Kinetic Mechanism for Conformational Transitions between Poly-L-prolines I and II: A Study Utilizing the Cis-Trans Specificity of a Proline-Specific Protease†

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ABSTRACT: The isomeric specificity of aminopeptidase P [Lin, L.-N., & Brandts, J. F. (1979) *Biochemistry 18*, 5037] was employed to study the mechanism of poly-L-proline transitions in solution. The rates of hydrolysis for the all-trans (II), the all-cis (I), and the partially cis-to-trans or trans-to-cis isomerized poly-L-proline were measured under conditions of high enzyme activity. The results confirm our previous suggestion that the cis-to-trans isomerization of poly-L-proline I, when dissolved in water, begins at the N-terminal end and proceeds in a step-by-step manner to the C terminus. Our data further show that the trans-to-cis isomerization of poly-L-proline II in 90% butanol–water starts instead at the C-terminal end and also occurs with a single trans-cis junction in the polyproline chain of M_r 6000. Thus, the intermediate states with H_2N-trans···trans-cis···cis-COOH configuration are stable in H_2O as well as in butanol during isomerization. Our data also show that the cis-to-trans isomerization of a Pro–Pro bond will occur at nearly the same rate whether the peptide bond to be isomerized is at the middle of the polypeptide chain or at the N-terminal end. Finally, the hydrolysis data show that more than 90% of the peptide bonds of polyproline II can be cleaved in the time range required for the isomerization of one peptide bond, arguing against an earlier suggestion that a small fraction of cis residues are randomly distributed in the peptide chain of poly-L-proline II in water. However, our data cannot rule out the possibility that a small fraction of cis residues might exist at or near the C-terminal end of poly-L-proline II in water.

It is well-known that poly-L-proline will undergo cis-to-trans or trans-to-cis conformational changes, depending on the property of solvents in which it dissolved. In solvents such as water, aliphatic acids, or benzyl alcohol, the all-cis form of poly-L-proline (I), a right-hand helix, will isomerize to the all-trans form of poly-L-proline (II), a left-hand helix. In contrast, poly-L-proline II, when dissolved in propanol or butanol solution, will isomerize to poly-L-proline I. The kinetic and thermodynamic properties of isomerization for poly-L-proline have been extensively studied by using optical and hydrodynamic methods and have been reviewed by several authors (Carver & Blout, 1967; Mandelkern, 1967; Von Hippel & Schleich, 1969). The fact that the kinetics are nearly zero order suggests an unzippering mechanism with only one cis-trans junction occurring in each molecule. However, these techniques are unable to demonstrate at which end of the peptide chain the isomerization starts. Recently, high-resolution NMR has been employed to investigate the cis-to-trans isomerization for poly-L-proline (Conti et al., 1969; Deber et al., 1970; Torchia & Bovey, 1971). Resonances corresponding to α-trans and α-cis protons were assigned and used to measure the rate of cis-to-trans isomerization in D_2O. The presence of an additional, small resonance in the α-cis region during isomerization, which was not found by previous NMR studies (Conti et al., 1969; Deber et al., 1970), was interpreted by Torchia & Bovey (1971) as evidence that the cis-to-trans isomerization starts at the C-terminal end of the chain. This suggested that the poly-L-proline chains with the H_2N-cis··· cis-trans···trans-COOH configuration, rather than the H_2N-trans···trans-cis···cis-COOH configuration, are stable in D_2O.

Another question regarding the conformation of poly-L-proline arises from the observation that the characteristic length parameters obtained from hydrodynamic measurements are much smaller than the values that are calculated from conformational energy maps for the all-trans polymers based on crystallographic pyrrolidine ring geometries (Mattice & Mandelkern, 1971; Clark et al., 1979). It has been pointed out that the existence of either a small fraction of cis residues in the all-trans polymer or a small population of residues in the low-energy region located at about $\psi = -50°$ will markedly reduce the calculated dimensional properties and bring them into close accord with the experimental results (Schimmel & Flory, 1967; Mattice et al., 1973; Ooi et al., 1974; Tanaka & Scheraga, 1975). With these arguments in mind, Wu et al. (1975) observed a small ^1H NMR resonance in the α-cis proton region for poly-L-proline (M_r ranged from 1800 to 97 000) in D_2O and argued that poly-L-proline in D_2O contains ca. 2 to 3% of cis residues. More recently, the same laboratory has demonstrated (Clark et al., 1979) by calculations that a small fraction of cis residues, randomly distributed along the polypeptide chain, has a dramatic effect on the characteristic hydrodynamic parameters. However, they found that the fraction of cis residues required to lower the characteristic axial ratio to the experimental values for poly-L-proline in D_2O is greater than that observed experimentally if ψ for the trans residues is restricted to the region between 90 and 220°. They further suggested that the conformational state near $\psi = -50°$ for the trans residue is probably accessible for poly-L-proline in water as well.

