Poly(2-acrylamido-2-methyl-1-propanamide) (PAMPA): A Neutral, Water-Soluble Synthetic Polymer with Double-Stranded Helix Conformation

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A new synthetic polymer, poly(2-acrylamido-2-methyl-1-propanamide) (PAMPA), is described. It combines the structural features of poly(leucine) and poly(glutamine), its molecular weight averages 42000 D, and it contains *ca.* 270 residues. A ^{13}C-NMR-tacticity study indicates that it consists of a mixture of syndiotactic (38%), heterotactic (48%), and isotactic (17%) polymer. PAMPA has a tendency to self-organize into a coiled-coil double-stranded helix structure in aqueous solution, as determined by *Fourier*-transform infrared spectroscopy (FT-IR). Molecular modeling of PAMPA shows that the helix formation is driven by repeated intramolecular H-bonds between nearest-neighbor amide groups, and that the structure is further stabilized by hydrophobic interactions between the two lateral Me groups. PAMPA has a neutral structure, is highly water-soluble, and demonstrates temperature stability.

1. Introduction – Molecular self-assembly plays a fundamental role in biological systems, as evidenced by proteins and polynucleotides. They exhibit intramolecular self-organization in aqueous solution by their spontaneous and reversible folding into well-defined conformations [1]. Understanding self-assembly and the associated noncovalent interactions is a central concern in structural biochemistry. The research on chemical systems with a tendency toward spontaneous self-organization is also emerging as a new strategy in chemical synthesis [2]. Nonbiological, simple synthetic polymers may provide alternative systems for investigating self-organization and allow researchers to understand particular interactions. Furthermore, the folding of linear polymers may provide synthetically simple tools to generate architectures that could potentially rival the biopolymers in their complexity and functionality.

Natural proteins carry out sophisticated chemical functions *via* folding into specific and compact conformations that are thermodynamically and kinetically stable. These tertiary folding structures provide 'active sites' comprising functional groups drawn from different regions of the linear polypeptide backbone. So far, a synthetic polymer that folds into a specific tertiary structure has not been described. Protein tertiary structure arises from the assembly of regular secondary structures (helices, sheets, and turns). The starting point for designing a synthetic polymer with the tendency to self-organize might, therefore, start with identification of a polymeric backbone with well-defined secondary-structural preferences.

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The folding of proteins involves complex interactions of a variety of noncovalent cohesive forces: H-bonds, electrostatic interactions involving charged groups and electrical dipoles, *Van der Waals* interactions, steric packing, and hydrophobic effects [3]. One of the most important secondary structures is the helix, which forms the basis of the more complicated structure. Duplex formation in nucleic acids and α-helix formation in proteins are the most prominent examples of helical supramolecular structures of biological origin. The α-helix as the most abundant secondary structure in proteins was first postulated by *Pauling et al.* [4]. It is generally believed that the primary reason for the self-assembly of polypeptides into a helix conformation in solution is intramolecular H-bonding of the backbone [5]. This helix conformation is further stabilized by the hydrophobic interactions of the side chains [6]. During the past decade, several types of synthetic polymers with secondary helical structure have been reported and have involved the engineering of H-bonds, whereas the backbones used have been closely related to peptides [7]. The most well-studied examples are the β-peptides consisting of β-amino acids. They have a rigid modified backbone and have a tendency to adopt a helical conformation. Other peptide-mimicking foldmers are vinylogous peptides and sulfonopeptides. Spontaneous self-assembly into double or triple helices of single-stranded oligo(2,2′-bipyridyl) has been observed, and this process is driven by the formation of a Cu^{I} complex [8]. More recently, a set of *meta*-linked phenylacetylene oligomers have been described, and they are claimed to collapse into helical conformations, which are solvent-dependent [9]. They represent examples of non-H-bonded helices. However, all the reported synthetic systems consist of only a small number of residues, and the longest oligomer so far has 18 residues. Natural proteins typically require more than 100 residues to display a stable tertiary structure. Polymers consisting of preorganized monomers also require *ca.* 40 residues for a stable tertiary structure. The synthesis of large molecules able to form stable tertiary structures poses a great synthetic challenge.

