J. Biochem. Mol. Biol. Vol. 29, No. 6 pp. 519~524 November 30, 1996

Kinetic Studies of Peptidylprolyl *cis-trans* Isomerase from Porcine Spleen

Soo Ja Kim* and Chan Lee[1]

Department of Chemistry, College of Liberal Arts and Sciences, Kyung Hee University, Seoul 130-701
[1]Department of Food Science and Technology, Chung-Ang University, Ansung 456-756, Korea
(Received July 24, 1996)

Abstract : Peptidylprolyl *cis-trans* isomerase (PPIase) catalyzes the *cis-trans* isomerization of prolyl peptide and facilitates the folding of cellular proteins and peptides. PPIase consists of two distinct immunophilins, each specifically binding to the immunosupressive drug cyclosporin A (CsA) or FK506, respectively. A PPIase was isolated and partially purified from porcine spleen. The molecular weight of porcine spleen PPIase was determined to be ~14,000 on the basis of SDS-PAGE. The purified enzyme was strongly inhibited by FK506, but not by CsA. The inhibition constant and the true concentration of enzyme preparations were determined by active site titration using the tight binding inhibitor FK506: $K_i = 18.7$ nM and $E_t = 172$ nM. The equilibrium ratio of conformer, [*cis*]/[*trans*], of prolyl peptide substrates (N-Suc-Ala-Xaa-Pro-Phe-*p*-NA) in anhydrous trifluoroethanol/LiCl solvent system varied from 0.24 to 0.85 depending on the nature of Xaa. Overall, in this solvent-salt system, the populations of the *cis* conformer of substrates in equilibrium are higher than in an aqueous solution so that the substantial error caused by high background absorption can be reduced. The reactivities of porcine spleen PPIase are shown to be highly sensitive to changes in the structure of substrates. Thus, k_{cat}/K_m value for the most reactive substrate (Xaa=Leu) is $4.007 + 10^6$ $M^{-1}s^{-1}$ and, is 2,636 fold higher than that for the least reactive peptide substrate tested, Xaa=Glu.
Key words : *cis-trans* isomerase, FK506-binding protein, kinetics, peptidylprolyl, substrate specificity.

Peptidylprolyl *cis-trans* isomerase (PPIase, EC. 5.2.1.8) discovered by Fischer and coworkers is an enzyme of novel class that catalyzes *cis-trans* isomerization of peptidylproly imide bonds in peptides and proteins (Fischer *et al.*, 1984). This enzyme has been detected in many organisms and all cellular compartments tested (Fischer, 1994) and is known to catalyze the refolding of denatured proteins *in vitro* (Lang and Schmidt, 1988; Troupschung *et al.*, 1990; Schönbrunner *et al.*, 1991) and to play an important role in folding of newly synthesized proteins *in vivo*, where the *trans*→*cis* isomerization of proline peptide bond is considered as the rate limiting step in some protein folding process (Brandts *et al.*, 1975; Schmidt *et al.*, 1993). PPIases are also shown to be the initial binding proteins (immunophilins) for cyclosporin A (CsA) and FK506 (Fischer *et al.*, 1989; Takahashi *et al.*, 1989), both potent immunosuppressive drugs. Therefore these enzymes are classified as two families of proteins, cyclophilin and FK506-binding protein (FKBP), depending on their specificity for the CsA and FK506, respectively. Their catalytic activities are inhibited by binding of the respective ligand, but they are not inhibited by crossed inhibitors (Harding *et al.*, 1989; Siekierka *et al.*, 1989). In tracing biological functions of these enzymes, a number of cellular binding proteins for PPIase were identified (Friedman *et al.*, 1993; Nadeau *et al.*, 1993; Johnson and Toft, 1994) which may include native substrate. In certain bacterial systems, the binding of FK506 has been shown to protect FKBP protein against proteolytic digestion (Lee *et al.*, 1992). Recently human cyclophilin-CsA and FKBP-FK506 complexes were reported to inhibit the signal transduction pathway for activating human T-cells (Fischer *et al.*, 1994). Nevertheless, a clear link between the acceleration of protein folding by PPIase and its role in the immunosuppression process has yet to be identified. The identification of possible endogeneous PPIase inhibitors would be an effective approach that leads to an understanding of the whole process and this can be achieved on the basis of knowledge of detailed catalytic mechanisms and the chemistry of the enzyme active site.