The isomeric specificity of aminopeptidase P can be utilized to obtain fairly definitive information on structural features of poly-L-proline. It has been demonstrated in a previous paper (Lin & Brandts, 1979b) that aminopeptidase P, a proline-specific exopeptidase, can only cleave the trans form of the Pro–Pro bond of poly-L-proline from the N-terminal end in a step-by-step manner irrespective of the size of polypeptide, while the cis form of peptide bond has to isomerize to the trans form before it can be cleaved. Our preliminary hydrolysis and CD data (Lin & Brandts, 1979b) argued against the suggestion that the cis-to-trans isomerization in water begins at

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the C-terminal end as suggested by Torchia & Bovey (1971). In this communication, more detailed and convincing evidence will be presented to support the argument that the cis-to-trans isomerization (i.e., I → II) in aqueous solution begins at the N-terminal end rather than at the C-terminal end. New evidence will also be provided to show that the trans-to-cis isomerization (i.e., II → I) in butanol starts at the C-terminal end. Thus, the intermediate states of poly-L-proline with the H_2N-trans···trans-cis···cis-COOH configuration are stable in aqueous solution as well as in butanol solution.

Finally, since high-activity aminopeptidase P can only cleave the trans form of the peptide bond, the presence of a small fraction of cis residues in poly-L-proline II in water, as suggested by Wu et al. (1975), would greatly slow down the hydrolysis rate and could be detected. As will be seen, our hydrolysis data argue against the idea that a few percent of cis residues are randomly distributed along the peptide chain of poly-L-proline II in water. Although our data cannot rule out the possibility that a small fraction of cis residues are present at or near the C-terminal end of poly-L-proline II, their existence in that location would not drastically affect its hydrodynamic properties.

Materials and Methods

Materials. Poly-L-proline (M_r ~6000; lot 58C-5039), glycyl-L-hydroxyproline (lot 93C-0070), L-prolylglycine (lot 34C-0240), and proline were obtained from Sigma Chemical Co. *N*-Cbz-L-prolyl-L-proline (lot G-1947) was the product of Vega Biochemicals. Except for poly-L-proline, these peptides and amino acids were directly used without further purification. *Escherichia coli*, strain B (ATCC No. 11303), was obtained from Grain Process Corp. (Muscatine, IA) as a frozen cell paste (three-fourths log growth phase, enriched media). Butanol (spectra grade) was purchased from Fischer Co. Chelex 100 (100–200 mesh, sodium form) was obtained from Bio-Rad Laboratories. Sephadex G-50 and G-200 and DEAE-Sephadex A-25 were purchased from Pharmacia. All other chemicals were reagent grade. All the solutions used for preparation of aminopeptidase P and for its assay were passed through Chelex 100 in order to remove trace amounts of heavy metals.

Preparation of Aminopeptidase P. Aminopeptidase P was isolated from *E. coli* (strain B). The preparation procedures have been described previously (Yaron & Berger, 1970; Lin & Brandts, 1979b). The specific activity of the purified enzyme was estimated to be ~80 units/mg. The purified aminopeptidase P was examined for possible contamination of other proline-specific enzymes [such as prolidase (EC 3.4.3.7), iminodipeptidase (EC 3.4.3.6), and proline iminopeptidase (EC 3.4.1.4)] by using glycyl-L-hydroxyproline and L-prolylglycine as substrates. No hydrolysis could be detected when the purified aminopeptidase P (5 units) and these two substrates (0.01 M) were incubated in Tris buffer at pH 8.0 at 37 °C for several hours. The requirement of a free amino group at the N-terminal end for the prepared aminopeptidase P activity was also examined by using *N*-Cbz-L-prolyl-L-proline as a substrate. No cleavage of the Pro–Pro bond could be detected.