Another main problem is that all synthetic systems using H-bonding disintegrate in aqueous or polar solvents that are able to compete for H-bonds [10]. A central question in supramolecular chemistry is how H-bond interaction and hydrophobic interaction can be built cooperatively into synthetic systems in order to generate *via* self-organization the helix conformation, which then remains stable in aqueous medium. Furthermore, the solubility of a synthetic polymer in polar media is an important issue. For instance, the natural poly(alanine) peptides, which adopt a helix conformation, are scarcely soluble in H_2O. Nature has provided us with excellent examples of H_2O-soluble and conformationally stable supramolecular structures that may be used as a model for the design of synthetic alternatives. Thus, efforts have been made to increase the H_2O solubility of poly(alanine) and other peptides *via* insertion of polar amino acids or *via* introduction of polar functional groups on the side chains of natural peptides [11]. Therefore, when designing preorganized helical synthetic polymer candidates, their aqueous solubility should be a major concern.

2. Results and Discussion. – 2.1. *Synthesis.* We hereby report a new synthetic polymer system: poly(2-acrylamido-2-methyl-1-propanamide) (PAMPA) (*Fig. 1*). From the chemical point of view, this homopolymer is homologous to a polypeptide, and its molecular architecture can be viewed as inherited from poly(leucine) and

