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Hydrophobicity of Amino Acid Residues: Differential Scanning Calorimetry and Synthesis of the Aromatic Analogues of the Polypentapeptide of Elastin

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SYNOPSIS

Relative hydrophobicities of aromatic amino acid residues are investigated by using differential scanning calorimetry (DSC) on 10 synthetic copolypentapeptides of poly(VPGVG) of elastin. Utilizing the hydrophobic-driven process of the inverse temperature transition exhibited by these polypentapeptides in aqueous solution, the relative hydrophobicities of Phe, Trp, and Tyr residues are determined by the critical temperature and heat of the transition. The DSC data for the aromatic residue containing copolypentapeptide aqueous solution indicate that tryptophan is the most hydrophobic amino acid residue, phenylalanine the third most hydrophobic on basis of transition temperature and the second on basis of transition heat. For tyrosine, significant differences are observed between the phenolic and the phenoxide anionic states. At pH 7, where tyrosine is protonated, it is found to be the second most hydrophobic amino acid residue on the basis of the transition temperature, whereas on the basis of the heat of transition, it is less hydrophobic than both tryptophan and phenylalanine. Changing the pH from pH 7 to pH 12, for example, for poly[0.8(VPGVG), 0.2(VPGYG)] in aqueous solution shifts the transition temperature from 7 to 49°C with a dramatically reduced heat. On the basis of both the transition temperature scale and the heat of transition, the hydroxylated tyrosine appears less hydrophobic than glycine. © 1992 John Wiley & Sons, Inc.

INTRODUCTION

The hydrophobic effect has been the subject of investigation for most of a century. The terms hydrophobic and hydrophilic were first used to describe phenomena in colloids.[1] Langmuir, Butler, Cohn, and Edsall, as well as other pioneers, studied the hydration of nonpolar solutes and amino acids.[2-5] Waugh[6] made the early suggestion of classifying amino acid residues as hydrophobic or hydrophilic. It is Kauzmann's classic review on proteins and polypeptides that inspired numerous scientists to further investigations.[7] Using Gibbs' free energy of transfer of the amino acids between water and an organic solvent such as methanol or acetone to measure the hydrophobic character, and extending the studies of Cohn and McKenzee in the 1930s,[3] Nozaki and Tanford formulated the first hydrophobicity scale.[8] Now the number of hydrophobicity scales is well above 40. These can be classified as either experimental scales or empirical scales based on calculations. On the experimental side, there are the scales derived (1) by measuring the distribution coefficients of the amino acid between an aqueous phase and a suitably chosen organic phase,[8] or vapor phase[9]; (2) by measuring the surface tension of amino acid solutions[10]; (3) by measuring the mobilities of the amino acids on chromatographic paper[11]; (4) by measuring the amount of water that does not freeze when an aqueous macromolecular solution is rapidly frozen and then equilibrated at −20 to −40°C[12]; and (5) by measuring the heat capacity of some tripeptides and model organic compounds.[13] Another relevant approach is determine the effect on protein stability of different amino acid

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residues by point mutation.[14] Since the nonpolar region of membranes differs from the inside of protein, hydrophobicity scales were developed, focusing on the peculiarities of the lipid–protein interaction.[15] A large number of the scales in the literature are statistical in origin. Despite the variations in computational methodology and technical definitions, these approaches utilize x-ray determined crystal structures and are all based on the common principle of the probability of occurrence of an amino acid residue on the surface or inside of the globular proteins.[16–22]

Differences exist among scales. Some lack values for all 20 amino acid residues. One scale places glycine in the most hydrophobic position[9]; another gives glycine the same value as cysteine, valine, leucine, isoleucine, and methionine.[12] In a recent scale that uses the interesting approach of measuring the heat capacities of amino acids,[13] only a few amino acids were measured directly, while the relevant data for other amino acids were either obtained by measurements on model compounds or taken from other methods.

As the residues in protein are covalently linked and incorporated moieties, they do not distribute randomly. It is not surprising, therefore, to see criticisms of the approach involving transfer free energy of amino acids and related compounds on the grounds that the data do not properly reflect the influence of chain connectivity, proximities of component chemical moieties such as the peptide linkage, and other interactions. In a recent paper, Ben-Naim criticized the concept of transfer free energy of an amino acid from water to an organic solvent.[23] He concluded "the currently used hydrophobicity scales, based on partition coefficients between water and an organic solvent, are inadequate measures of the contribution of side chains being transferred from water to the interior of the protein."

Since the main purpose of developing an hydrophobicity scale is to understand protein structure and mechanism, a scale developed directly on polypeptides would seem to be more appropriate than those developed on other small molecules. In this regard, however, it is impossible to carry out investigations of the hydrophobicity scale using homopolymers of the amino acid residues in water because of solubility problems and structural variations. The polypentapeptide of elastin, poly(VPGVG), on the other hand, provides a unique model system for such investigations by a "host–guest" technique. The elastin-based polypentapeptide, poly(VPGVG), exhibits an inverse temperature transition, i.e., the polypeptide molecules become intramolecularly and intermolecularly more structured on raising the temperature of aqueous solutions. This has been demonstrated by using a variety of methods.[24–26] Particularly interesting are the DSC studies carried out on poly(VPGVG) and its more hydrophobic analogues, poly(IPGVG) and poly(LPGVG),[27] in which in aqueous solutions the polypeptides undergo an endothermic transition. The transition temperature of poly(VPGVG) is higher than that of poly(IPGVG) and that of poly(LPGVG), both of which are regarded as more hydrophobic than poly(VPGVG) from the hydrophobicity of Nozaki and Tanford.[8] The heat of the transition for poly(VPGVG) is less than that of its more hydrophobic analogues. It becomes possible therefore, by DSC, to develop an hydrophobicity scale based on the thermodynamic properties of the inverse temperature transition of the polypentapeptides by using the polypentapeptide of elastin, poly(VPGVG), as a host polymer. By choosing a suitable residue position in the pentamer for substitution, it becomes possible to study the effect of the substitution of a guest residue at that site for a series of guest/host ratios.

