the temperature range 30 to −78°, as the spectra given in Figure 2 show.

The determination of stereoregularity of styrene-β,β-d_2 polymers, including the atactic polymer, using proton nmr spectroscopy, is in progress. It shows good agreement with the determination by ^{13}C nmr spectroscopy, and will be reported in the near future.

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The γ Turn. Evidence for a New Folded Conformation in Proteins

As the number of known protein structures has increased, it has become apparent that "hairpin bends," in which the direction of the polypeptide chain changes by 180°, occur relatively frequently.[1,2] It has also been suggested[1,2] that these bends may be important in determining the folding of the polypeptide chain. Venkatachalam[3] studied the allowed conformations of tripeptides and concluded that chain reversal could be achieved by four classes of bend which he designated types I, I′, II, and II′. The conformations of these bends has been discussed in detail elsewhere,[1-5] and need not be reviewed here, except to restate the conclusion that a reversal of the polypeptide chain direction could be achieved through a bend containing *at least four* α-carbon atoms. These so-called β bends have been commonly observed in globular proteins, notably at points where the polypeptide chain folds back and forth to form an extended antiparallel β structure.

Recently, Némethy and Printz[5,6] have proposed a new class of bend, containing only *three* α-carbon atoms. They propose the name γ turn, or 1–3 turn, for this new conformation, and discuss its relation to the β turn.

The purpose of this note is to present evidence which shows rather convincingly that a "γ turn" exists in the thermostable protease thermolysin, whose atomic structure has recently been determined.[7-9]

The segment of the protein which is of interest in the present context is Ser(25)–Thr(26)–Tyr(27). The apparent secondary structure in the vicinity of these residues is shown in Figure 1. This figure is essentially part of Figure 10 of ref 9, but drawn in more detail. Recently we have calculated an improved electron density map of thermolysin, based on five isomorphous heavy-atom derivatives. Study of this map has confirmed our initial interpretation of the conformation of the polypeptide chain in the vicinity of residues 25–27. The conformational angles[10] and hydrogen-bond distances for these residues, measured from our current model of the thermolysin structure, are listed in Table I. As will be discussed

TABLE I

Conformational angles, deg							
—Observed for thermolysin[a]—				—Predicted for the γ turn[b]—			
	φ	ψ	ω[c]		φ	ψ	ω
Ser(25)	−148	92	(180)	Ala(1)	172	128	−170
Thr(26)	86	−57	(180)	Ala(2)	68	−61	172
Tyr(27)	−114	148	(180)	Ala(3)	−131	162	

Hydrogen-bond lengths (H···O), Å			
Observed		Predicted[b]	
$N_{25}H_{25}\cdots O_{27}C_{27}$	1.8	$N_1H_1\cdots O_3C_3$	1.82
$N_{27}H_{27}\cdots O_{25}C_{25}$	1.6	$N_3H_3\cdots O_1C_1$	1.78

[a] The observed conformational angles have an estimated standard error of ±15°. Both the angles and bond lengths are subject to refinement of the thermolysin crystal structure. [b] Reference 6. [c] Reference 20.

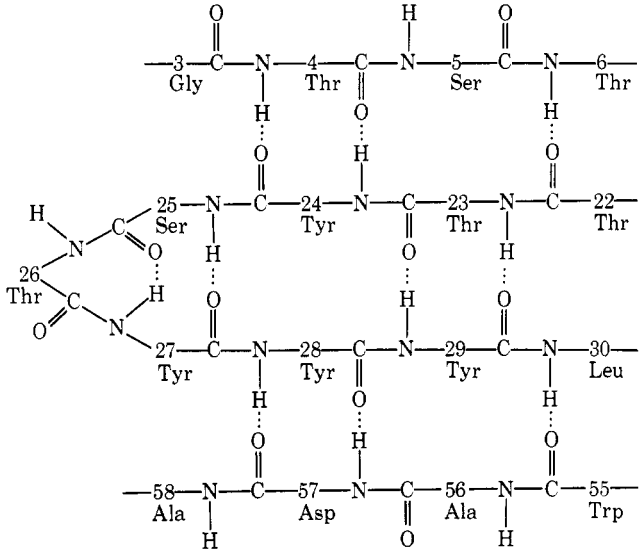

Figure 1. Secondary structure of thermolysin in the vicinity of the γ turn.

below, the proposed backbone conformation at Thr(26) is somewhat unusual, and we will therefore summarize the evidence that our interpretation of the electron density map at this point is essentially correct. First, the electron density in this part of the molecule is quite well defined, as evidenced by the fact that we were able to correctly identify[8,9] over 60% of the 80 amino-terminal residues of thermolysin, without reference to the chemically determined amino acid sequence.[11] In the vicinity of residues 25–27, density corresponding to all the backbone carbonyl groups and to the amino acid side chains can be seen. Secondly, the antiparallel β structure in the vicinity of these residues (Figure 1) tends to confirm their apparent conformation. For example, the apparent hydrogen bond (N_4—$H_4\cdots O_{24}$) (subscripts refer to residue numbers) tends to confirm the orientation of the peptide between C_{24}^{α} and C_{25}^{α}, and the hydrogen bond (N_{28}—$H_{28}\cdots O_{57}$) tends to confirm the orientation of the peptide between C_{27}^{α} and C_{28}^{α}. Finally, our confidence in interpretation of the "γ turn" is strengthened by the fact that it was made without foreknowledge of the prediction of Nemethy and Printz.[6]

The side chains of Ser(25), Thr(26), and Tyr(27) are all exposed to solvent; in fact, Thr(26) is at an "apex" of the molecule at the end of its longest diagonal. The hydrogen bond (N_{25}—$H_{25}\cdots O_{27}$) appears to be at least partly shielded

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from the solvent by the side chains of Tyr(29) on one side and Tyr(24) and Tyr(28) on the other. Access of solvent to hydrogen bond (N_{27}—$H_{27}\cdots O_{25}$) seems to be hindered partly by C^{β} of Thr(26) and also by the phenolic oxygen of Tyr(29) which appears to be within hydrogen-bonding distance of O_{25}.