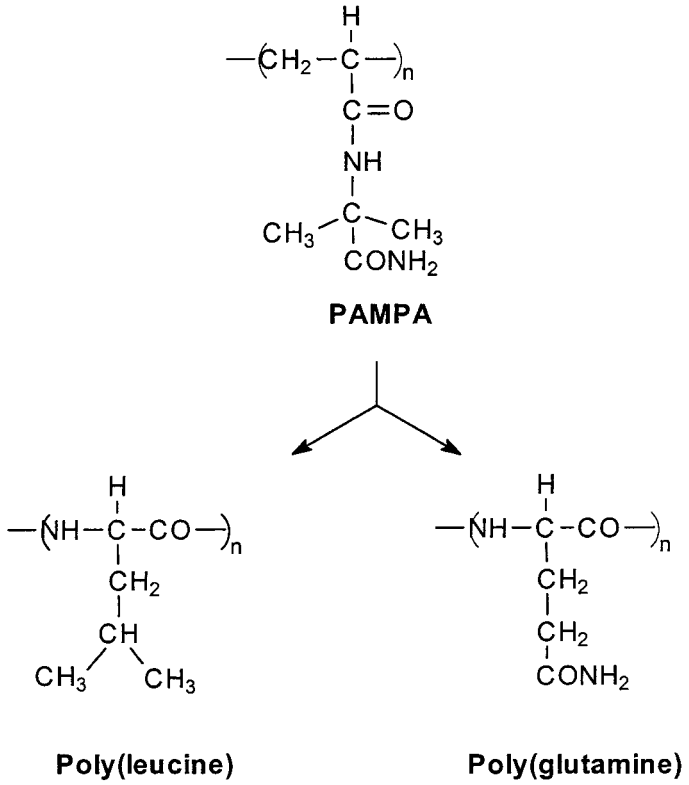


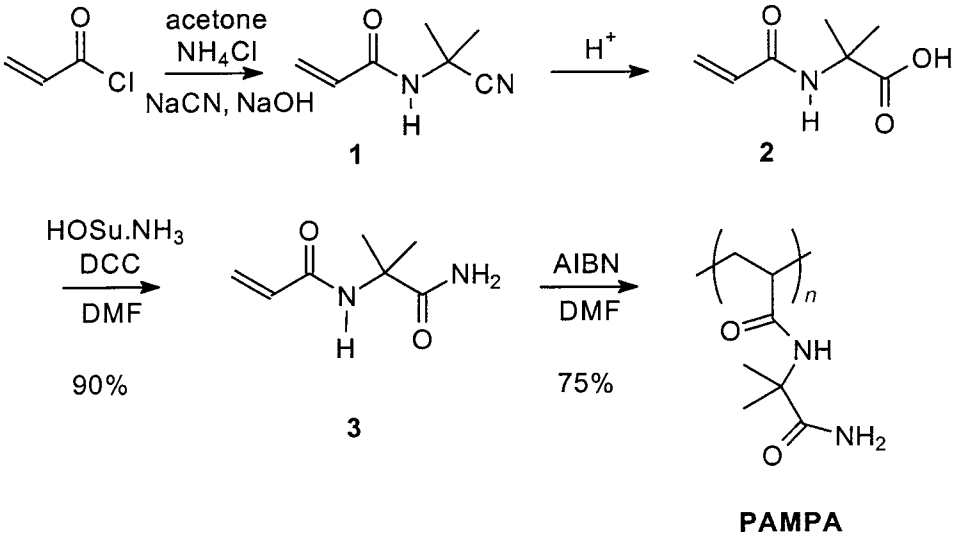
Fig. 1. *The structure of PAMPA*

poly(glutamine). Leucine and glutamine are known to be helix-forming residues. An important difference between PAMPA and polypeptides or synthetic peptide-mimicking oligomers is that PAMPA has a nonpolar substituted poly(ethylene) backbone and includes peptide bonds in its side chain, while the latter have peptide groups on their backbone.

PAMPA was prepared as shown in the *Scheme*. The known acid **2** [12] was generated from a mixture of acryloyl chloride and acetone in the presence of NaCN and NH_4Cl, followed by acid hydrolysis of the resulting *N*-cyano amide **1**. Amonolysis with the ammonium salt of *N*-hydroxysuccinimide [13] in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) then gave the monomer amide **3**. Polymerization was carried out in DMF at 70°, initiated by a catalytic amount of 2,2′-azobis(2-methylpropanenitrile) (AIBN) [14]. Purification with *Sephadex G-50* and H_2O as eluent to remove monomer and DMF, followed by dialysis against H_2O, afforded PAMPA as a light white fiber. PAMPA is completely miscible with H_2O, DMF, and DMSO. Its molecular weight averages *ca.* 42000 D, as determined by gel-permeation chromatography (GPC) with proteins as standard.

2.2. *FT-IR Conformational Analysis.* Synthetic polyacrylamide polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) undergo temperature-, salt-concentration-,

Scheme. *Synthesis of PAMPA*



or solvent-induced transitions. As such, they show behavior similar to natural proteins and have, therefore, been used as simple models to study the physical behavior of proteins [15]. Due to the similarity between the constitutions of PAMPA and a polypeptide, the techniques for determining the secondary structure of proteins were applied to investigate the structural motif of the PAMPA system. Since the PAMPA polymer is a racemic system, circular dichroism (CD) is not suited to study its secondary structure. We, therefore, turned to *Fourier*-transform infrared spectroscopy (FT-IR), a technique that has been extensively used to study the different types of conformations of peptides [16–19], to compare the spectral patterns of synthetic PAMPA with those of natural proteins and to investigate the secondary structure of PAMPA.

FT-IR Measurements of PAMPA were taken in D_2O as solvent. Apart from the polyethylene backbone of PAMPA, the main mid-IR absorption of the PAMPA side chains is expected in the region between 1600 and 1700 cm^{-1}, where the polyethylene backbone does not absorb. In this region, the absorption is almost completely (80%) due to the C=O stretching vibration. There is a minor contribution of the N–H bending. *Fig. 2* shows the slightly resolution-enhanced spectrum of the C=O stretching band of PAMPA ($k = 1.5$) as well as the *Gaussian* bands that contribute to it. The three Gaussians have their maximum at 1623, 1634, and 1651 cm^{-1}, respectively. Their respective contribution to the overall structure is 18, 36, and 46%. Normally, the band position at 1651 cm^{-1} is typical of a classical α-helix, while the bands at 1623 and 1634 cm^{-1} are usually assumed to indicate β-sheets and random-coil, or disordered, structure.