Of the five positions in VPGVG, only the fourth position can function as a variable for all of the naturally occurring amino acid residues and modifications thereof. For example, the Ala^1 and Ala^5 analogues exhibit irreversible precipitations on raising the temperature through the transition rather than forming reversible viscoelastic phases. That position four is flanked by Gly residues is partly responsible. It is also important that the β-spiral structure is flexible with the residue-four side chain at the surface and that these polypentapeptides can be crosslinked to form dominantly entropic elastic matrices with energy barriers for mobility being but a kcal or two per mole residue. These properties serve to make the polypentapeptide system uniquely suited for studying this aspect of protein folding. At the very least, this scale is functionally what is required to design elastic transductional matrices. The ECEPP/2 and CHARMm calculations have shown that the β-spiral of poly(VPGVG) is well suited to accommodate the aromatic residues at position four. In fact, even the Glu(NAD), where NAD is the nicotinamide adenine dinucleotide, has been incorporated successfully into this structure.

This is the first of a series of studies that report the syntheses, differential scanning calorimetry, and other relevant physical characterizations of a series of polypentapeptides of the general formula poly[f_V(VPGVG),f_X(VPGXG)] where f_V and f_X are mole fractions with $f_V + f_X = 1$ and X is the

guest residue. In particular, the present report contains data on the aromatic residues, i.e., X = Trp(W), Phe(F), and Tyr(Y).

Differential scanning calorimetry (DSC) provides direct, model-independent determinations of the transition properties, such as enthalpy, entropy, and critical temperature in thermally induced structural and phase transitions of biomolecules.[28] DSC studies of the inverse temperature transition of the elastin-based polypeptides add another experimental dimension in providing a functional relative hydrophobicity scale.

MATERIALS AND METHODS

Calorimetry

The studies were performed using the Micro 1710 differential scanning calorimeter (Hart Scientific, Provo, UT). The instrument includes four removable cells; one is used for reference and the other three can be used for samples. The thermopile response as a function of energy input was calibrated by the built-in calibration heater of the instrument. For each cell, a temperature-dependent calibration constant was determined every 10°C from −5 to 105°C and fit by the least-squares method to a second-order polynomial in temperature. The polynomials are used in the experiment to convert the measured electrical signal to the power required on heat absorption. The detailed calibration and checking of the performance of the instrument has been presented elsewhere.[27]

Peptide Syntheses

It has been found in previous studies that the highest molecular weights were obtained when the pentamer permutation with Pro at the carboxyl terminus (GVGVP) and *para*-nitrophenol were used for carboxyl activation.[29] Accordingly, the structures are accurately written as poly[f_V(GVGVP), f_X(GXGVP)]. Except for two or three residues at the end of the high polymer, this is the same as poly[f_V(VPGVG), f_X(VPGXG)]. The reason for continuing to use the VPGVG sequence in general discussions is the occurrence of a conformational feature, the $Val^1C{=}O\cdots HNVal^4$ β-turn.

The peptides Boc-Val-Pro-OBzl and Boc-Gly-Val-Gly-Val-Pro-OBzl were prepared as previously described.[29,30] These peptides will be introduced as necessary in the subsequent syntheses. In general, the Boc-Val-Pro-OBzl was crystallized, on a large scale (100 g), from ethyl ether with petroleum ether to yield a pure product. Also, Boc-Val-Gly-OBzl and Boc-Gly-Val-Gly-OBzl were purified by precipitation, on a large scale, from ethyl acetate with petroleum ether to yield a pure product. The other oligopeptides were purified of extractions, then concentrated to a very minimal amount of chloroform and precipitated with ethyl ether which was followed by ether washes and petroleum ether washes. The pentapeptides were then characterized by ^{13}C-nmr before polymerization to verify structure and purity. Thin-layer chromatograph (tlc) was performed on silica gel plates obtained from Whatman, Inc., with the following solvent systems:
R_f^1: $CHCl_3$,CH_3OH,CH_3COOH; 95 : 5 : 3;
R_f^2: $CHCl_3$,CH_3OH,CH_3COOH; 90 : 10 : 3;
R_f^3: $CHCl_3$,CH_3OH,CH_3OOH; 85 : 15 : 3.

Syntheses of the Phe-Containing Polypentapeptides. *Boc-Phe-Gly-OBzl (I).* Boc-Phe-OH (Boc: *tert*-butyloxycarbonyl; OBzl: benzyl ester; 15.0 g, 0.056 mol) was dissolved in dimethylformamide (DMF) (50 mL) and cooled to 0°C. Then N-methylmorpholine (NMM; 6.2 mL) was added. The solution was cooled to −15 ± 1°C and isobutyl chloroformate (IBCF; 7.3 mL) was added slowly under stirring while maintaining the temperature at −15°C. After stirring the reaction mixture for 10 min at this temperature, a precooled solution of Tos-Gly-OBzl (Tos: *p*-toluenesulfonyl; 19.0 g, 0.056 mol) and NMM (6.2 mL) in DMF (50 mL) was added slowly and the reaction mixture was stirred overnight at room temperature. The reaction mixture was subjected to reduced pressure until 50% of the reaction mixture remained, and then it was poured into about 500 mL of ice-cold saturated $KHCO_3$ solution and stirred for 30 min. Then 250 mL of 90% NaCl solution was added. The precipitant was filtered, washed with water, 10% citric acid, and water, and dried to obtain 20.30 g (yield 87.1%) of I. R_f^1 0.45, R_f^2 0.70, R_f^3 0.91.

Boc-Gly-Phe-Gly-OBzl (II). Compound I (8.0 g, 0.019 mol) was deblocked with 4.0 N HCl/dioxane for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried (yield 100%). Boc-Gly-OH (3.39 g, 0.019 mol) was dissolved in 25 mL of DMF and cooled to −15°C. 1-Hydroxybenzotriazole (HOBt; 3.20 g, 0.021 mol) and 1-ethyl-3-dimethylaminopropyl carbodiimide (EDCI; 4.01 g, 0.021 mol) were added. After 20 min a precooled solution of HCl-H·Phe-Gly-OBzl and NMM (2.09 mL, 0.019 mol) was added, and the reaction mixture stirred overnight at room temperature. The DMF

was removed under reduced pressure and the residue was extracted into $CHCl_3$. The $CHCl_3$ extract was washed with water, 10% citric acid, 5% $NaHCO_3$, water, dried over Na_2SO_4, and the solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried down to obtain 8.67 g (yield 95.3%) of II. R_f^1 0.62, R_f^2 0.84.