In the present study, a PPIase was isolated and partially purified from porcine spleen and some of its kinet-

*To whom correspondence should be addressed.
Tel : 82-02-961-0241, Fax : 82-02-966-3701
E-mail : sjkim@nms.kyunghee.ac.kr

ic properties and substrate binding properties were studied.

Materials and Methods

Materials

N-Succinylalanylalanylprolylphenyalanyl-*p*-nitroanilide (N-Suc-Ala-Ala-Pro-Phe-*p*-NA), recombinant human FK506-binding protein (hFKBP), α-chymotrypsin, phenylmethylsulfonyl fluoride (PMSF), Trizma base, acrylamide, N,N'-methylenebisacrylamide were purchased from Sigma (St. Louis, USA). Tetrapeptide substrates, 1-7 for PPIase assays were prepared by Bachem Bioscience (Heidelberg, Germany). Trifluoroethanol (TFE) and tetrahydrofuran (THF) were obtained from Aldrich Chemicals (Milwaukee, USA). FK506 and CsA were provided by E. Lee (Wheatridge, USA). Sephadex G-75 and CM-Trisacryl M were purchased from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). NANOpure deionized water of conductivity no less than 18.0 Mohm-cm at 25℃ was used throughout this experiment.

Enzyme purification

Peptidylprolyl *cis-trans* isomerase was isolated and purified by the method of Kofron *et al.* (1991) with the following modifications. The porcine spleen was homogenized in 1:4 (w/v) lysis buffer (0.025 M Tris-HCl, pH 7.6, 0.1 M NaCl, 5 mM 2-mercaptoethanol and 1 mM PMSF). The homogenate was centrifuged at 8,000×*g* for 20 min and the supernatant was heated to 60℃ for 20 min. The mixture was centrifuged at 20,000×*g* for 30 min. The proteins from the supernatant was precipitated by 80% saturation with ammonium sulfate. The precipitate was dissolved in a small amount of lysis buffer and applied to a Sephadex G-75 column (1.6×75 cm) pre-equilibrated in 0.05 M Tris-HCl buffer, pH 7.6, and was eluted by the same buffer. The PPIase activity of each fraction was determined using a coupling enzyme assay method (Fischer *et al.*, 1984; Harding *et el.*, 1989; Siekierka *et al.*, 1989) with N-Suc-Ala-Ala-Pro-Phe-*p*-NA as the substrate for the PPIase catalyzed reaction. The fractions which had the highest enzyme activity were pooled and concentrated and dialyzed against 0.0l M acetate buffer, pH 5.4 using Amicon ultrafiltration apparatus with YM-10 membrane. The final mixture in 0.01 M acetate buffer was applied to a CM-Trisacryl M column (1.2×25 cm) equilibrated with 0.01 M acetate buffer, pH 5.4 and was eluted by linear gradient of 0~0.3 M NaCl in the same buffer. Fractions with high enzyme activity were collected, concentrated and stored at 4℃. The molecular weight of porcine spleen PPIase was determined to be ~14,000 on the basis of SDS-PAGE. The enzyme preparation was purified 250 fold as compared to the ammonium sulfate precipitates.

Substrate preparation

Synthetic peptide substrates (1-7) were dissolved in anhydrous TFE or THF which contained 0.47 M LiCl to give 5.5 mM solution. The *cis/trans* equilibrium constant and the percentage of *cis* isomer of each peptide preparation were determined as follows: first, absorption at 390 nm of the reaction mixture without substrate and PPIase was zeroed and, after mixing with substrate, the absorption was read at 390 nm. This measures the amount of *trans* peptide in equilibrium mixture under the conditions applied. Since the rate for reequilibration of reaction mixture upon addition of substrate is slower than the α-chymotrypsin catalyzed hydrolysis of preexisting *trans* isomer, there is an initial burst which is follwed by slower increase in *p*-nitroaniline production. Then PPIase was added and allowed to react to completion (about 1 min). When there was no further change in absorption, the absorption corresponding to the total *p*-nitroaniline released was measured. The subtraction of the first measured value from the second absorption gave the amount of *cis* isomer in the sample.