However, the absorption pattern analysis of the C=O stretching band of PAMPA shows striking similarity with the amide-I band (1600–1700 cm^{-1}) of double-stranded coiled-coil helical proteins, such as desmin and tropomyosin [17]. *Heimburg et al.*

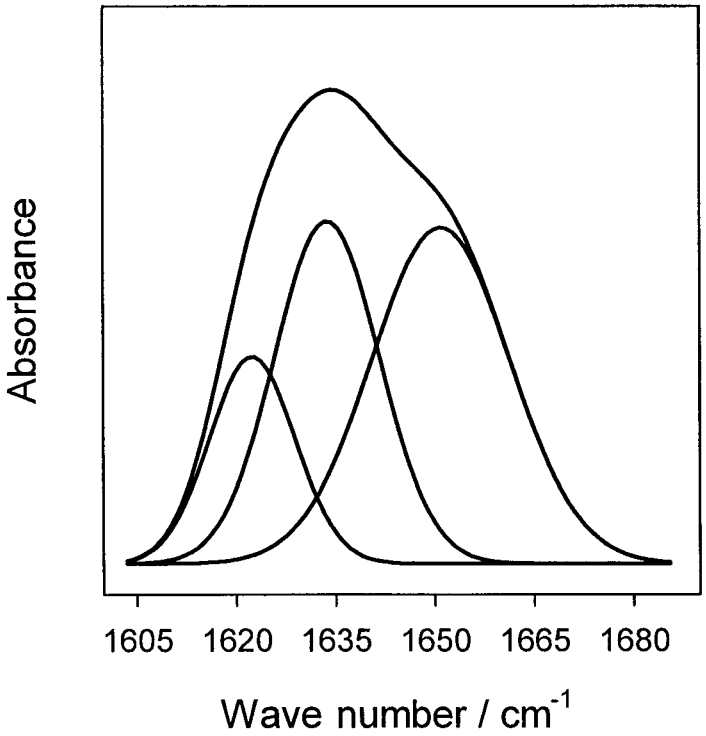


Fig. 2. *The IR C=O stretching band* (1600–1700 cm^{-1}) *of PAMPA in* D_2O *at 25°*. The band is slightly resolution-enhanced ($k = 1.5$). The three *Gaussians* underneath are the contributing components with maxima at 1623, 1634, and 1651 cm^{-1}.

showed that a coiled-coil helix structure is characterized by three bands at 1628 (28%), 1640 (40%), and 1653 (32%) cm^{-1}, respectively. All three bands are assigned to a helix structure. Their findings are confirmed by normal-mode calculations of coiled-coil models [18]. It is argued that the lower wave number *Gaussians* are due to the strong H-bonding of the backbone C=O groups with the solvent in addition to the intrahelical H-bonds. The similarity between PAMPA and coiled-coil helix proteins might suggest that PAMPA adopts a similar structure in solution. Considerations about the function of the Me groups for structural organization of PAMPA support this conclusion. Indeed, the coiled-coil tertiary structure of natural proteins is formed *via* hydrophobic interactions of the side chains of the double-stranded α-helices. As PAMPA has two hydrophobic Me groups on the side chain, it is expected to exhibit a tendency to form a coiled-coil helical structure. The fact that the maxima of the three *Gaussians* for PAMPA do not exactly match those found for the proteins investigated is explained by the dependence of their position on the relative alignment and the number of H-bonds [19].

The temperature stability of PAMPA was investigated. It was found that PAMPA does not undergo a major cooperative transition up to 95°. Only a shift of the wave number of the C=O stretching-band maximum can be observed (*Fig. 3*). This

