Systemin Has the Characteristics of a Poly(L-proline) II Type Helix

Arazdordi Toumadje and W. Curtis Johnson, Jr.*

*Biochemistry & Biophysics Department
Oregon State University
2011 Agriculture and Life Science Building
Corvallis, Oregon 97331-7305*

Received April 17, 1995

One goal for plant pathologists is to reduce the damage to plant tissue by insects. This can be achieved by controlling predator population, but plants use their own natural defense mechanism to reduce the damage by insects. Tomato plants, for example, produce chemical signals in their leaves that activate the synthesis of proteinase inhibitors in their tissues, which in turn disrupt the digestion of the feeding insects.[1] The search for a systemic inducing signal for the proteinase inhibitor I and II genes in tomato leaf extracts led Ryan's group at Washington State University to discover an 18-mer peptide called systemin.[2,3] Native systemin has been sequenced, and synthesized systemin is as effective as the native peptide, so there are no unusual features.[2] Systemin is the only oligopeptide hormone-like molecule that has been found in plants. Here we investigate the secondary structure of this unique peptide in solution.

The sequence of systemin is AVQSK PPSKR DPPKM QTD. The most interesting features in this sequence are the double prolines at positions 6 and 7, and 12 and 13. These prolines suggest a left-handed, 3_1, poly(L-proline) II (PPII) secondary structure for at least the central portion of the peptide. Recently, oligopeptides containing proline repeats that are ligands for Src homology 3 (SH3) have been shown to have a 3_1 helicity.[4–6] On the other hand, sequences PPSK and DPPK are predicted by the Chou–Fasman method[7] to be β turns. We searched the Protein Information Resource data base of 67 423 proteins and found that PPSK was represented 235 times while DPPK was represented 191 times. However, of these 426 proteins, only two have their structure in the Brookhaven Data Bank. The two proteins, mengo encephalocarditis virus coat protein[8] and polio virus (type 1, Mahony strain),[9] both have the sequence DPPK, and in both cases this sequence is part of a 3_1 helix. Overall, there has been an increase in recognition of left-handed 3_1 structure in proteins. Recently, the conformation of 80 globular proteins was analyzed for helical structures and 96 segments of four or more residues of PPII were identified.[10]

NMR studies report that systemin has no stable secondary structure in aqueous solution.[11] However, the 3_1 helix is so extended that it does not have any special NOEs that distinguish it from an unordered peptide (Prof. Jean S. Baum, personal communication). Therefore, the NMR studies are consistent with a 3_1 structure for systemin. Circular dichroism (CD) is particularly sensitive to secondary structure. Characteristic of PPII as a 3_1 helix is a small positive CD band at about 228 nm with a $\Delta\epsilon$ of +1.4 and a large negative band at about 205 nm with $\Delta\epsilon$ of −18; collagen as a 3_1 helix has a small positive band at about 220 nm with a $\Delta\epsilon$ of +2.5 and a large negative band at about 200 nm with a $\Delta\epsilon$ of −24.[12] Repeating charged polypeptides as the so-called "random coil" have a strong preference for the 3_1 region of conformational space, and their CD has the 3_1 shape, but with a $\Delta\epsilon$ of +1.5 at 217 nm and a $\Delta\epsilon$ of about −10 at 197 nm at ambient temperature.[13–17] In contrast, truly unordered polypeptides with a dynamic structure that samples all of the available conformational space have a similar but somewhat different shape. Their hallmark is a negative band at about 225 nm with a $\Delta\epsilon$ of about −1 and a negative band at 200 nm with a $\Delta\epsilon$ of about −5.[10–17] Thus the shape and magnitude of the CD differentiates between truly unordered polypeptides and those with a preference for the 3_1 structure.