Assay of peptidylprolyl *cis-trans* isomerase catalyzed reaction

The coupling enzyme assay method for PPIase activity determination suggested originally by Fischer *et al.* (1984) depends upon the isomer specific proteolytic activity of α-chymotrypsin and was modified by Kofron *et al.* (1991) so that the steady state kinetic parameters could be measured accurately. Kern *et al.* (1995) used dynamic NMR spectroscopy for the noncoupling assay of pig kidney cyclophilin.

Standard PPIase assay mixture contained 0.05 M Tris-HCl buffer, pH 8.0, α-chymotrypsin, 1.67 mg (90 U), peptide substrate 0.11 mM, TFE 2% (v/v), LiCl 9.4 mM and PPIase in total volume of 0.5 ml. Reaction was initiated by adding substrate to the equilibrated reaction mixture and changes in absorption at 390 nm were measured at 25℃ using Shimadzu UV 3101 PC Spectrophotometer. The first order rate constants (k_{obs}) were determined by exponential curve fit to the reaction progress curves or by the logarithmic linear transformation. Effect of inhibitors (peptide analogs, FK506 and CsA) and nonenzymic spontaneous isomerization rate constants were also determined in a similar manner.

Substrate specificity

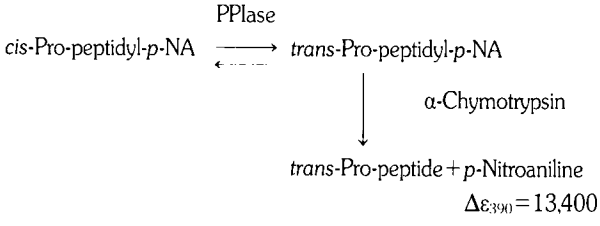
k_{cat}/K_m for the PPIase catalyzed isomerization with seven different tetrapeptide substrates varying in amino

acid Xaa in N-Suc-Ala-Xaa-Pro-Phe-*p*NA were calculated from the observed first order rate constants of enzyme catalyzed reactions and uncatalyzed reactions of the same peptides.

Results and Discussions

Assay of PPIase catalyzed *cis-trans* isomerization of prolyl peptides

The coupling enzyme assay method described by Fischer *et al.* (1984) and Harrison and Stein (1990a) involved the coupling of the reversible PPIase reaction with the irreversible, α-chymotrypsin catalyzed hydrolysis of C-terminal amide bond of peptide liberating *p*-nitroaniline. In this reaction, α-chymotrypsin has the isomer selectivity and it cleaves the Phe-*p*NA amide bond of *trans* isomer of substrate only. In the presence of high concentrations of α-chymotrypsin, the *trans* isomer in equilibrium mixture can be hydrolyzed in the burst phase and rate limiting slower hydrolysis would follow this. The PPIase catalyzed reaction rate can be determined from the absorption at 390 nm ($\Delta\varepsilon = 13{,}400$) or some other wave lengths by this slower *p*-nitroaniline liberation.



The isomerization of prolyl peptide bond is a facile reaction and enzyme catalyzed isomerization is accompanied by a significant degree of uncatalyzed, spontaneous isomerization. When the concentration of substrate is very low ($[S] \ll K_m$), the enzyme catalyzed isomerization can be expressed by first order reaction kinetics and the observed first order rate constants (k_{obs}) would thus include both of the enzymatic and nonenzymatic rate constants;

$$v = k_{obs}[S] = k_{ne}[S] + \{(k_{cat}/K_m)[E]\}[S] \quad (1)$$

$$k_{obs} = k_{ne} + (k_{cat}/K_m)[E] \quad (2)$$

where k_{obs} is the observed first order rate constant, k_{ne} stands for the rate constant for nonenzymatic isomerization, [S] represents the concentration of *cis*-peptide and $(k_{cat}/K_m)[E]$ is the pseudo first order rate constant for PPIase catalyzed *cis-trans* isomerization of prolyl peptide.