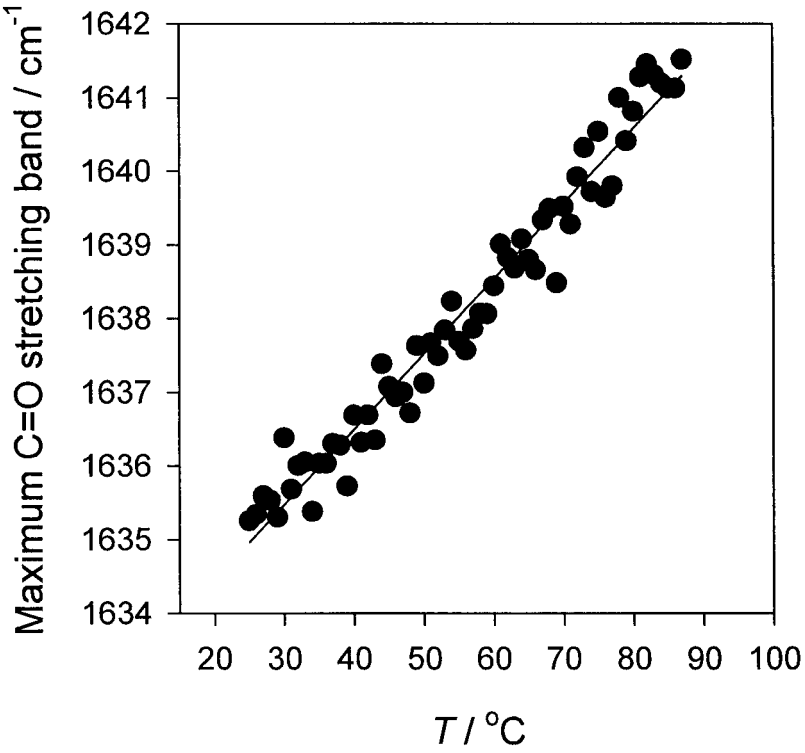


Fig. 3. *Temperature dependence of the maximum of the IR C=O band of PAMPA*

finding is confirmed by differential scanning calorimetry (DSC, unpublished data). In contrast, other poly(acrylamide) compounds such as PNIPAM undergo transitions at low temperature (39° for PNIPAM). These transitions have been characterized as coil-globule transitions [15][20]. Also, isolated natural α-helices are marginally stable in aqueous solution. They are formed rapidly, but their rate of unraveling is just as high. The absence of such a temperature transition, as well as the fact that PAMPA shows significant temperature stability, might be interpreted as further evidence for its stable double-helical structure.

2.3. *^{13}C-NMR Tacticity Study.* Although the tacticity assignment of poly(acrylamide) is rather controversial, ^{13}C-NMR is considered to be the most useful technique for this purpose. As the polymerization of the monomer was carried out under radical conditions, PAMPA is expected to consist of a mixture of isomers of different tacticity. Its tacticity was studied by ^{13}C-NMR (125 MHz) in D_2O as solvent at room temperature. The signals of the two C=O C-atoms (at 182 and 179 ppm) show small splittings and cannot be used for the interpretation of the structure of the backbone, while the signals of CH_2 C-atoms that appear at 37 ppm give a complex pattern. However, the signals of CH C-atoms appear as three distinct peaks at 44.9, 44.0, and 43.0 ppm, with relative areas of 35, 48, and 17%, assigned to syndiotactic (rr), heterotactic (mr), and isotactic (mm) polymers, respectively. The stereoregularity of

PAMPA was assigned by comparison of the CH C-atom pattern with the one of the known poly(acrylamide) [21] and poly(acrylic acid) [22]. As a general rule, the low-field and high-field peaks are assigned to syndiotactic and isotactic sequences, respectively. The fact that the syndiotactic polymer is favored over the isotactic polymer in this case might be due to the bulkiness of the side-chain group and to preorganization into a helical structure driven by the internal H-bonding (see below). The observation that PAMPA appears as a polymeric mixture prevents its structure determination by X-ray-diffraction studies.