Boc-Gly-Phe-Gly-OH (III). Compound II (8.57 g, 0.018 mol) in glacial acetic acid (85 mL) was hydrogenated in presence of 10% palladized charcoal catalyst at 40 psi. The catalyst was filtered off and the solvent removed under reduced pressure. The residue was triturated with ether, filtered, washed with ether, and dried to obtain 6.89 g (yield 99.5%) of III. R_f^2 0.30, R_f^3 0.62.

Boc-Gly-Phe-Gly-Val-Pro-OBzl (IV). Boc-Val-Pro-OBzl (7.20 g, 0.018 mol) was deblocked with HCl/dioxane and coupled to compound III (6.67 g, 0.018 mol) using EDCI with HOBt as described for the preparation of II to obtain 10.90 g (yield 91.9%) of IV. R_f^2 0.43, R_f^3 0.68.

Boc-Gly-Phe-Gly-Val-Pro-ONp (V). Compound IV (10.55 g, 0.016 mol) was hydrogenated as in the preparation of III and reacted with bis(4-nitrophenyl carbonate (bis-PNPC; 1.5 equivalent) in pyridine (85 mL). When the reaction was complete, as determined by tlc, the solvent was removed under reduced pressure. The residue was taken into $CHCl_3$. The $CHCl_3$ was washed with water, 10% citric acid, 5% $NaHCO_3$, water, dried over Na_2SO_4. The solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried to obtain 7.9 g (yield 72.1%) of V. $R_f^1$0.39, $R_f^2$0.55, $R_f^3$0.72.

Boc-Gly-Val-Gly-Val-Pro-ONp (VI). Boc-Gly-Val-Gly-Val-Pro-OBzl (15.20 g, 0.029 mol) was hydrogenated and reacted with bis-PNPC (1.5 equivalent) in pyridine (85 mL) as described in the preparation of V to obtain 16.16 g (yield 86.5%) of VI. R_f^1 0.38, R_f^2 0.63, R_f^3 0.75.

Poly[0.8(Gly-Val-Gly-Val-Pro),0.2(Gly-Phe-Gly-Val-Pro)] (VII). Compound V (0.255 g, 3.7×10^{-4} mol) and compound VI (0.949 g, 1.5×10^{-3} mol) were deblocked together using trifluoroacetic acid (TFA), and a one molar solution of the TFA salt in DMSO was polymerized for 18 days using 1.6 equivalent of NMM as base. The polymer was dissolved in water, dialyzed using 3500 mol wt. cutoff dialysis tubing and lyophilized. The product was base treated with 1N NaOH (2 equivalent per pentamer), dialyzed using 50 kD mol wt. cutoff dialysis tubing for one week and lyophilized to obtain 0.49 g (yield 64.3%) of VII.

Poly[0.9(Gly-Val-Gly-Val-Pro),0.1(Gly-Phe-Gly-Val-Pro)] (VIII). Compound V (0.133 g, 1.9×10^{-4} mol) and compound VI (1.117 g, 1.7×10^{-3} mol) were treated as compound VII to obtain 0.58 g (yield 73.7%) of VIII.

Syntheses of the Tyr-Containing Polypentapeptides. *Boc-Gly-Val-Pro-OBzl (IX).* Boc-Val-Pro-OBzl (6.50 g, 0.016 mol) was deblocked with HCl/dioxane and coupled to Boc-Gly-OH (2.78 g, 0.016 mol) using EDCI with HOBt as described in the preparation of II. Since the product is an oil, the peptide was not triturated with ether. It was dried to obtain 7.28 g (yield 99.3%) of IX. R_f^1 0.58, R_f^2 0.69.

Boc-Tyr(2,6-Cl_2-Bzl)-Gly-Val-Pro-OBzl (X). Compound IX (4.20 g, 0.009 mol) was deblocked with HCl/dioxane and coupled to Boc-Tyr-OH (3.96 g, 0.009 mol) using EDCI with HOBt as described in the preparation of II to obtain 6.46 g (yield 91.5%) of X. R_f^1 0.41, R_f^2 0.67.

Boc-Gly-Tyr(2,6-Cl_2-Bzl)-Gly-Val-Pro-OBzl (XI). Compound X (6.36 g, 0.008 mol) was deblocked using HCl/dioxane and coupled to Boc-Gly-OH (1.42 g, 0.008 mol) using EDCI with HOBt as described in the preparation of II to obtain 6.02 g (88.3%) of XI. R_f^2 0.52, R_f^3 0.67.

Boc-Gly-Tyr(2,6-Cl_2-Bzl)-Gly-Val-Pro-ONp (XII). Compound XI (5.90 g, 0.007 mol) was hydrogenated and reacted with bis-PNPC (1.5 equivalent) in pyridine (50 mL) as described in the preparation of V to obtain 4.30 g (yield 70.1%) of XII. R_f^1 0.40, R_f^2 0.71, R_f^3 0.89.

Poly[0.8(Gly-Val-Gly-Val-Pro),0.2(Gly-Tyr(2,6-Cl_2-OBzl)-Gly-Val-Pro)] (XIII). Boc-Gly-Val-Gly-Val-Pro-ONp (1.593 g, 2.5×10^{-3} mol) and compound XII (0.407 g, 6.1×10^{-4} mol) were treated as compound VII to obtain 1.60 g (yield 81.3%) of XIII.

Poly[0.9(Gly-Val-Gly-Val-Pro),0.1(Gly-Tyr(2,6-Cl_2-OBzl)-Gly-Val-Pro)] (XIV). Boc-Gly-Val-Gly-Val-Pro-ONp (2.610 g, 24.0×10^{-3} mol) and compound XII (0.390 g, 4.5×10^{-4} mol) were treated as compound VII to obtain 1.24 g (yield 62.92%) of XIV.

Poly[0.8(Gly-Val-Gly-Val-Pro),0.2(Gly-Tyr-Gly-Val-Pro)] (XV). Compound XIII [1.0 g, 0.002 mol (based on pentamers)] was deblocked using HF : DMS (1 : 3, v/v)[31] at 0°C for 1 h. It was triturated with ether and then dissolved in water, dialyzed using 3500 mol wt. cutoff dialysis tubing, lyophilized, base treated, dialyzed using 50 kD mol wt. cutoff dialysis tubing and lyophilized to obtain 0.792 g (yield 85.2%) of XV.