Fig. 1A represents the typical reaction progress curves for the *cis-trans* isomerization of Suc-Ala-Ala-Pro-Phe-*p*-NA in the presence of (a) PPIase, (b) enzyme plus FK506 and (c) neither enzyme nor inhibitor. At 25℃,

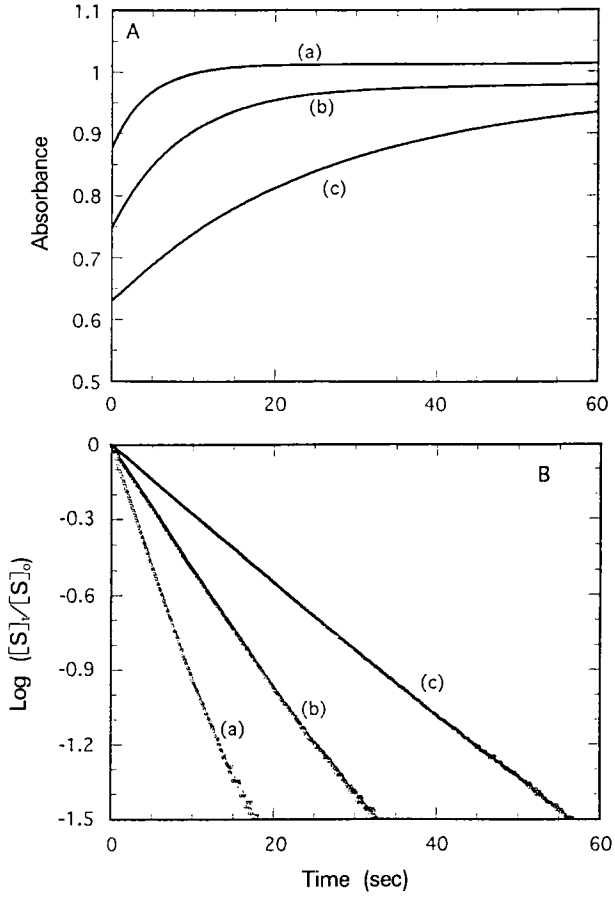


Fig. 1. (A) Representative progress curves for the *cis-trans* isomerization of Suc-Ala-Ala-Pro-Phe-pNA. In the presence of porcine spleen PPIase (a), in the presence of PPIase and FK506 (b) and nonenzymatic spontaneous isomerization (c). Reactions were carried out in 0.05 M Tris-HCl buffer, pH 8.0, at 25℃. [α-chymotrypsin] = 0.167 mg, [Suc-Ala-Ala-Prp-Phe-pNA] = 0.16 mM, [PPIase] = 172 nM, [FK506] = 71.4 nM. (B) Logarithmic analysis of data (A). $[S_o] = [cis]$ at zero time, $[S_t] = [cis]$ at t sec.

there are abrupt changes in absorption very early, probably during the mixing time. This abrupt change in absorbance reflects the rapid reaction of *trans*-substrate with a α-chymotrypsin. The remaining *cis*-isomer of substrate does not react with α-chymotrypsin. The first order PPIase catalyzed and nonenzymatic conversion of *cis* to *trans* peptide is the rate limiting step for the whole coupled reaction and thus appears as slow exponential increase in absorbance in the progress curves. These data reveal much larger change in absorption and faster reaction as compared to Harrison and Steins (1990a) experiment where the change in absorption due to remaining *cis*-peptide was only 0.13 and the initial burst phase lasted about 10 seconds after mixing with substrate. Fig. 1B represents the results of logarithmic analysis of data A. The linearities in the early part in this graph indicate that the first order reaction rate calculation is not affected by burst phase