Treatment of Poly-L-proline. Poly-L-proline purchased from Sigma Chemical Co. was in the trans form and contaminated with low molecular weight species. The procedures for removing low molecular weight species and for converting into the all-cis form were described in a previous paper (Lin & Brandts, 1979b).

Circular Dichroic Measurements. A Cary 60 spectropolarimeter with a 6002 CD accessory was employed to follow the rate of isomerization. The procedures for measuring the cis-to-trans isomerization in water have been reported earlier (Lin & Brandts, 1979b). Trans-to-cis isomerization of poly-L-proline II was carried out in 90% butanol–10% water solution since poly-L-proline II is not sufficiently soluble in 100% butanol. The experimental procedures were as follows. Five milligrams of poly-L-proline II was dissolved in 2.5 mL of H_2O, and then 22.5 mL of butanol was added and the solution was mixed thoroughly with a vibrator. An aliquot of the sample was intermittently scanned from 280 to 200 nm by using a cell of 1-mm path length. The ellipticity values at 217.5, 215, 212.5, and 210 nm were used to quantitate the percentage of cis and trans forms at various times. The ellipticity value for the all-trans form was determined by extrapolating to zero time. Since the all cis form of poly-L-proline cannot be obtained in 90% butanol–10% water solution (see Figure 3), the sample equilibrated in butanol–water for 12 days at room temperature was dried in vacuo and an equivalent volume of 100% butanol (the equilibrated sample contains more than 50% cis peptide bonds and can be readily dissolved in 100% butanol) was added. The ellipticity values for the all-cis form of poly-L-proline were then determined by scanning the sample after standing at room temperature for 4 days.

Assay for the Rate of Hydrolysis of Poly-L-proline Catalyzed by Aminopeptidase P. The acid ninhydrin colorimetric method (Troll & Lindsley, 1955; Sarid et al., 1959) was employed to measure the rate of hydrolysis. The experimental procedures were very similar to those described earlier (Lin & Brandts, 1979a,b). For the cis-to-trans isomerization, the kinetic studies of hydrolysis for poly-L-proline were carried out for the all-trans form, the all-cis form (metastably present as the initial state), and the partially cis-to-trans isomerized forms obtained by incubating the all-cis form in H_2O at 23 °C for various times before adding to the enzyme. Aliquots of the sample (in 90% butanol–10% water) used to measure the CD change were also used for kinetic studies of hydrolysis in order to better correlate the hydrolysis data with CD change for the partially isomerized poly-L-proline. The procedures were as follows. A 2.5 mL amount of poly-L-proline solution incubated in 90% butanol–10% water at 23 °C for times up to 8 days was withdrawn into a test tube and dried in vacuo. Then hydrolysis studies were immediately carried out as usual by adding aminopeptidase P solution. The sample for measuring the hydrolysis of the completely trans-to-cis isomerized poly-L-proline was that obtained after incubation in 100% butanol, as described above.

Results

As in previous work (Lin & Brandts, 1979a,b), all hydrolysis studies were carried out at a high ratio of aminopeptidase P activity to substrate concentration to ensure that the rate of hydrolysis for the trans form of the Pro–Pro bond is much faster than the rate of isomerization. The time dependence of hydrolysis by aminopeptidase P for the all-trans form, the all-cis form (metastably present as the initial state), and the partially cis-to-trans isomerized forms of poly-L-proline at 23 °C is shown in Figure 1. The rate of cis-to-trans isomerization at 23 °C (in terms of percent trans) obtained from the CD change when the all-cis form of poly-L-proline was dissolved in H_2O is also plotted in Figure 1 for comparison with the hydrolysis data. Hydrolysis of all three forms of poly-L-proline was done with the same ratio of enzyme activity to poly-L-proline concentration (75 units/mg) and in the same buffer. In addition, hydrolyses for the all-trans and all-cis forms were also carried out with a lower ratio of enzyme activity to substrate concentration (25 units/mg). The data of Figure 1 clearly show that the rates of hydrolysis for the all-trans form