Poly[0.9(Gly-Val-Gly-Val-Pro),0.1(Gly-Tyr-Gly-Val-Pro)] (XVI). Compound XIV [1.0 g, 0.002 mol (based on pentamers)] was treated the same as compound XV to obtain 0.501 g (yield 52.0%) of XVI.

Syntheses of the Trp-Containing Polypentapeptides. *Boc-Gly-Val-Pro-OBzl (XVII).* Boc-Val-Pro-OBzl (32.0 g, 0.079 mol) was deblocked with HCl/dioxane and coupled to Boc-Gly-OH (13.8 g, 0.079 mol) using IBCF as described in the preparation of I. Since the product is an oil, the solvent was removed completely from the reaction mixture. The residue was taken in $CHCl_3$ and washed with water, 10% citric acid, 5% $NaHCO_3$, water, dried of Na_2SO_4. The solvent was removed under reduced pressure and the peptide was dried to obtain 24.0 g (yield 89.2%) of XVII. R_f^1 0.58, R_f^2 0.69.

Boc-Trp-Gly-Val-Pro-OBzl (XVIII). Compound XVII (12.0 g, 0.026 mol) was deblocked with HCl/dioxane and coupled to Boc-Trp-OH (7.40 g, 0.024 mol) using IBCF as described for the preparation of I. The peptide was purified on a silica gel column, 60–200 mesh, with ethyl acetate as the eluting solvent to obtain 13.0 g (yield 83.7%) of XVIII. R_f^2 0.57, R_f^3 0.72.

Boc-Gly-Trp-Gly-Val-Pro-OBzl (XIX). Compound XVIII (1.10 g, 0.002 mol) was deblocked with HCl/dioxane that contained 1.5% 1,2-ethanedithiol and coupled to Boc-Gly-OH (0.30 g, 0.002 mol) using EDCI with HOBt as described in the preparation of II to obtain 1.01 g (yield 84.0%) of XIX. R_f^1 0.38, R_f^2 0.59, R_f^3 0.68.

Boc-Gly-Trp-Gly-Val-Pro-ONp (XX). Compound XIX (1.0 g, 0.001 mol) was saponified in methanol (25 ml) using 1N NaOH (1.8 equiv.) and reacted with 4-nitrophenol (0.3 g, 0.002 mol) using dicyclohexylcarbodiimide (DCC) (0.25 g, 0.001 mol) and 4-dimethylaminopyridine (0.030 g, 0.00009 mol). The reaction mixture was taken in $CHCl_3$ and washed with water, 10% citric acid, 5% $NaHCO_3$, water, and dried of Na_2SO_4. The solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried to obtain 0.37 g (yield 35.9%) of XX. R_f^1 0.48, R_f^2 0.65, R_f^3 0.75.

Poly[0.86(Gly-Val-Gly-Val-Pro),0.14(Gly-Trp-Gly-Val-Pro)] (XXI). Boc-Gly-Val-Gly-Val-Pro-ONp (0.53 g, 8.2×10^{-4} mol) and compound XX (0.10 g, 1.34×10^{-4} mol) were deblocked using TFA containing 6% 1,2-ethanedithiol. A one-molar solution of the TFA salt in DMSO was polymerized 15 days using 1.6 equivalent of NMM as base. The polymer was dissolved in water, dialyzed against 3500 mol wt. cutoff dialysis tubing, and lyophilized. The product was base treated with 1.0N NaOH (2 equivalent per pentamer) and dialyzed against 50 kD mol wt. cutoff dialysis tubing for one week and lyophilized to obtain 0.27 g (yield 73.7%) of XXI.

Poly[0.9(Gly-Val-Gly-Val-Pro),0.1(Gly-Trp-Gly-Val-Pro)] (XXII). Boc-Gly-Val-Gly-Val-Pro-ONp (1.0 g, 1.5×10^{-3} mol) and compound XX (0.13 g, 1.7×10^{-4} mol) were treated the same as compound XXI to obtain 0.49 g (yield 69.9%) of XXII.

These peptides were checked at every step using tlc and were checked with ^{13}C-nmr spectra at least at the pentamer stage and the final product. The compounds treated with HF for deprotection had spectra run before and after final deprotections. The final products were also verified by 400-MHz proton nmr spectroscopy. Figure 1 presents examples of ^{13}C- and proton nmr spectra for the aromatic residue containing copolypentapeptides along with that of the host polypentapeptide. The absence of extraneous peaks verifies the synthesis, and the ratio of relevant peaks verified the incorporation ratios. Figure 2 contains representative chromatograms from high performance liquid chromatography (HPLC) amino acid analyses for the case of f_X approximately 0.10. While the accuracy depends on many factors, generally, it can be considered for the determination of f_X to be in the range of ± 0.01 for both nmr and HPLC analyses.

Preparation of the Polypentapeptides

Low-conductivity H_2O (with a resistance greater than 13 MΩ) was used as solvent for the synthetic polypeptides studied. This is referred to as type I water. In all the experiments, each weighed amount of sample was dissolved in the amount of H_2O required to obtain the concentration, 20 mg/mL used for all the polypeptides. The sample in water was maintained at low temperature (2°C) for about 12 h to ensure that the sample was completely dissolved in the water. The samples were preheated up to 70°C with a scan-rate 8.2°C/h (0.136°C/min) such that the polypeptide systems will have passed once through the inverse temperature transition before the data were recorded on the second run. The inverse temperature transition is found to be essentially reversible, but the reverse process is slow. The reported scans were begun after the samples had reequilibrated at 2°C for ~ 90 h. The scan rate used was again 8.2°C/h (0.136°C/min). As the tyrosine side chain contains an hydroxyl group with a nominal pKa of 10, experiments for the tyrosine containing polypentapeptides were done at two pH values, namely, pH 7 and 12. The pH 7 solution was obtained by dissolving the polypeptides in type I water, without pH adjustment, and the pH 12 solution was obtained by pH adjustment using 1N NaOH. A baseline scan with solvent followed each DSC scan involving sample. The sample scan and baseline scan were run at the same scan rate, and against the same reference cell, the content of which remained the same for the two runs. The reported