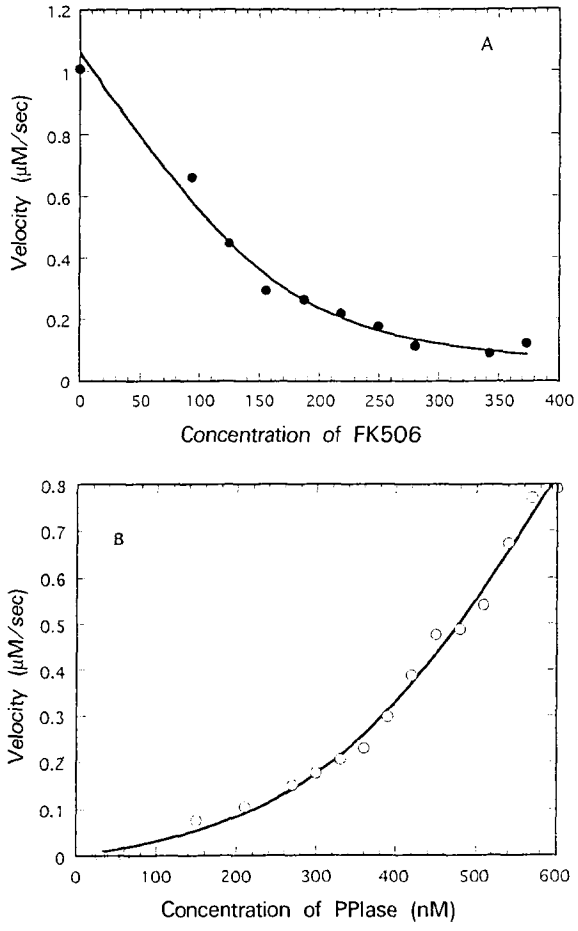


Fig. 2. Active site titration of PPIase by tight binding inhibitor, FK506. (A) Reactions were carried out in 0.05 M Tris-HCl buffer, pH 8.0 at 25℃. In final 0.5 ml assay mixture contained 0.167 mg α-chymotrypsin, 0.035 mM Suc-Ala-Ala-Pro-Phe-*p*NA, 0~373 nM FK506 and 172 nM PPIase. Steady state velocities determined from $(k_{obs}-k_{ne})[S]$, were plotted against varying FK506 concentration and fitted to Eq. (3) by nonlinear regression analysis. (B) Reactions were carried out with varying concentration of PPIase of 0~600 nM. [FK506]=311.4 nM, [Suc-Ala-Ala-Pro-Phe-pNA]=0.035 mM.

rate even though the analysis included the very first points at zero time. It is a contrast to the aforementioned Harrison and Steins experiment where they had to abort data of initial 20 seconds to avoid the inclusion of burst rate and that would leave considerably shorter reaction time to be analyzed. In an analogous experiment, CsA at the concentration of more than one hundred times of [FK506] had no effect on the PPIase activity, indicating that porcine spleen PPIase is a FKBP rather than a cyclophilin.

Determination of enzyme concentration and K_i for FK506 by active site titration using tight binding inhibitor

Enzyme concentration of the partially purified porcine spleen PPIase preparation was determined by active site titration using tight binding inhibitor, FK506. Enzyme activities were measured with the substrate N-Suc-Ala-Ala-Pro-Phe-*p*NA in the presence of inhibitor, and analyzed using the rate Eq. (3) described by Williams and Morrison (1979) for the enzyme reaction in the presence of tight binding inhibitor.

$$v=(1/2)\ R\{[(K_{iapp}+I_t-\alpha E_t')^2+4K_{iapp}\alpha E_t']^{1/2}-(K_{iapp}+I_t-\alpha E_t')\} \quad (3)$$

In Eq. (3), R represents the reciprocal of the steady state rate in the absence of inhibitor; K_{iapp} denotes the apparent inhibition constant; I_t is the total inhibitor; E_t' is the total protein used and α represents the fraction of total protein that reacts with inhibitor as enzyme, i.e.

$$E_t=\alpha E_t' \quad (4)$$

In Fig. 2A and B, the steady state velocities of PPIase catalyzed reactions, $(k_{obs}-k_{ne})$, obtained from different fixed concentrations of I_t and E_t' are plotted against I_t or E_t', and analyzed by nonlinear least square fit to Eq. (3). K_{iapp} and α can be determined from the best fit line equation and E_t from Eq. (4). Assuming FK506 be a competitive tight binding inhibitor and $[S] \ll K_m$ (K_m was estimated as >20 mM by Harrison and Stein, (1990a); as 0.5 mM by Park *et al.* (1992)), K_{iapp} is equal to the true dissociation constant according to Eq. (5)

$$K_{iapp}=K_i\ (1+S/K_m) \quad (5)$$

K_i of 18.7 nM calcuated here (and E_t=172 nM) is lower than by Harding *et al.*, (1989), K_i=50 nM (E_t=670 nM) worked with same substrate, but an order of magnitude higher than by Harrison and Stein (1990 b), K_i=1.7 nM (E_t=50 nM), working with 12 times more reactive Suc-Ala-Leu-Pro-Phe-*p*-NA at 10℃. The cause of the discrepancy is presumably attributed to the amount of enzyme used for inhibition study. In the present study, relatively large amount of enzyme was used to get higher activity as compared to nonenzymatic reaction. This influenced favorably the calculation of first order rate constant as shown above, but this could give a poor estimation of K_i.