2.4. *Molecular Modeling.* FT-IR Experiments (see above) have indicated that PAMPA might be able to adopt a double-stranded helical structure. Given the structural complexity of the double-stranded helical structure, we have performed molecular modeling of the single-stranded helical structure of PAMPA with MacroModel. A 14-mer was used for the modeling, and the resulting single-helix structure of syndiotactic, heterotactic, and isotactic PAMPA is presented in *Fig. 4*. All three types of PAMPA can self-assemble into a helical conformation. Intramolecular H-bonds between nearest-neighbor amide groups assure the coherence of the resulting supramolecular structure. In protein helices (α and 3_{10}), H-bonding between nearest-neighbor backbone amide groups is unfavorable. However, the presence of conformationally repeating units caused by the eight-membered H-bonding ring drives PAMPA to helicalization. PAMPA may then adopt a regular helical structure in which *ca.* 5 residues make up a full turn. In this conformation, the secondary structure is optimally preorganized for the formation of the intracatenate H-bonding patterns. The globular structures of the three types of helices are very similar. The isotactic polymer is the most compact one and has only one type of H-bond. The CO$\cdots$H distance is 1.75, and the helix turn is 9.5 Å. In the case of the syndiotactic polymer, there are two alternating types of H-bonds, one is 1.8 and the other 2.1 Å, and the helical turn is longer (10.9 Å) than that of the isotactic isomer. The heterotactic polymer is in fact a mixture of syndiotactic and isotactic isomers. It has three types of H-bonds: the most stable one is 1.8 Å, similar to the one found in the isotactic isomer, and the other two are comparable to those of the syndiotactic isomer. The heterotactic helix has a turn of 10 Å. The three helical conformations are further stabilized by hydrophobic interactions between the two lateral Me groups. This hydrophobic effect is believed to be essential for the helix structure to be stable in aqueous solution and also leads to the formation of a double-stranded coiled-coil helical structure. Furthermore, the H_2O solubility of the polymer is increased by the presence of an additional amide group at the end of the side chains. This amide group does not interfere with the internal H-bonding upon helix formation. This general model of PAMPA corresponds to the specific patterns of polar and nonpolar groups found in a polypeptide chain. Polar groups on the surface form H-bonds with H_2O, and polar groups localized internally are involved in intramolecular H-bonding. The nonpolar side chains are removed from H_2O and assembled into hydrophobic cores.

3. Conclusion. – In summary, a synthetic polymer that presumably adopts a stable double-stranded helical conformation in water is reported. In contrast to the backbone of natural peptides, PAMPA has a poly(ethylene) backbone and two amide functional groups in its side chain. Due to this constitutional organization, intramolecular eight-