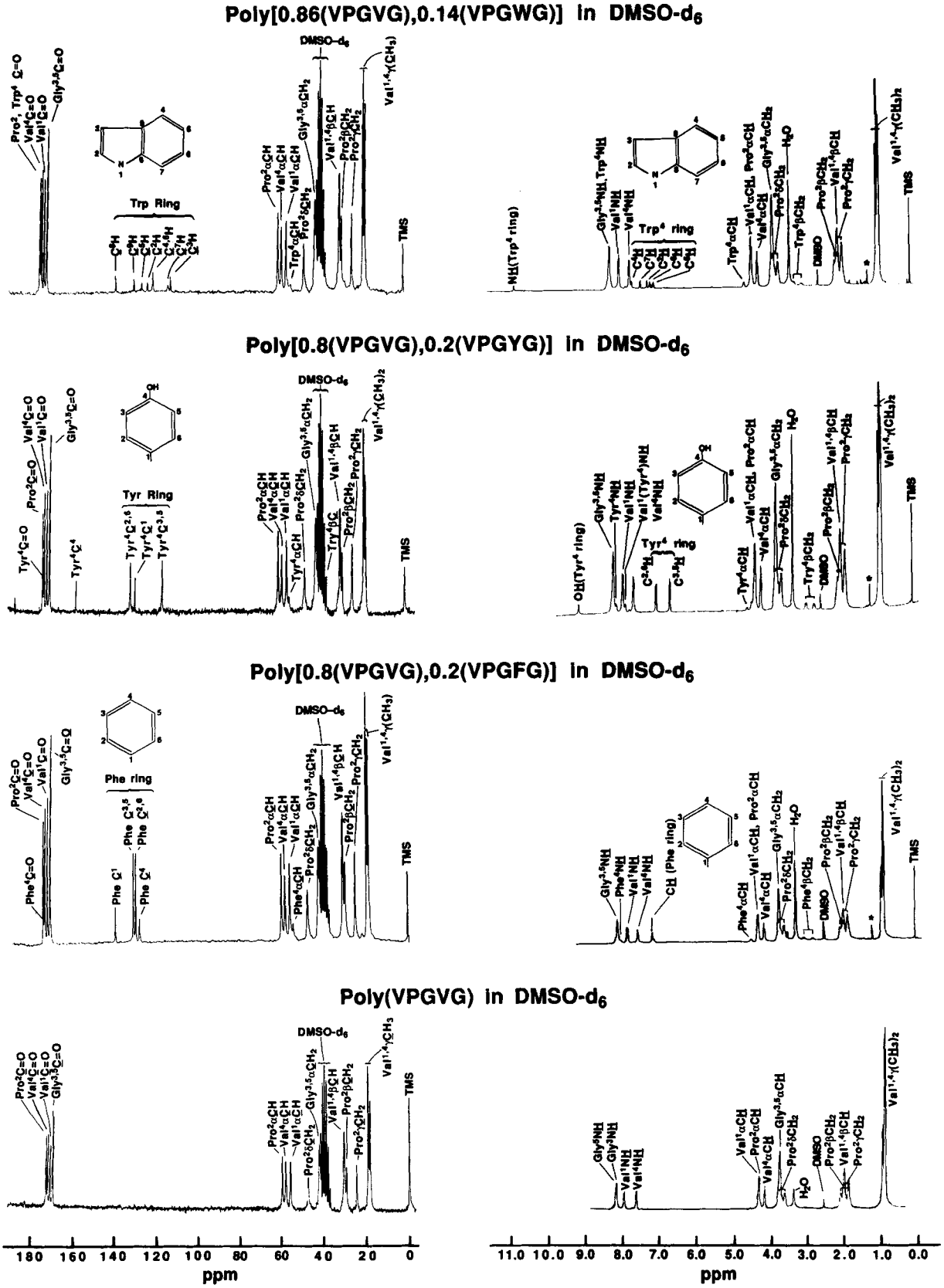


Figure 1. Examples of the 400-MH ^{1}H-nmr spectra (right) obtained on a Bruker WH-400 spectrometer and of the ^{13}C-nmr spectra obtained on a FX-100 spectrometer for the aromatic containing copolypentapeptides of the poly(VPGVG).