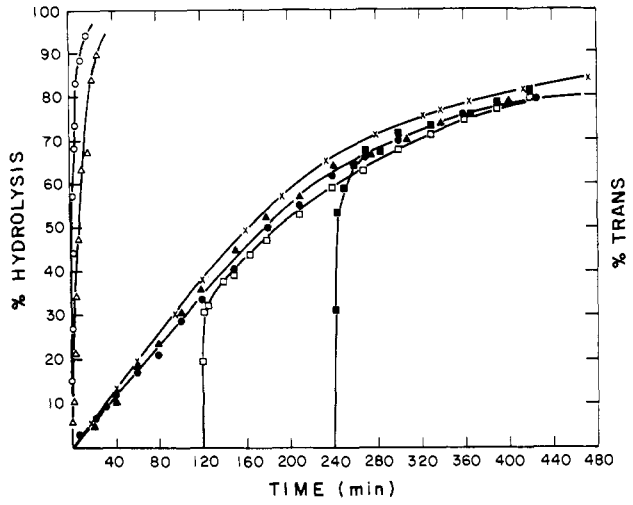

FIGURE 1: Rate of hydrolysis and cis-to-trans isomerization for poly-L-proline at 23 °C. Open circles and open triangles represent the degree of hydrolysis (left ordinate) vs. time when poly-L-proline was in the all-trans form. Filled circles and filled triangles represent the degree of hydrolysis vs. time when the initial state of poly-L-proline was the all-cis form. Open squares and filled squares represent the degree of hydrolysis vs. time for the partially cis-to-trans isomerized poly-L-prolines which were obtained by incubating the all-cis form of poly-L-proline in water for 2 and 4 h, respectively, at 23 °C before addition to aminopeptidase P solution. The ratio of aminopeptidase P activity to substrate concentration was 75 units/mg except for the triangles where the ratio of enzyme activity to substrate concentration was 25 units/mg. The concentration of poly-L-proline was ~0.038% in 1.3 mL of 0.05 M veronal buffer containing 2×10^{-4} M DTT, 3.5×10^{-3} M manganous chloride, and 1.4×10^{-2} M sodium citrate at pH 8.6. Times signs represent the rate of cis-to-trans isomerization (right ordinate) of poly-L-proline (0.013%) obtained from CD data when the all-cis form was dissolved in water.

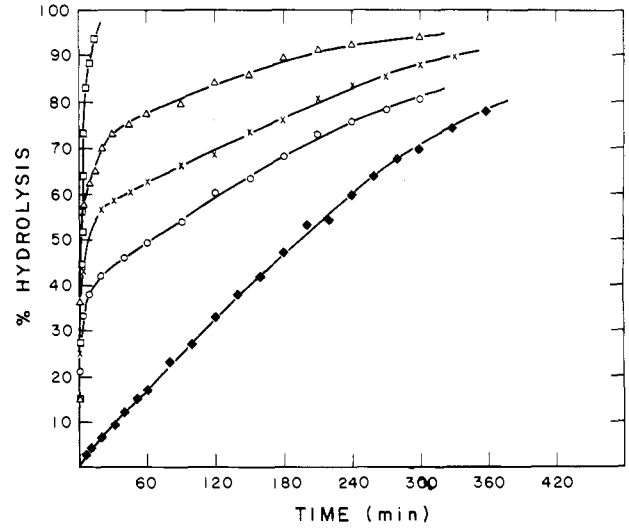

FIGURE 2: Rate of hydrolysis for poly-L-proline catalyzed by aminopeptidase P at 23 °C. Open and filled squares represent the degree of hydrolysis vs. time when the initial state of poly-L-proline was the all-trans and the all-cis form, respectively. Triangles, times signs, and circles represent the degree of hydrolysis vs. time for the partially trans-to-cis isomerized poly-L-prolines which were obtained by incubating the all-trans form of poly-L-proline in 90% butanol–10% water for 24 h, 48 h, and 8 days, respectively. The ratio of enzyme activity to substrate concentration was 75 units/mg. The concentrations of poly-L-proline and buffer solution were the same as those of Figure 1.

of poly-L-proline are fast and depend on aminopeptidase P activity. In less than 5 min, 80% of proline was released from poly-L-proline with the ratio of enzyme activity to substrate concentration at 75 units/mg, while it takes more than 18 min to cleave the same amount of proline with the ratio of enzyme activity to substrate concentration at 25 units/mg. In contrast, the rate of hydrolysis for the all-cis form (initial state) is very slow and independent of aminopeptidase P activity (Figure 1). At the two different enzyme concentrations, it took ~7 h to release 80% of the proline. The kinetic pattern of hydrolysis for the all-cis form of poly-L-proline is very similar to that of the cis-to-trans isomerization measured from CD change. These data confirm our previous suggestion (Lin & Brandts, 1979b) that aminopeptidase P can only cleave the trans form of the Pro–Pro bond from the N-terminal end in a step-by-step manner and the slow cleavage of poly-L-proline preequilibrated as the cis form is rate-limited by the cis-to-trans isomerization.

