# STUDIES ON THE PROTONATION OF POLYPEPTIDES IN STRONG ORGANIC ACIDS

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The helical conformation of a variety of polypeptides can be broken down into a randomly coiled form upon the addition, in increasing amounts, of strong organic acids ( such as Dichloroacetic acid (DCA) or Trifluoro acetic acid (TFA))to a solution of the polypeptide in inert solvents like chloroform or ethylene dichloride (EDC) (Doty, Holtzer, Bradbury, and Blout, 1956; G.D.Fasman, 1962). This helix-coil interconversion has been studied by a variety of physical techniques, notably by optical rotatory dispersion (ORD), and is found to occur co-operatively ( Urnes and Doty, 1961).

Recently, Klotz and coworkers (Hanlon, Russo, and Klotz, 1963; Hanlon and Klotz, 1965; Stake and Klotz,1965; Hanlon, 1966), on the basis of near infrared spectra and conductivity work, have suggested that some polypeptides, e.g., Poly-gamma-benzyl-L-glutamate (PBLG), are protonated at the peptide groups in the presence of DCA or TFA. On the basis of these results, these authors have concluded that the changes observed in the ORD behavior in these systems need not reflect the breakdown of the helix due to the destruction of the interpeptide hydrogen bonds.

However, the nuclear magnetic resonance studies of Stewart, Mandelkern, and Glick (1967) on PBLG dissolved in chloroform-TFA solvent mixture reveal that the strong acid does not protonate the peptide groups, but rather participates in competitive hydrogen bonding with these groups, and the destruction of the helix by the addition of TFA is caused by this process.

For the past year or so, we have been conducting ORD experiments on these polypeptide systems in solvent mixtures containing strong acids such

as TFA, with special emphasis on the helix-coil transition region. The ORD data that have so far been reported on these systems in this region are restricted to wavelength ranges removed from the peptide group absorption bands in the ultraviolet, and the estimates of the amount of helical structure in the polymer have been based on analysis of these visible-near ultraviolet ORD data by the Moffitt Yang equation (Urnes and Doty, 1961). We report here the ORD patterns in the Cotton effect regions of PBLG at several TFA solvent compositions. The main reason behind the investigation of the Cotton effect zone is as follows: if indeed protonation of the peptide groups occurs upon the addition of the organic acid TFA, one would expect to find changes in the positions and/or intensities of the n- $pi^*$ (2220 A) and the pi- $pi^*$ (2060 and 1900 A) absorption bands of the amide chromophore in the helical polypeptide conformation. These changes in the characteristics of the amide bands cannot be easily seen in the ultraviolet absorption spectrum of the polypeptide because: (a) the n- $pi^*$ band of polypeptides is very weak and is located only with great difficulty, and (b) TFA itself would start absorbing in this region and thereby make absorption measurements unreliable. On the other hand, the magnitudes of the Cotton effects in the ORD experiments, due to these transitions, are large in helical polypeptides, and the extrema of the Cotton effects appear at longer wavelengths than the absorption maxima themselves. Thus the effect of TFA on these Cotton effects are measured more conveniently, and yield essentially the same kind of information. Also, any effect of protonation of the peptide groups by TFA would be expected to be more dramatically reflected in the Cotton effect zones than in the visible or near ultraviolet part of the rotatory dispersion.

It should be pointed out here that while this work was nearly completed, Quadrifoglio and Urry (1967) have reported independently on the circular dichroism of PBLG in the transition region, with similar conclusions.

Figure 1 gives the experimental ORD profile of PBLG (Pilot Chemical Co.,Mass.) in the peptide absorption region. Results are presented for

the n- pi* range for solutions of the polymer in EDC-TFA solvent mixtures of varying compositions. It can be seen that until the TFA composition reaches about 10 %(volume by volume), the Cotton effect trough at 2330 A of the n- pi* band is not affected to a great extent. The trough residue