The agreement between the backbone conformational angles and hydrogen bond lengths observed for the "γ turn" in thermolysin and those predicted by Némethy and Printz[6] (Table I) is quite good, particularly for the crucial conformation at the second α-carbon. Conformations lying in this region of energy diagrams (near $\phi = 60°, \psi = -60°$) were designated as "disallowed" for residues other than glycine in early studies,[12,13] but more recent calculations[14-17] have suggested that this conformation might be allowed, or even favored. The conformational angles of (86°, −57°) observed for Thr(26) in thermolysin may be compared with the conformations at energy minima which have been predicted to occur at (∼60°, ∼−60°),[14,15] (69°, −69°),[16] and (80°, −60°).[17] The conformation is characterized by a bent hydrogen bond (N_3—$H_3\cdots O_1$) which contributes favorably to the conformational energy, and by close approaches ($C_2^{\beta}\cdots O_1$) and/or (N_3—$H_3\cdots O_1$), which were originally considered too short to be allowed.[12,13] There is precedent for the occurrence of this conformation in globular proteins. For example, in lysozyme,[18] Phe(38) has been reported to have the conformation (112°, −27°), and in ribonuclease S[19] Lys(37) was reported to have a conformation near (77°, −45°). On the other hand, participation of a residue with this conformation in a γ turn has not been heretofore reported. It will be noted in Table I that the biggest discrepancy between the observed conformations[20] and those predicted by Némethy and Printz[6] is (−40°, +36°) for ($\Delta\phi_1$, $\Delta\psi_1$). Since these differences are approximately equal and opposite, they tend to offset each other in such a way that the observed course of the polypeptide chain is quite similar to that predicted theoretically (see Figure 1 of ref 6). The predicted values for (ϕ_1, ψ_1) lie just outside the low-energy region of most conformational maps, whereas the observed value for Ser(25) lies inside the "fully allowed" region.[12]

It may be noted that model-building experiments show that another polypeptide chain reversal is possible by utilizing three α-carbon atoms, C_1^{α}, C_2^{α}, C_3^{α}, with dihedral angles approximately (70°, −170°), (−86°, 57°), and (−155°, −60°). In this case the hydrogen bonds are (N_3—$H_3\cdots O_1$), as in the γ turn, and (N_4—$H_4\cdots O_0$) [*cf.* $N_1H_1\cdots O_3$], the former arising from conformational angles inverse to those at C_2^{α} in the γ turn. This alternative three-α-carbon chain reversal might therefore be designated the "inverse γ turn," or the "inverse 1–3 turn." Note that the γ turn and inverse γ turn are not to be confused with the "γ helix"[21] which has the conformation (84°, 78°).

The occurrence of a γ turn in thermolysin suggests that it may be found in other proteins, and should therefore be considered as a potential structural feature along with β turns, β structure, and helices. Furthermore, the γ turn and conformations near to (60°, −60°) should also be considered as possible conformations in structural predictions for both macromolecules and smaller peptides.

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Microstructure Analysis of Poly(propylene oxide) by ^{13}C Nuclear Magnetic Resonance Spectroscopy

The microstructure analysis of a polymer provides indispensable information on the mechanism of the polymerization reaction. The ^1H nmr analysis of the microstructure of poly(propylene oxide-α-d_1) developed in this laboratory[1] provided some information on the mode of action of catalysts used in the polymerization.[2] Unfortunately, the application of this method is limited to the dyad structure.

The application of ^{13}C nmr spectroscopy, which results in chemical shifts larger than those by ^1H nmr has been developed for vinyl polymers. ^{13}C nmr analysis of poly(propylene oxide) described in this paper provides new information about the triads of methine carbons in the main chain, and is in disagreement with the analysis made by Schaefer.[3]

Five different samples of poly(propylene oxide) whose microstructures had been analyzed by ^1H nmr of their monodeuterio derivatives[1,2] were prepared using three kinds of catalyst: I and II, amorphous polymers prepared with *tert*-BuOK and $Al(C_2H_5)_3$–H_2O (molar ratio 1:1) catalysts, respectively; and the crude (III), crystalline (IV), and amorphous polymers (V) prepared using $Zn(C_2H_5)_2$–H_2O (molar ratio 1:0.8) freeze-dried catalyst. Natural-abundance ^{13}C nmr spectra at 25.1 MHz were measured in deuteriobenzene at 30° with proton noise decoupling using a Varian XL-100-15 spectrometer equipped with a standard VFT-100X Fourier transform system. Internal field frequency stabilization of the spectrometer was provided by a lock signal from tetramethylsilane dissolved in the solvent. The peak area ratio was determined by using a Du Pont 310 curve resolver.

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