Hydrolysis data for two partially isomerized poly-L-proline samples (Figure 1) show the same kinetic pattern. These samples were initially in the all-cis form and preequilibrated for either 2 or 4 h before adding the enzyme. A fast and slow phase are seen. The rates of hydrolysis for the fast phase are comparable to those of the all-trans form, while the rates for the slow phase are nearly identical with those of the all-cis form. However, the relative amplitudes of the two kinetic phases are different and proportional to the period of time the sample was incubated in H_2O before addition to the aminopeptidase P solution. Poly-L-proline which was incubated in H_2O for 2 h has a fast phase of 30%, while that incubated for 4 h has a fast phase of 60%. It is also interesting to note that the percentage of proline released from the all-cis form of poly-L-proline which was continuously hydrolyzed for 2 or 4 h is also ca. 30 and 60%, respectively. Furthermore, the fast-phase amplitudes for the hydrolysis of two, partially cis-to-trans isomerized forms are nearly identical with the total percentage of the trans form observed from the CD change when the all-cis form of poly-L-proline was dissolved in H_2O for 2 and 4 h. Thus, all the isomerized trans residues of poly-L-proline must be in the N-terminal end while the remaining cis residues are in the C-terminal end, indicating that the cis-to-trans isomerization begins at the N-terminal end.

The kinetics for the poly-L-proline II → I conversion have also been studied, in addition to those for the I → II reaction discussed above. As detailed under Materials and Methods, the partially trans-to-cis isomerized poly-L-proline was obtained in 90% butanol–10% water solution and the completely trans-to-cis isomerized poly-L-proline was obtained in 100% butanol. These poly-L-proline solutions had been dried in vacuo before hydrolysis studies were carried out. Figure 2 shows the kinetic data for hydrolysis of poly-L-proline preequilibrated as the completely trans-to-cis isomerized (i.e., 100% cis) form, the all-trans form, and the partially trans-to-cis isomerized form. Even though all hydrolyses were measured with the same ratio of enzyme activity to substrate concentration (75 units/mg), quite different kinetic patterns were observed. As expected from the previous results, only a slow phase or a fast phase was observed when poly-L-proline was preequilibrated as the all-cis form or the all-trans form, while both a fast and slow phase were seen for the hydrolysis of the partially isomerized poly-L-proline. The kinetic patterns of hydrolysis for the all-cis and all-trans forms shown in Figures 1 and 2 are identical within experimental error. These results are expected when the isomerization of poly-L-proline is completely reversible. Figure 2 also shows that the amplitude of the fast phase accounts for about 69, 55, and 40% of the residues for the partially trans-to-cis isomerized poly-L-proline when the all-trans form of poly-L-proline was dissolved in 90% butanol–10% water for 24 h, 48 h, and 8 days, respectively. The fast phase can again be attributed to the cleavage of the trans form of the peptide bond in poly-L-proline.