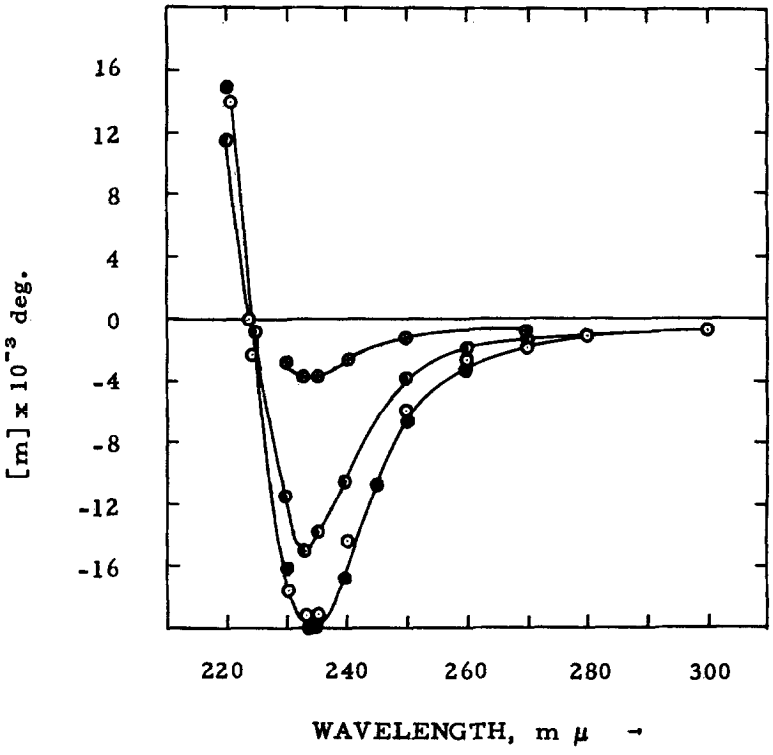


FIG. 1: ULTRAVIOLET ROTATORY DISPERSION OF PBLG:

● : in pure EDC; ○ : in 95% EDC - 5% TFA
◐ : in 90% EDC - 10% TFA; ⊕ : in 85% EDC - 15% TFA

molar rotation decreases from - 19500° in pure EDC to - 15800° in the solvent composition of 19 % TFA. By the time the TFA content has increased to 15 %, the Cotton effect trough is virtually vanished, with a rotation value of - 4000° . When the solvent contains 20 % TFA, the randomly coiled conformation of PBLG is obtained.

PBLG undergoes a helix-coil transition in the solvent composition range

of about 10-15 % TFA. At TFA proportions less than 10 %, the solvent supports the helical conformation of the polypeptide, which breaks down co-operatively upon an increase of the acid component in the solvent mixture. The data in Figure 1 adequately represent this behavior. At TFA compositions below 10 %, e.g., say at 5 % TFA, the Cotton effect extremum is virtually unchanged when compared to that of the complete helix situation in pure EDC. If any appreciable degree of protonation had occurred around this composition of TFA in the solvent, one would have expected to find the rotation profile changed, as either a wavelength shift, or a magnitude change, or both. The absence of any such change seems to indicate that either the protonation does not occur, or that even if it does, the rotatory properties are not grossly affected thereby. On the basis of our main argument mentioned above, we believe that TFA does not prptonate the amide groups of the polypeptide at least in these compositions of the solvent mixture. Hanlon (1966) has tried to build molecular models of PBLG where some of the peptides are protonated, and suggested that the helical register can be maintained by accomodating some of the protonated peptides via a highly distorted system of hydrogen bonding. That such a modified structure would exist stably at appreciable degrees of protonation is, however, questionable. Also, if the protonation were appreciable, we would expect corresponding modifications in the rotatory characteristics, and we did not find these here. Watanabe, Yoshioka, and Wada (1964) have suggested that in TFA containing solvents, PBLG gets protonated at the chain ends. If this were true, this represents a very small fraction of the peptide residues and would not be reflected in the ORD easily.

It is worth pointing out that competitive hydrogen bonding between the strong acid and the peptide residue cannot be ruled out. Apparently the ORD results quoted here are insensitive to this possibility, until the transition itself is started. The results of our experiments suggest, then, that before the helix-coil interconversion stage, no protonation of the peptide residues in PBLG occurs in presence of TFA, as could be detected by optical rotatory dispersion.

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