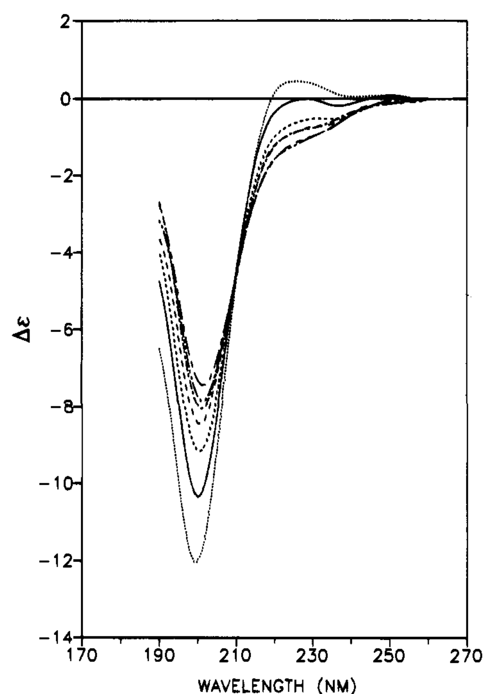

Figure 1. CD spectra of systemin in 10 mM phosphate buffer measured at 5 °C (•••), 20 °C (—), 40 °C (- - -), 60 °C (— —), 70 °C (— -), 80 °C (— - -), and 85 °C (— —).

Figure 1 shows the CD of systemin as a function of temperature. At 5 °C we see the characteristic 3_1 spectrum, but with lower intensity. It is a rare peptide that has significant α, β, or turn structure in aqueous buffer, so it is reasonable to analyze this CD as a combination of 3_1 helix and truly unordered CD. Although we do not know the precise 3_1 helix CD that corresponds to systemin, it is reasonable to use both PPII and collagen as models. Peptide $(TSDSR)_3$ is used as the model for the unordered structure. Figure 2 shows that systemin has the proper shape and intensity corresponding to 58% PPII or 36% collagen. Presumably the 3_1 helix for the systemin sequence has maxima and minima at wavelengths intermediate

* To whom correspondence should be addressed.

(1) Green, T.; Ryan, C. A. *Science* **1972**, *175*, 776–777.
(2) Pearce, G.; Strydom, D.; Johnson, S.; Ryan, C. A. *Science* **1991**, *253*, 895–898.
(3) Pearce, G.; Johnson, S.; Ryan, C. *J. Biol. Chem.* **1993**, *268*, 212–216.
(4) Gout, I.; Dhand, R.; Hiles, I. D.; Fry, M. J.; Penayotou, G.; Das, P.; Truong, O.; Totty, N. F.; Hsuan, J.; Booker, G. W.; Campbell, I. D.; Waterfield, M. D. *Cell* **1993**, *75*, 25–36.
(5) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933–945.
(6) Musacchio, A.; Saraste, M.; Wilmanns, M. *Nature Struct. Biol.* **1994**, *1*, 546–551.
(7) Chou, P. Y.; Fasman, G. D. *Adv. Enzymol.* **1978**, *47*, 45–148.
(8) Luo, M.; Vriend, G.; Kamer, G.; Minor, I.; Arnold, E.; Rossmann, M. G.; Boege, U.; Scraba, D. G.; Duke, G. M.; Palmenberg, A. C. *Science* **1987**, *235*, 182–191.
(9) Filman, D. J.; Syed, R.; Chow, M.; Macadam, A. J.; Minor, P. D.; Hogle, J. M. *EMBO J.* **1989**, *8*, 1567–1579.
(10) Adzhubei, A. A.; Sternberg, M. J. E. *J. Mol. Biol.* **1993**, *229*, 472–493.
(11) Russell, D. J.; Pearce, G.; Ryan, C. A.; Satterlee, J. D. *J. Protein Chem.* **1992**, *11*, 265–274.
(12) Jenness, D. D.; Sprecher, C.; Johnson, W. C., Jr. *Biopolymers* **1976**, *15*, 513–521.
(13) Tiffany, M. J.; Krimm, S. *Biopolymers* **1968**, *6*, 1379–1382.
(14) Krimm, S.; Mark, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *60*, 1122–1129.
(15) Drake, A. F.; Siligardi, G.; Gibbons, W. A. *Biophys. Chem.* **1988**, *31*, 143–146.
(16) Woody, R. W. *Adv. Biophys. Chem.* **1992**, *2*, 37–79.
(17) Dukor, R. K.; Keiderling, T. A. *Biopolymers* **1991**, *31*, 1747–1761.