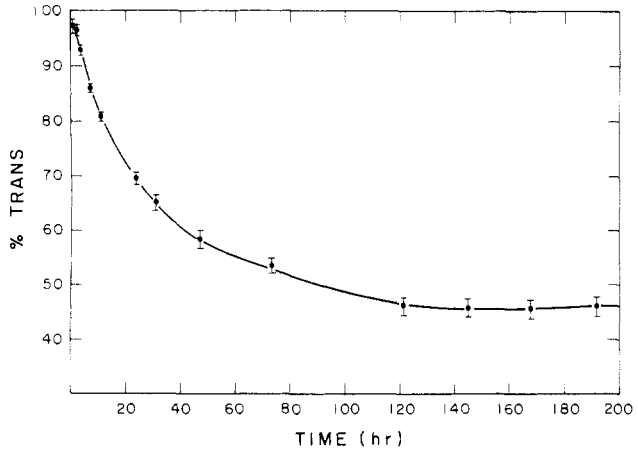

FIGURE 3: Rate of trans-to-cis isomerization of poly-L-proline in 90% butanol–10% water solution at 23 °C as measured by CD data. The initial state of poly-L-proline was the all-trans form. The data were the averaged values obtained at 217.5, 215.0, 212.5, and 210 nm by assuming a linear relationship between CD and helicity. The vertical bars represent the maximum deviation of each measurement at the various wavelengths. Sample concentration was 0.2 mg/mL.

If the trans residues are on the amino-terminal end in the partially isomerized samples, then the amplitude of the fast phase of hydrolysis should be identical with the percentage of trans form observed from CD data under the same experimental conditions. The kinetics for the trans-to-cis isomerization, obtained from CD data, are presented in Figure 3. These data show that the isomerization proceeds very slowly and never reaches the all-cis form. The percent of cis form in the final equilibrated poly-L-proline was estimated to be 54%. Figure 3 shows that poly-L-proline has 70, 57, and 46% of the trans form of the peptide bond when the all-trans form of poly-L-proline was dissolved for 24 h, 48 h, and 8 days. These data are identical within error with the percentage of the fast-phase amplitude seen for hydrolysis (Figure 2). These results then show quite conclusively that all the remaining trans residues are in the N-terminal segment and all the isomerized cis residues are in the C-terminal segment. Thus, the trans-to-cis isomerization in butanol solution must start from the C-terminal end.

As was pointed out in the beginning of the paper, some investigators (Wu et al., 1975) have suggested that the all-trans form of poly-L-proline does in fact contain a few percent of cis residues and that these may be distributed nearly randomly along the chain, producing points of flexibility which have marked effects on hydrodynamic properties. In order to investigate this suggestion, we have conducted additional studies on the all-trans form under conditions of lower temperature and higher enzyme concentration, where isomerization should be very slow and where cleavage of a trans bond should be relatively fast.

Figure 4 shows the rate of hydrolysis for the all-trans form at 6 °C. The ratio of enzyme activity to substrate concentration was 200 units/mg. In spite of the higher enzyme concentration, the rate of hydrolysis at 6 °C was ~3 times slower than that at 23 °C (75 units/mg), undoubtedly reflecting a positive activation energy for hydrolysis. The time required for the cis-to-trans isomerization of a single Pro–Pro bond is ~10 times slower at 6 °C than at 23 °C and can be estimated as 70 min. The data of Figure 4 show that over 90% of the peptide bonds can be cleaved in 35 min at 6 °C. In other words, nearly 100% of the peptide chain can be cleaved in times which are shorter than that required for the isomerism of a single cis residue.

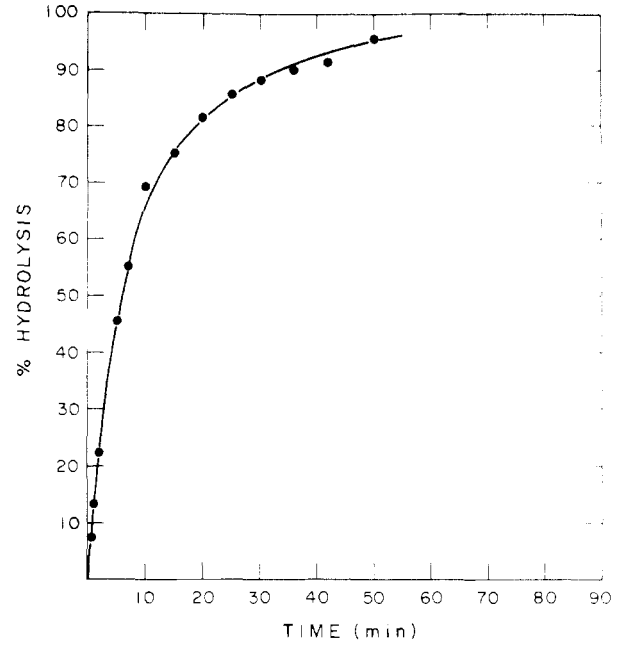

FIGURE 4: Rate of hydrolysis for the all-trans form of poly-L-proline catalyzed by aminopeptidase P at 6 °C. The ratio of enzyme activity to substrate concentration was 200 units/mg. The concentration of poly-L-proline was 0.03% in 1.3 mL of 0.07 M veronal buffer containing 2×10^{-4} M DTT, 3.5×10^{-3} M manganous chloride, and 1.4×10^{-2} M sodium citrate at pH 8.6.

