100 Mc./sec. Nuclear Magnetic Resonance Study of the Helix–Coil Transformation in Polypeptides

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THE high-resolution proton n.m.r. spectra of a number of polypeptides have been studied under various concentration ratios of deuterochloroform (helix-supporting solvent) and trifluoroacetic acid (random-coil-supporting solvent).[1,2] Since polypeptides serve as protein model compounds, such studies are of considerable importance and may contribute significant new information to the knowledge of protein conformations. It has been shown that certain changes in spectral features can be correlated with the variation of the Moffitt parameter, b_0.[2] These changes have, therefore, been interpreted as resulting from the helix–coil transformation. Although these transformations have been studied by various physical means, the mechanism involved and the types of forces which are operative in causing these transformations are not yet clear. Hanlon *et al.*[3] have suggested that some polypeptides are protonated at the amide moiety, whereas Stewart *et al.*[4] have concluded on the basis of n.m.r. data that trifluoroacetic acid (T.F.A.) hydrogen-bonds to the helix, thus effecting the helix–coil transformation. However, a recent circular dichroism study by Quadrifoglio and Urry[5] has indicated that there is no evidence of protonation of the amide bond before the transformation takes place and also that, if hydrogen bonding of T.F.A. to the helix does exist, it does not greatly alter the helical polypeptide co-ordinates.

Poly-L-leucine (P.L.L.) and poly-(β-methyl-L-aspartate) (P.M.A.) are examples of right- and left-handed helical screw senses, respectively. In the case of a right-handed helix such as P.L.L., the conformation of the amino-acid residue is usually such that optimum hydrogen-bonding between the first and fourth residues is allowed. The conformation corresponding to a left-handed helix is instead such that the C=O and N–H bonds may not be properly oriented for optimum hydrogen-bonding.[6] Furthermore, previous studies have shown that interactions between nonbonded atoms, which are clearly different for right- and

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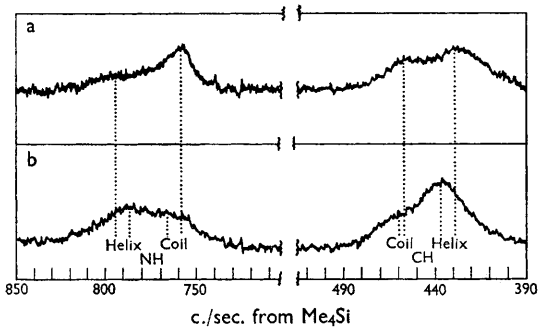
left-handed helices, are important in determining helical conformational potential energies and the thermodynamics of the helix–coil transformation. P.L.L. and P.M.A. are examples of right- and left-handed helices, respectively. The above differences in the hydrogen-bonded and the nonbonded interactions between P.L.L. and P.M.A. should produce some differences in their spectral features.

The 100 Mc./sec. spectra of P.L.L. and P.M.A. were investigated in detail over the entire $CDCl_3$–T.F.A. concentration range. Representative spectra of solutions of intermediate helical contents are shown in the Figure, where one observes



FIGURE

100 *Mc./sec. n.m.r. spectra of* (a) *poly-(β-methyl* L-*aspartate) in a* 5% *solution of T.F.A. in* $CDCl_3$ *and* (b) *poly-*L*-leucine in a* 42% *solution of T.F.A. in* $CDCl_3$. *The coil* α-CH *proton resonances occur downfield from the helix* α-CH *resonance, whereas the coil* NH *proton resonances occur upfield from the helix* NH *resonances.*

separate peaks for the helical and coiled conformations for both the peptide NH and the α-CH protons. Separate resonances corresponding to helical and coiled conformations have not been observed before, probably since all previous studies have been carried out at 60 Mc./sec., where the chemical shifts are reduced by the factor 3/5. It is therefore possible to obtain lower limits to the lifetimes, τ, of the protons in the two conformations since $\tau \geqslant (2\pi\delta)^{-1}$, where δ is the chemical shift between a given proton in a helical and a coiled conformation. The value of $(2\pi\delta)^{-1}$ is of the order of 10^{-2}. Furthermore, as has been previously pointed out, an upper limit of 10 sec. can be assigned to τ, since the spectral changes are observed immediately after mixing.

Since separate peaks for the helical and coiled conformations are observed, it is possible to estimate directly the "conformational purity" of the various solutions and furthermore, to correlate b_0 directly with the helical content of the polypeptide. In the two examples studied, the change in b_0 was found to correlate quite well with helical content within experimental error. A b_0 of *ca.* −420 corresponded to 80—85% helical content at 30% T.F.A. The results clearly show that the gradual change in b_0 does represent a conformational change and is not due to solvent or other similar effects.

It was previously shown by Stewart *et al.*[2] that the T.F.A. proton does not undergo an unusually large downfield shift which would be indicative of protonating to the helix. Further confirming evidence of lack of protonation is indicated by the fact that neither the helical NH proton line-widths nor the frequencies change appreciably as a function of T.F.A. concentration. These results also tend to indicate that T.F.A. is not hydrogen-bonded to the amide groups of the helix.

The coil α-CH proton resonance occurs at lower magnetic field than the helical α-CH proton resonance. Furthermore, the shift difference between the left-handed helix (P.M.A.) and the coil is larger than the shift difference between the right-handed helix (P.L.L.) and the coil, in agreement with the theoretical predictions of Sternlicht and Wilson.[7]

In contrast to the α-CH resonance, the coil NH proton resonance occurs at higher magnetic field than the helical NH proton resonance. Such a phenomenon might imply a rupture of the intramolecular peptide hydrogen bond without subsequent formation of an intermolecular solvent–NH hydrogen bond. Increasing the acid concentration would then cause the breaking of the intramolecular hydrogen bonds, thus allowing the helix–coil transformation to occur as follows.

$$\underset{\text{(HELIX)}}{\rangle N\text{–}H \cdots O = C\langle} + CF_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}OH \rightarrow CF_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}OH \cdots O = \underset{\text{(COIL)}}{C\langle\rangle N\text{–}H}$$

The T.F.A. monomer is supplied by the equilibrium

$$(CF_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}OH)_2 \rightleftharpoons 2\, CF_3\text{–}\overset{\overset{O}{\|}}{C}\text{–}OH$$

The helix–coil transformation is controlled by such factors as the nonbonded interaction energies, and the relative stabilities of the various hydrogen bonds which are formed and broken, as well as the

acidity and monomer–dimer equilibrium constant of the acid used to elicit the transformation.

Further investigation into the relative importance of the various factors controlling the helix–coil transformation, as well as the kinetics involved, are currently in progress.

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