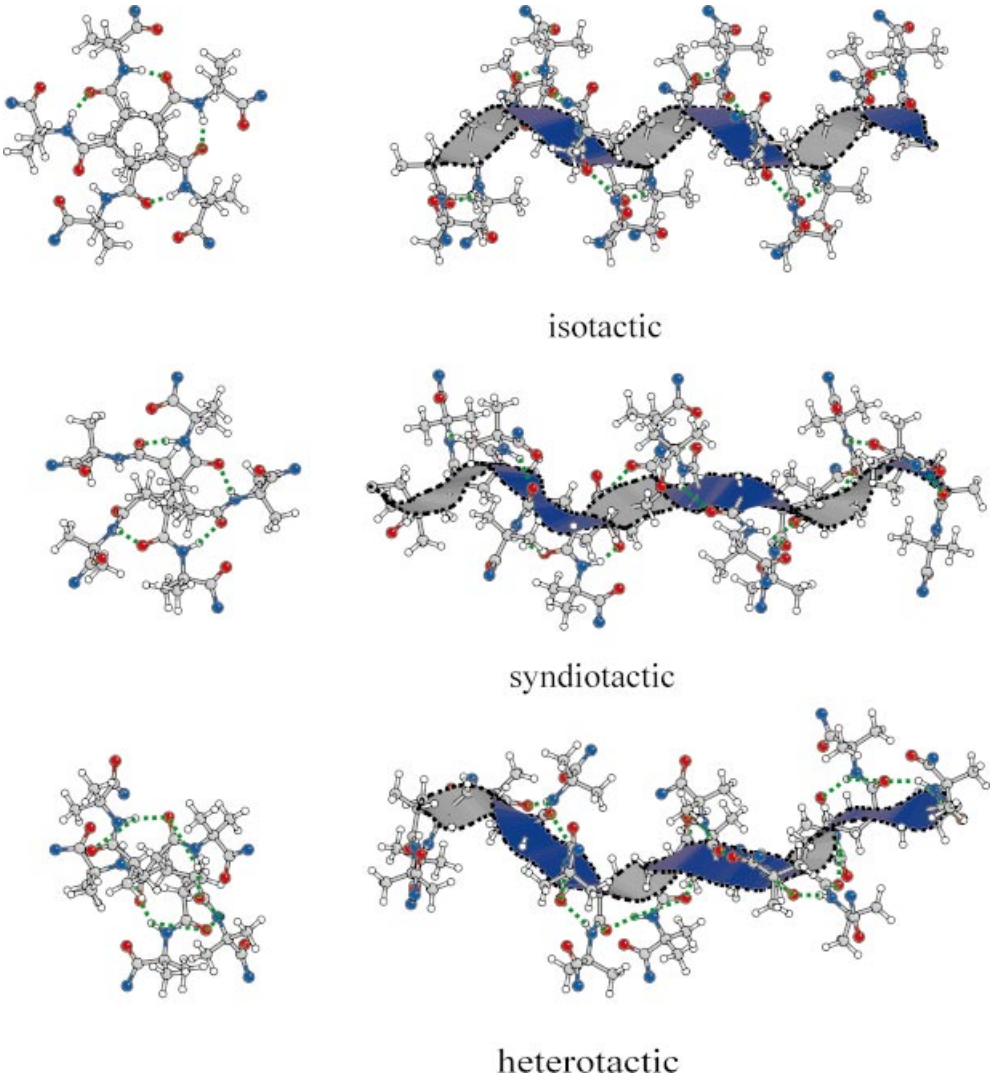


Fig. 4. *Computed model of the proposed helix structure of isotactic* (top), *syndiotactic* (middle), *and heterotactic* (bottom) *PAMPA*

membered rings stabilized by one H-bond between nearest-neighbor amide groups make up the conformational repeating units of PAMPA. This eight-membered H-bonding motif for helical formation has also been observed in a peptide-mimicking synthetic system [23].

In proteins, the double-stranded or coiled-coil helical structure consists of two helices wrapped around each other with a left-handed super twist. Regularly repeated hydrophobic leucine groups are responsible for the double-stranded-helix formation. Some synthetic polymers can also form a double-stranded helical structure. For

example, the crystal-structure analysis of poly(methyl methacrylates) showed that either isotactic [24], syndiotactic [25], or mixtures of isotactic and syndiotactic stereocomplexes [26] adopt double-stranded helices. The two helical chains are held together mainly by nonbonding *Van der Waals* interactions between the Me end groups. Because PAMPA combines the structural features of a poly(leucine) peptide and a poly(glutamine) peptide, it has the tendency to adopt a double-stranded helical conformation. The 2-methyl-1-propanamide substituent of the aforementioned eight-membered ring confers, as well as stabilization of the helical conformation, H_2O solubility. The two Me groups on the side chain create a hydrophobic face, which is essential for the stability of the double helical structure. The presumed coiled-coil helical structure stabilizes the helix conformation of the polymer. PAMPA is additionally a neutral polymer, and the external amide groups on the side chain are exposed to the environment and secure the aqueous solubility of the polymer. PAMPA may be considered a polymer system of unnatural origin that is capable of adopting a stable tertiary structure in aqueous solution.

Experimental Part

General. N,N′-Dicyclohexylcarbodiimide (DCC) and 2,2′-azobis(2-methylpropanenitrile) (AIBN) were obtained from commercial suppliers and used without further purification. DMF was dried over CaH_2 and distilled under vacuum. Air-sensitive reactions were performed under N_2. Acid **2** was synthesized according to the *Heilman*'s method [12], and the ammonium salt of *N*-hydroxysuccinimide ($NH_3 \cdot$HOSu) was prepared according to the literature procedure [13]. *Sephadex G-50* (superfine) was purchased from *Pharmacia Biotech*. M.p.: *Büchi-Tottoli* apparatus; uncorrected. ^{1}H- and ^{13}C-NMR spectra: *Varian Gemini* 200-MHz and *Varian Unity* 500-MHz spectrometers.