0002-7863/95/1517-7023$09.00/0 © 1995 American Chemical Society

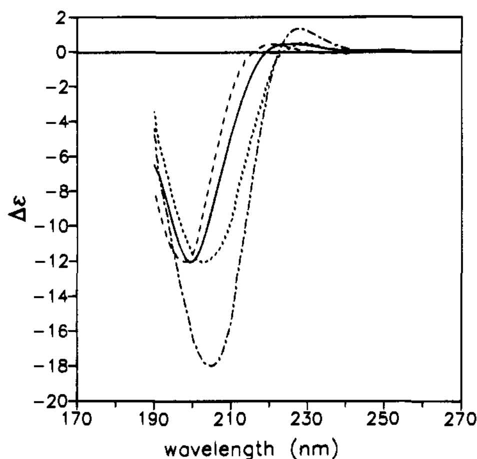

Figure 2. Comparison of the CD spectra for systemin at 5 °C (—), polyproline II redrawn from ref 12 (- • -), 58% PPII and 42% $(TSDSR)_3$ as an unordered peptide (• • •), and 36% collagen and 64% $(TSDSR)_3$ (— — —).

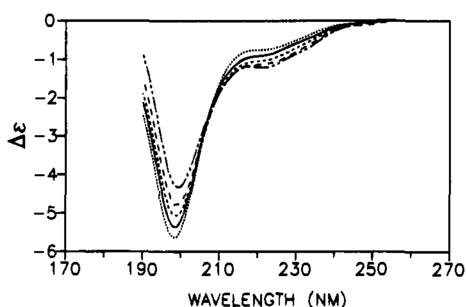

Figure 3. CD spectra of $(TSDSR)_3$ in 10 mM phosphate buffer measured at 5 °C (• • •), 20 °C (—), 40 °C (- -), 60 °C (— —), and 80 °C (— - -).

between the two models. Although CD cannot tell the difference between 36–58% of systemin in a static 3_1 structure or a population average of 3_1 and unordered structure, the static structure is consistent with expectations based on its sequence and the precedent of the SH3 ligands. Raising the temperature decreases the intensity of the CD dramatically, as the thermal agitation destroys 3_1 structure in favor of the dynamic unordered peptide. A similar effect of temperature involving the disappearance of 3_1 conformation in the repetitive domain of maize glutelin-2 peptides, $H(VHLPPP)_nOH$, has also been reported.[18] At 85 °C the CD has roughly the intensity expected for 100% unordered peptide.

For comparison, the CD of a truly unordered peptide, $(TSDSR)_3$, is shown under identical conditions in Figure 3. While the magnitude of the CD for this peptide decreases slightly as the temperature is raised to produce a more dynamic molecule, the effect is far less than seem for systemin, which has a high tendency to assume the 3_1 helix. The CD of $(TSDSR)_3$ at 5 °C is much less intense than the CD of systemin at 5 °C.

Certain organic solvents tend to induce secondary structure in peptides.[19-25] Figure 4 compares the CD of systemin in

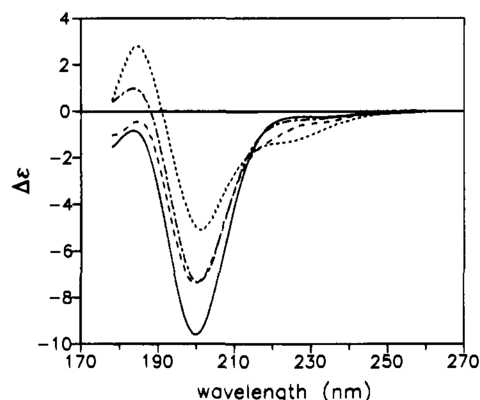

Figure 4. CD spectra of systemin in 10 mM phosphate buffer (—), 2 mM SDS (— —), 25 mM SDS (— -), and 100% TFE (- -) at ambient temperature.