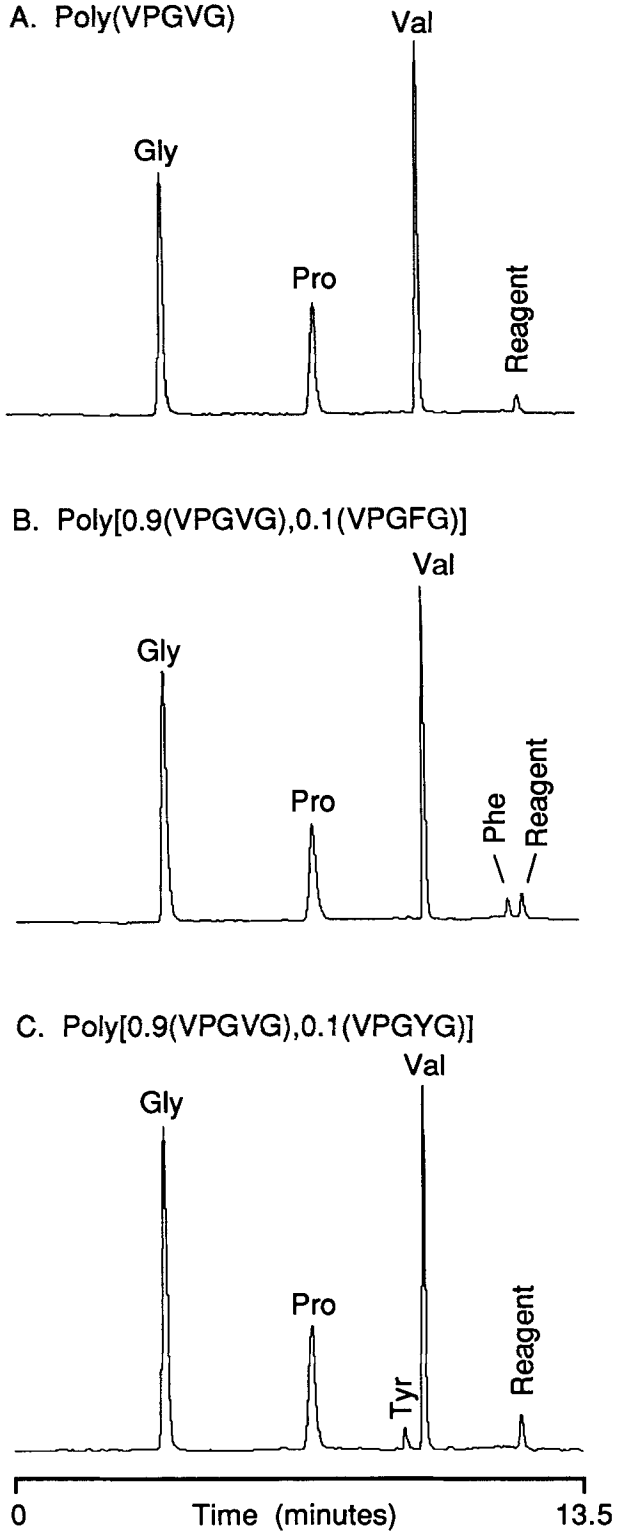


Figure 2. HPLC chromatograms for (A) poly(VPGVG), (B) poly[0.9(VPGVG),0.1(VPGFG)] and (C) poly[0.9(VPGVG)],0.1(VPGYG)]. Even with the smallest value for f_X ($\simeq$0.1), it is demonstrated that the aromatic peak can be determined and quantified. The Trp residue is destroyed during the acid hydrolysis. Its quantification was achieved from the nmr spectrum.

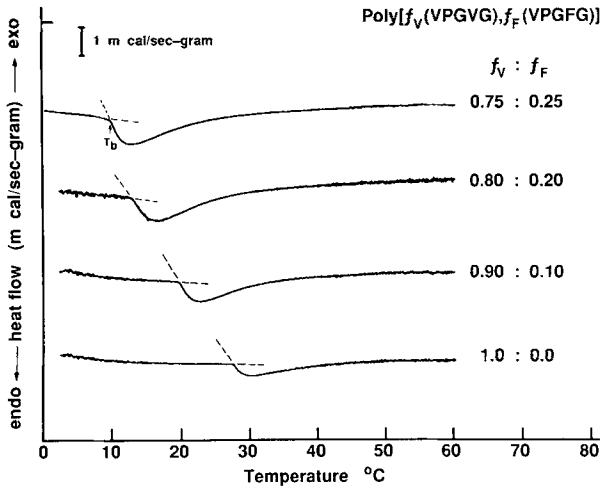


Figure 3. DSC thermograms for the phenylalanine containing copolypentapeptides of the poly(VPGVG) in H_2O at the scan rate of 8.2°C/h (0.136°C/min) and the peptide concentration of 20 mg/mL.

curve is the result of subtracting the baseline run from the sample run.

The heat, ΔQ (ΔH) and entropy, ΔS, of the transition were obtained by integrating the DSC scans with time as the integrand variable, because the DSC scans were recorded as a function of time. A straight line, drawn from 2°C below the onset temperature of the transition at which the thermoprofile departs abruptly from the pretransition baseline to a point tangent to the curve on the high temperature side, is used as the baseline.[16]

The temperature dependence of the transitions can be characterized by the temperature of maxi-

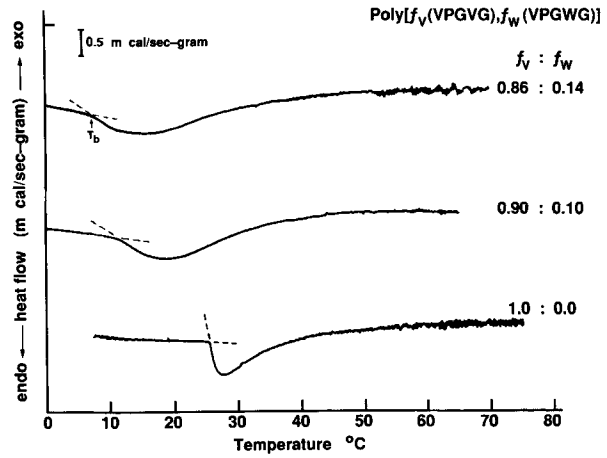


Figure 4. DSC thermograms for the tryptophan containing copolypentapeptides of the poly(VPGVG) in H_2O at the scan rate of 8.2°C/h (0.136°C/min) and the peptide concentration of 20 mg/mL.

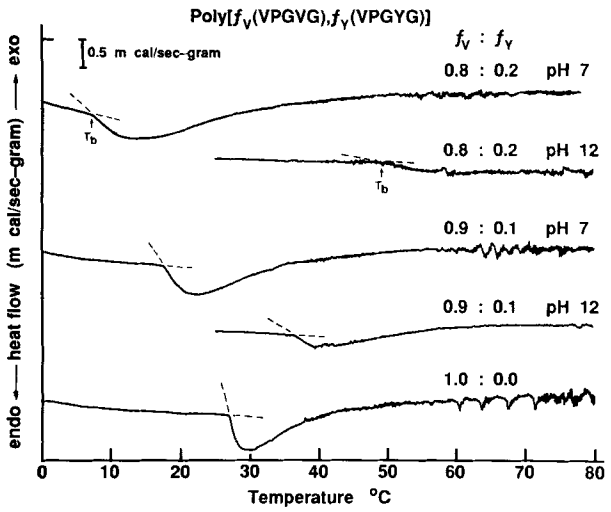


Figure 5. DSC thermograms for the tyrosine containing copolypentapeptides of the poly(VPGVG) in H_2O at the scan rate of 8.2°C/h (0.136°C/min) and the peptide concentration of 20 mg/mL.

mum heat absorption (T_m), the temperature for the onset of the transition (T_b), and another characteristic temperature defined as $T_c = \Delta H/\Delta S$.

RESULTS AND DISCUSSION

Figure 3 presents the DSC thermograms for poly[f_V(VPGVG),f_F(VPGFG)], Figure 5 the poly[f_V(VPGVG),f_Y(VPGYG)], and Figure 4 the poly[f_V(VPGVG),f_W(VPGWG)], in their different $f_V : f_X$ ratios. The corresponding thermodynamic data are reported in Table I.