Discussion

The purified aminopeptidase P was shown to require an N-terminal prolyl residue for hydrolysis to occur (see Materials and Methods). The hydrolysis data in Figure 1 show that aminopeptidase P can cleave poly-L-proline much faster when it is preequilibrated as the all-trans form than when the initial state of poly-L-proline is the all-cis form. It was also shown that the rate of hydrolysis for the all-trans form of poly-L-proline is nearly proportional to the enzyme activity while a higher concentration of aminopeptidase P has no detectable effect on the rate of hydrolysis of the all-cis form (initial state), indicating that the rate-limiting step in cleavage of the cis form involves a step other than hydrolysis per se. Since the kinetics for hydrolysis of the all-cis form are nearly identical with the kinetics for the cis-to-trans isomerization, observed from CD change, it seems certain that aminopeptidase P can only cleave the trans form of the Pro–Pro bond at the N-terminal end, while the slow hydrolysis for the all-cis form of poly-L-proline is rate limited by the cis-to-trans isomerization.

If the above is true, the only conclusion which is consistent with the hydrolysis data is that the cis-to-trans isomerization of poly-L-proline in water begins at the N-terminal end and proceeds in a residue-by-residue manner to the C terminus. If isomerization started at the C-terminal end, as suggested by Torchia & Bovey (1971) from NMR data, we would expect to see no hydrolysis until all the CD change had occurred rather than seeing the parallel change in CD and hydrolysis as was observed. The fact that 100% of the trans residues are available for cleavage by the aminopeptidase can only mean that all the isomerized trans residues are in the N-terminal side of the chain while all the remaining cis residues are on the C-terminal side and that only a single trans-cis junction point occurs in each chain. Thus, the cis-to-trans isomerization of poly-L-proline in water starts from the N-terminal end.

In contrast to that of the cis-to-trans isomerization in aqueous solution, the trans-to-cis isomerization of poly-L-proline II in 90% butanol–10% water apparently begins at the

C-terminal end. Both fast and slow phases are also seen for the hydrolysis of the partially trans-to-cis isomerized poly-L-proline (Figure 2), indicating that all the remaining trans residues are in the N-terminal side and all the isomerized cis residues are in the C-terminal side. The quantitative agreement between the total number of the trans residues (from CD) and the amplitude of the fast phase of hydrolysis again shows clearly that the trans-to-cis isomerization proceeds from the C-terminal end with a single trans-cis junction point per chain. Accordingly, the stable intermediate states of poly-L-proline must be H_2N-trans···trans-cis···cis-COOH in both water and in 90% butanol–10% water. This conclusion is in direct contradiction to that suggested by Torchia & Bovey (1971) from NMR data in D_2O.

Our data show that the presence of aminopeptidase P does not affect the rate of cis-to-trans isomerization (Figure 1). Thus, the rate of isomerism is the same for residues in the middle or on the N-terminal end. The rates of the cis-to-trans isomerization seen from both hydrolysis and CD change (Figure 1) appear to follow the zero-order kinetics only for the first 60% of isomerization. This can probably be attributed to the fact that poly-L-proline still has some molecular weight heterogeneity in spite of having been fractionated on a Sephadex G-50 column. It has been reported earlier (Kurtz & Harrington, 1966; Harrington & Sela, 1958) that the optical rotation of poly-L-proline in aqueous solution changed in an approximately linear fashion with time for a sample with a molecular weight of ~3000 but not for a sample with a molecular weight of 5000, which is consistent with our observations.