aqueous solution with systemin in 2,2,2-trifluoroethanol (TFE) and sodium dodecylsulfate (SDS), both below and above the micelle concentration. At the nonmicellar concentration of 2 mM SDS, which tends to induce β-strand structure in many peptides,[23-25] the CD of systemin is virtually unchanged from aqueous solution. Micellar SDS at 25 mM mimics the membrane environment and often induces peptides to become α-helical.[20] Here again the CD of systemin is only slightly changed. In most peptides, 100% TFE induces a large amount of α-helical structure,[21-25] but the CD in this solvent shows only about 12% α-helix as judged by the negative intensity of the 222 nm band. The inability of these organic solvents to induce either α-helix or β-strand structure in systemin supports the idea that this peptide already has a stable structure in aqueous solution, the 3_1 helix.

In summary, the proline pairs in the sequence, the fact that DPPK is found in 3_1 helices in proteins, the CD in aqueous buffer, and the precedent of SH3 ligands all point to a poly(L-proline) II type, 3_1 structure for a substantial population or the central portion of systemin.

The peptide sequence of systemin was synthesized with an Applied Biosystems Model 431A peptide synthesizer and analyzed by the staff of the Central Service Laboratory at OSU. The peptide was purified on a Hewlett-Packard HPLC Model 1050 Ti-series using a semipreparative C_{18} reversed-phase column from VYDAC. Analytical HPLC under the same conditions exhibited a single peak with no detectable impurities in the peptide preparation. Amino acid analysis verified composition and accurately determined the peptide concentration.

Stock solutions were prepared in 10 mM sodium phosphate buffer, pH 7.0, in SDS solution, or in 100% TFE. CD spectra were measured in 1 mm or 50 μm cells using a JASCO J-720, calibrated at two points with (+)-10-camphorsulfonic acid.[26] All data are in $\Delta\epsilon$ (M^{-1} cm^{-1}) on a per amide basis. CD spectra are repeatable with virtually no error. However, the ability of systemin samples to form the 3_1 helix increases somewhat with age, leading to a standard deviation for CD intensity of about 6%. The extinction coefficient for normal absorption of the amide bond at 190 nm was determined to be 8692 M^{-1} cm^{-1} in phosphate buffer and 6261 M^{-1} cm^{-1} in 100% TFE.

Acknowledgment. It is a pleasure to thank Sonya M. Baker for recognizing the poly(L-proline) II helix of DPPK in the crystal data. This work was supported by PHS Grant GM21479 from the National Institutes of Health.

JA951217+

(18) Rabanal, F.; Ludevid, M. D.; Pons, M.; Girald, E. *Biopolymers* **1993**, *33*, 1019–1028.
(19) Jirgensons, B. *Biochem. Biophys.* **1977**, *473*, 352–358.
(20) Gierasch, L. M. *Biochemistry* **1989**, *28*, 923–930.
(21) Sonnichsen, F. D.; Van Eyk, J. E.; Hodges, R. S.; Sykes, B. D. *Biochemistry* **1992**, *31*, 8790–8798.
(22) Buck, M.; Radford, S. E.; Dobson, C. M. *Biochemistry* **1993**, *32*, 669–678.
(23) Wu, C. C.; Yang, J. T. *Mol. Cell. Biochem.* **1981**, *40*, 109–122.
(24) Wu, C. C.; Ikeda, K.; Yang, J. T. *Biochemistry* **1981**, *20*, 566–570.
(25) Waterhouse, D. V.; Johnson, W. C., Jr. *Biochemistry* **1994**, *33*, 2121–2128.
(26) Chen, G. C.; Yang, J. T. *Anal. Lett.* **1977**, *10*, 1195–1207.