence for interactions between sequences 1–10 and 11–24.

Mass Spectral Characteristics of Poly(4,4′-isopropylidenediphenyl carbonate)

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ABSTRACT: The mass spectra have been obtained for 4,4′-isopropylidenediphenol (IDP), the diphenyl carbonate of IDP (I), the biscarbonate of IDP (II), the cyclic trimer (III) and tetramer (IV) carbonates of IDP, and poly(4,4′-isopropylidenediphenyl carbonate) (V). Fragments of mass up to *m/e* 762 corresponding to the cyclic trimer have been observed at sample probe inlet temperatures of 250–400°. The spectra show characteristic patterns which correlate with the loss of methyl groups and carbon dioxide and with hydrogen rearrangement reactions which occur during the loss of aryloxy groups from aryl ethers and during the loss of carbon dioxide. These patterns are consistent with those observed for diphenyl carbonate by others and for the model compounds I and II. They are confirmed by metastable patterns and precise mass measurements.

The mass spectral characteristics of poly(4,4′-isopropylidenediphenyl carbonate) (Lexan resin) have been determined and correlated with those of diphenyl carbonate,[1,2] 4,4′-isopropylidenediphenol (IDP), the diphenyl carbonate of IDP, the carbonate of isopropylidenediphenol, and the cyclic trimer and tetramer IDP carbonates. The techniques used have been previously described in reports on the mass spectral characteristics of poly(*p*-xylylene),[3] poly(pivalolactone),[4] and poly(2,6-dimethyl-1,4-phenylene ether).[5] Fragments of mass up to *m/e* 762 have been observed and the degradation patterns correlated with those of related structures.[6–15]

Experimental Section

The poly(4,4′-isopropylidenediphenyl carbonate), the acyclic dimer, and the cyclic trimer[16] and tetramer[16,17] were kindly supplied by Dr. Arnold Factor of the General Electric Co. Research and Development Center. This polymer is terminated with a monofunctional phenol, has a residual hydroxyl content of less than 0.05 wt %, has an intrinsic viscosity of 0.49 dl/g, and is a typical unstabilized commercial (G.E.) polymer. Diphenyl carbonate (DPI-655) was used as received. 4,4′-Isopropylidenediphenol (DPI-6767) was used as received, after careful recrystallization (mp 157°) from benzene, and as the residue left after essentially complete dissolution in hot benzene. The diphenyl carbonate of 4,4′-isopropylidenediphenol (previously undescribed) was prepared by Mr. K. S. Kim using the Schotten–Baumann technique and was recrystallized twice from ethanol, mp 102–103°.

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