Recent NMR data (Wu et al., 1975) have been interpreted as evidence for the presence of ca. 2 to 3% cis residues in the all-trans form of poly-L-proline in D_2O. It has also been shown by calculations (Clark et al., 1979) that the presence of 2 to 3% cis residues, if randomly distributed along the chain, could markedly lower the characteristic length for poly-L-proline and bring it nearly in accord with the experimental value. Our hydrolysis data argue strongly against the suggestion that cis residues, if they exist in polyproline II, are randomly distributed along the chain. If this were the case, aminopeptidase P would cleave rapidly only to the first cis residue so that all trans residues on the C-terminal side of the first cis residue would be "protected" from rapid cleavage and would only be cleaved in a slow reaction with a time constant similar to that for cis → trans isomerization. Thus, with a 2% cis content (i.e., ~1 residue/chain on the average for the poly-L-proline used in this study) distributed randomly, one would expect rapid cleavage of only 50% of the residues since the average position of the single cis residue will be in the center of the chain. However, we find that (at 6 °C) 90% of the residues are cleaved in 35 min even though the expected time for isomerization of a single cis residue is 70 min, from CD data. If small amounts of cis residues were located near the C-terminal end, this would be consistent with our hydrolysis data and with the NMR data but would not explain the hydrodynamic properties of poly-L-proline. These could, however, be reconciled by assuming a small population of the trans state near $\psi = -50°$ in the conformational map (Clark et al., 1979). This is certainly more consistent with the hydrolysis data than is the presence of randomly distributed cis residues.[1]

In summary, the present study provides strong evidence to support the argument that the cis-to-trans isomerization of poly-L-proline in aqueous solution begins at the N-terminal end while the trans-to-cis isomerization of poly-L-proline in 90% butanol–10% water starts at the C-terminal end. The intermediate states of poly-L-proline with the H_2N-trans··· trans-cis···cis-COOH configuration are stable in aqueous solution as well as in butanol solution during isomerization. Each isomerizing chain has only a single trans-cis junction point for the sample used here (M_r ~6000). Our evidence also argues against the assumption that a small fraction of cis residues are randomly distributed along the polypeptide chain for poly-L-proline in water, although the data cannot rule out the possibility that a small fraction of cis residues are located at or near the C-terminal end of the peptide chain.

References

Carver, J. P., & Blout, E. R. (1967) *Treatise Collagen 1*, 441.

Clark, D. S., Dechter, T. T., & Mandelkern, L. (1979) *Macromolecules 12*, 626.

Conti, F., Piatelli, M., & Viglino, P. (1969) *Biopolymers 7*, 411.

Deber, C. M., Bovey, F. A., Carver, J. P., & Blout, E. R. (1970) *J. Am. Chem. Soc. 92*, 6191.

Harrington, W. F., & Sela, M. (1958) *Biochim. Biophys. Acta 27*, 24.

Kurtz, T., & Harrington, W. F. (1966) *J. Mol. Biol. 17*, 440.

Lin, L.-N., & Brandts, J. F. (1979a) *Biochemistry 18*, 43.

Lin, L.-N., & Brandts, J. F. (1979b) *Biochemistry 18*, 5037.

Mandelkern, L. (1967) *Biol. Macromol. 1*, 675.

Mattice, W. L., & Mandelkern, L. (1971) *J. Am. Chem. Soc. 93*, 1769.

Mattice, W. L., Nishikawa, K., & Ooi, T. (1973) *Macromolecules 6*, 443.

Ooi, T., Clark, D. S., & Mattice, W. L. (1974) *Macromolecules 7*, 337.

Sarid, S., Berger, A., & Katchalski, E. (1959) *J. Biol. Chem. 234*, 1740.

Schimmel, P. R., & Flory, P. T. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 52.

Tanaka, S., & Scheraga, H. A. (1975) *Macromolecules 8*, 623.

Torchia, D. A., & Bovey, F. A. (1971) *Macromolecules 4*, 246.

Troll, W., & Lindsley, T. (1955) *J. Biol. Chem. 215*, 655.

Von Hippel, P. H., & Schleich, T. (1969) *Biol. Macromol. 2*, 501.

Wu, C. C., Komoroski, R. A., & Mandelkern, L. (1975) *Macromolecules 8*, 635.

Yaron, A., & Berger, A. (1970) *Methods Enzymol. 19*, 521.

[1] As pointed out by a reviewer, there is also a possibility that a small amount of randomly distributed cis residues might not be detected by the hydrolysis study if the rate of cis-to-trans isomerization for these isolated cis residues is much faster than that observed in the I → II transition. Although there is no reason to expect that this will be the case, there is also no way it can be absolutely ruled out.