All of the three aromatic amino acid containing polypentapeptides show an endothermic phase transition on increasing the temperature, as does the host polypentapeptide, poly(VPGVG). The copolypentapeptides, however, exhibit a broader transition than poly(VPGVG). This could be interpreted as the result of the disturbance of the β-spiral tertiary structure. On the basis of theoretical calculations, however, the aromatic substitutions result in very good β-spiral structures such that the broad transitions may arise from the quarternary structures of the polypeptides.

In terms of the mole fraction of the aromatic residues to that of valine, the transition temperatures T_b show a linear dependence to the extent of substitution reported. This is plotted for T_b vs f_X in

Table I DSC Data in Water of the Inverse Temperature Transition Exhibited by the Aromatic Residue Containing Copolypentapeptides poly [f_V (VPGVG), f_X (VPGXG)], where X = Trp, Phe, Tyr[a]

Compound	$f_V : f_X$	T_b[b] (°C)	T_m[b] (°C)	T_c[b] (°C)	ΔQ (cal/g)	ΔH (kcal/mol[c])	ΔS (cal/mol[c])
Poly[f_V (VPGVG), f_X (VPGXG)]							
X = Trp	1.0 : 0.0	25.4	27.4	30.99	3.05	1.25	4.11
	0.9 : 0.1	10.9	18.6	21.27	4.17	1.74	5.91
	0.86 : 0.14	7.2	15.2	19.45	4.31	1.82	6.22
	(0.0 : 1.0)[d]	−108	−59	−54			
X = Phe	1.0 : 0.0	27.4	29.6	33.30	2.91	1.18	3.89
	0.9 : 0.1	19.6	22.0	26.09	3.82	1.58	5.28
	0.8 : 0.2	12.9	16.0	20.43	4.58	1.92	6.54
	0.75 : 0.25	9.8	12.4	17.04	5.05	2.13	7.34
	(0.0 : 1.0)[d]	−43	−38	−31			
X = Tyr							
pH 7	1.0 : 0.0	27.2	29.7	33.67	2.99	1.22	3.98
	0.91 : 0.09	18.1	22.4	27.26	3.52	1.46	4.86
	0.86 : 0.14	12.5	18.0	21.93	3.87	1.62	5.49
	0.8 : 0.2	7.3	14.2	18.43	4.45	1.88	6.45
	(0.0 : 1.0)[d]	−74	−49	−44			
pH 12	0.91 : 0.09	36.4	[e]	44.31	1.43	0.60	1.89
	0.8 : 0.2	48.7	[e]	56.64	0.75	0.31	0.94
	(0.0 : 1.0)[d]	143					

[a] Reproducibility of the transition temperature is within 0.1°C for the Phe- and 0.5°C for the Tyr- and Trp-containing polypeptides.
[b] T_b: on set temperature of transition; T_c: $\Delta H/\Delta S$; T_m: temperature of maximum heat absorption.
[c] Per mole of pentamer.
[d] Extrapolation of $f_X = 1$.
[e] Too ill-defined.

Figure 6 and the values are given in Table I. The DSC-derived heat of transition increases with increasing mole fraction of the aromatic guest residues. In general, the heat increases with decreasing transition temperature as the transition temperature decreases with increasing mole fraction of the aromatic amino acid residue. For the Phe-containing copolypentapeptides, the dependencies of heat on mole fraction of guest residue and on the transition temperature are quite linear. But for the Tyr- and Trp-containing copolypentapeptides, the accuracy of heat obtained by integrating a broad DSC transition profile plus insufficient number of data points do not merit a quantitative description.

Tyrosine is generally taken to be less hydrophobic than phenylalanine because it contains a phenolic hydroxyl group.[32] Tyrosine, however, has been placed at different positions in different hydrophobicity scales, varying from position 2 to 14 of the 20 amino acid residues.[8-22] This may be understood in part on the basis of the DSC data reported here. As presented in Figure 6 and Table I, at pH 7 where the tyrosine hydroxyl group is protonated, the inverse temperature transition of the tyrosine containing copolypentapeptide progressively shifts to a lower temperature by increasing tyrosine composition in the copolypentapeptides. On the basis of transition temperature it is the second most hydrophobic amino acid residue, i.e., more hydrophobic than phenylalanine and less hydrophobic than tryptophan, whereas based on the heat of the transition

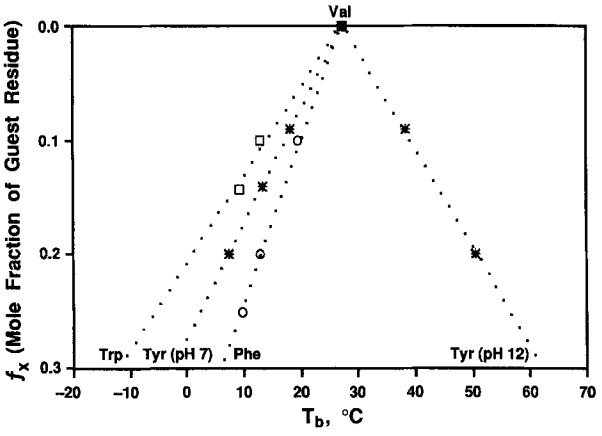


Figure 6. The dependence on molar fraction of guest residue f_X of the temperature T_b of an inverse temperature transition for the aromatic residue containing copolypentapeptides, poly[f_X(VPGXG),f_V(VPGVG)]. The linear dependence on f_X is used to extroplate the transition temperature to the value of $f_X = 1$ to give a comparison of hydrophobicities. The temperatures thus obtained are T_b(Phe) = −43°C, T_b(Trp) = −108°C, T_b(Tyr, pH 7) = −74°C, and T_b(Tyr, pH 12) = 143°C.