2-Methyl-2-(prop-2-enamido)propanamine (**3**). To a mixture of *2-methyl-2-(prop-2-enamido)propanoic acid* (**2**; 1.57 g, 10 mmol) and $NH_3 \cdot$HOSu (2.64 g, 20 mmol) in DMF (50 ml) at 0° was added DCC (4.12 g, 20 mmol) in portions. The mixture was stirred at 0° for 1 h, allowed to warm to r.t., and stirred for 2 d. The mixture was filtered and washed with CH_2Cl_2 (150 ml) and AcOEt (150 ml). The filtrate was concentrated, the residue was absorbed on silica gel and eluted with CH_2Cl_2/MeOH 50:1, then 20:1, to afford **3** (1.4 g, 90%). White solid. M.p: 171–172°. ^{1}H-NMR ((D$_6$)DMSO, 200 MHz): 1.36 (*s*, 6 H); 5.54 (*dd*, $J = 9.9$, 2.3, 1 H); 6.02 (*dd*, $J = 17.1$, 2.3, 1 H); 6.29 (*dd*, $J = 17.1$, 9.9, 1 H); 6.82 (*s*, 1 H); 7.06 (*s*, 1 H); 8.00 (*s*, 1 H). ^{13}C-NMR ((D$_6$)DMSO): 25.1 (*q*); 55.9 (*s*); 124.9 (*t*); 132.5 (*d*); 164.0 (*s*); 176.2 (*s*). ES-MS (i-PrOH/H_2O 1:1): 179 ([M + Na]$^+$), HR-MS: 179.0794, ([M + Na]$^+$, $C_7H_{12}N_2O_2Na$: 179.0797).

Poly(2-methyl-2-(prop-2-enamido)propanamide) (PAMPA). A soln. of **3** (2.22 g, 14.22 mmol) in dry DMF (15 ml) was flushed with N_2 for 15 min, and AIBN (24 mg, 0.142 mmol, 0.01 equiv.) was added. The reaction flask was placed in a preheated bath (70°) for 7 h and then cooled to r.t.. With a pipette, the mixture was dropped into dry Et_2O (150 ml), and the resulting pellets were collected and washed two times with dry Et_2O. The pellets were dissolved in H_2O (15 ml), and the soln. was passed through *Sephadex G-50* (30 g), eluting with H_2O. The collected product was lyophilized to give PAMPA as a light white fibre (1.62 g, 73%). The tacticity of PAMPA was determined by ^{13}C-NMR (125 MHz, D_2O). M.p. > 240° (dec.). ^{1}H-NMR (CD_3OH, 500 MHz): 1.47–2.24 (2 br. *s*, 9 H); 6.80 (*s*, 1 H); 7.33 (*s*, 1 H); 8.03 (*s*, 1 H). ^{13}C-NMR (D_2O, 125 MHz): 27.3 (4 peaks, *q*, Me); 37.4 (*m* + *t*, CH_2); 43.0; 44.0; 44.9 (3 peaks, *d*, CH); 59.2 (*s*, C); 178.6 (*s*, CONH); 182.4 (*s*, $CONH_2$).

FT-IR Measurement. PAMPA was dissolved in D_2O (50 mg/ml), and the soln. was allowed to stand overnight to ensure complete H/D exchange. The IR spectrum was obtained with a *Bruker IFS66* FT-IR spectrometer equipped with a liquid-N_2-cooled broad-band mercury-cadmium-telluride solid-state detector using a CaF_2 cell with a *Teflon* spacer of 50 μm (*Graseby Specac*). 250 Interferograms were co-added after registration at a resolution of 2 cm^{-1}. *Fourier* self-deconvolution and fitting were done with a program developed by *Heremans* and co-workers [27]. The deconvolution parameters used are a *Lorentzian* of 20 cm^{-1} half-bandwidth, a resolution enhancement factor (*k* value) of 1.5, and a triangular square apodization function.

Molecular Modeling. The modeling was performed with MacroModel V5.0 [28]. The surrounding H_2O was mimicked by means of the GB/SA solvation method, and the solvent accessible surface area was calculated by means of the DMS program of MidasPlus [29]. All figures were created with a modified version of Molscript [30].

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