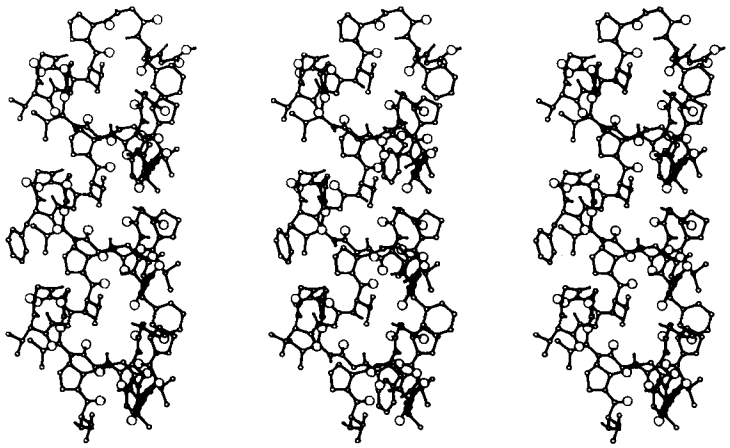
(see Table I), tyrosine is less hydrophobic than both phenylalanine and tryptophan. Increasing the pH value to 12, however, shifts the transition to a much higher temperature with a dramatically decreased heat; these values are in direct proportion to the tyrosine content in the polypentapeptide. At this pH where the tyrosine ring hydroxyl group is deprotonated, poly[0.8(GVGVP),0.2(GYGVP)] has a transition temperature of 49°C in comparison to a value of 37°C for poly[4(GVGVP),(GGGVP)],[33] indicating, on the basis of transition temperature, that the tyrosinate residue is less hydrophobic than glycine. A similar comparison is found using the heats of the transitions; at pH 12, poly-[0.8(VPGVG),0.2(VPGYG)] exhibits a ΔH of 0.31 kcal/mole whereas poly[0.8(VPGVG),0.2(VPGGG)] exhibits a higher ΔH of 0.42 kcal/mole.

A scale using the critical temperature of the inverse temperature transition has been reported[33] for 8 residues. Since the question in general is whether or not the polypeptide or protein is folded or unfolded at a chosen temperature, using temperature as the index becomes a practical choice.

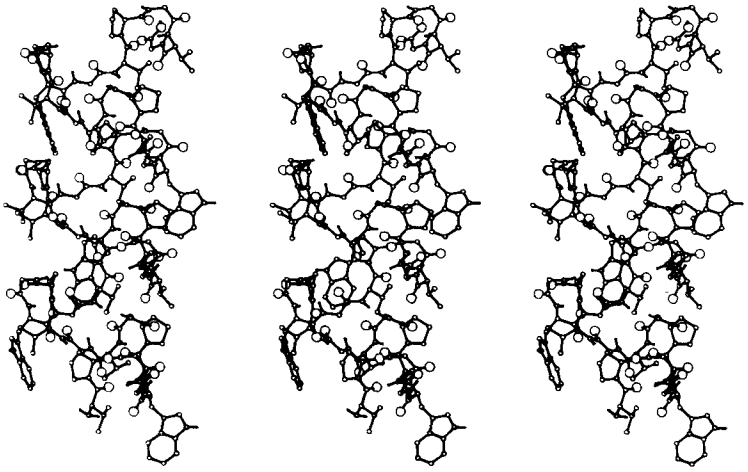
The scales based on small molecules (i.e., blocked amino acid residues) are subjected to the critique that the peptide linkage, which is integral to the expression of the hydrophobicity of an amino acid residue in a polypeptide or protein, is not included. This is true for the majority of the experimentally determined scales. On the other hand, scales based on the partition of amino acid residues inside or on the surface of the proteins are complicated by the conformational issue and because they provide no insight as to the physical forces involved. Accordingly, the scales so obtained are not true relative indices of the "phobia of hydration." Furthermore, there is the issue of preferred conformation on substitution. The "hydrophobia" does not mean that the residue with the phobia will definitely stay inside proteins, the conformational factor must come into play at this stage as well, of course, as the transition temperature for hydrophobic folding. Tryptophan may be less frequently buried in some classes of proteins not because of a lesser phobia of water, but rather because of its bulkiness.

This study is based on the polypentapeptide, poly(VPGVG), which forms a loose helical structure called a β-spiral rather than a more compact globular structure or a rigid helix or sheet. There is more freedom to accommodate changes of the side chain to a great extent without altering the overall molecular structure of this dynamic, elastic polypentapeptide. Starting from the structure of a stretched conformation of the poly(VPGVG), energy minimization using the ECEPP/2 program[34] results in

A. Poly[(VPGVG),(VPGFG)]



B. Poly[(VPGVG),(VPGWG)]



C. Poly[(VPGVG),(VPGYG)]

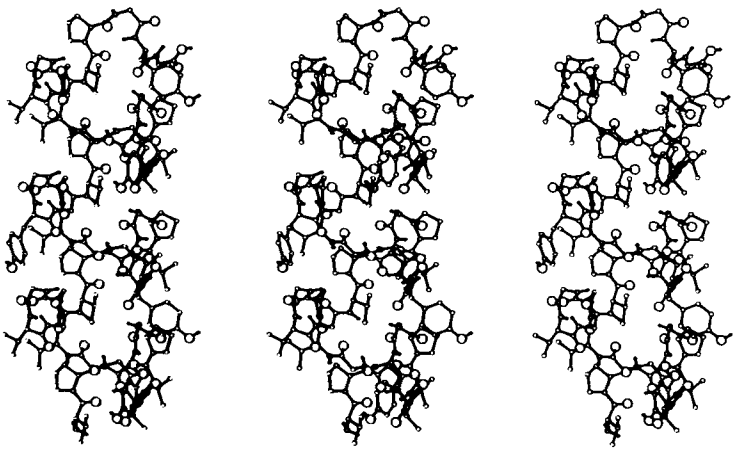


Figure 7. Stereoplots of the possible molecular structures for three hybrid polypentapeptides.

the correct structure for poly(VPGVG). Similar structures have been obtained for poly(VPGFG), poly(VPGYG), and poly(VPGWG) starting from the same backbone conformation. Three structures obtained for the hybrid polypeptides by the same procedure are shown in Figure 7.[35] It is reasonable therefore to assume that the copolypentapeptides used in this study retain the β-spiral structure of poly(VPGVG). Accordingly, it is not unreasonable to expect that the comparison of the DSC data of guest aromatic residues in poly(VPGVG) is not significantly compromised by perturbations of the β-spiral conformation.

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