Substrate specificity of porcine spleen PPIase

Synthetic prolyl peptide substrates exist in equilibrium of *cis-trans* conformer in solution. Since PPIase catalysis depends on the population of *cis* isomer in peptide substrate, *cis-trans* equilibrium constant or the percentage of *cis* isomer in an equilibrium mixture of peptide substrate is very important in carrying out PPIase catalyzed reaction. In aqueous solution *cis*-prolyl

Table 1. *cis/trans* Equilibrium constants of prolyl peptide in TFE/LiCl solution

Peptide No.	Structure of substrates[a]	K_{eq}=*cis/trans*[b]	Percent *cis* peptide
1	N-Suc-Ala-Gly-Pro-Phe-*p*NA	0.24± 0.004	19.42± 2.40
2	N-Suc-Ala-Glu-Pro-Phe-*p*NA	0.76± 0.046	43.02± 1.89
3	N-Suc-Ala-His-Pro-Phe-*p*NA	0.32± 0.021	23.69± 0.68
4	N-Suc-Ala-Lys-Pro-Phe-*p*NA	0.51+ 0.055	33.80± 2.15
5	N-Suc-Ala-Ala-Pro-Phe-*p*NA	0.85± 0.046	45.92± 0.37
6	N-Suc-Ala-Ile-Pro-Phe-*p*NA	0.41+ 0.013	29.04± 0.66
7	N-Suc-Ala-Leu-Pro-Phe-*p*NA	0.29± 0.018	28.20± 0.94

[a]Peptide substrates were dissolved in anhydrous TFE containing 0.47 M LiCl.
[b]Refer to text for the determination of *cis* and *trans* Data are average of 3~5 determinations.

Table 2. Substrate specificity of porcine spleen peptidylprolyl *cis-trans* isomerase toward N-Suc-Ala-Xaa-Pro-Phe-*p*NA[a]

Peptide No.	Xaa	k_{ne} (10^{-2} s^{-1})	k_{cat}/K_m (10^3 $M^{-1}s^{-1}$)	Relative activity
1	Glu	2.94± 0.22	1.52± 0.61	1.00
2	Gly	4.67± 0.09	2.19± 1.38	1.44
3	His	4.72± 0.68	204.50± 14.85	134.54
4	Lys	6.07± 0.59	274.15± 11.53	180.36
5	Ala	5.36± 0.67	303.73± 69.29	302.21
6	Ile	4.43± 0.79	2290.80± 541.60	1507.10
7	Leu	6.11± 0.51	4007.23± 858.40	2636.40

[a]Reaction mixture contained 0.05 M Tris-HCl buffer, pH 8.0, α-chymotrypsin 5 mg, peptide substrate 0.16 mM, TFE 2% (v/v), LiCl 9.4 mM at 25℃. $k_{cat}/K_m=(k_{obs}-k_{ne})/[E]$, where k_{obs} is the observed first order rate constant; k_{ne} represents the rate constant for nonenzymatic isomerization; [E] is the total PPIase which is determined by active site titration by tight binding inhibitor using FK506.

peptide represents only about 10%. Therefore almost all the substrate added are hydrolyzed in rapid burst phase and we can follow the PPIase catalyzed isomerization with the rest 10% substrate which is further complicated by the uncatalyzed spontaneous isomerization reaction. It is not possible to raise the substrate concentration too high because of the low solubility of the *p*-nitroaniline, the product. Harrison and Stein (1990a) studied the equilibrium constant in several different peptide substrates and calculated K_{eq}=[*cis*]/[*trans*] in the range of 0.060~0.235 depending on the Xaa in the substrate N-Suc-Ala-Xaa-Pro-Phe-*p*Na at 10℃. Kofron *et al.* (1991) reported that *cis-trans* equilibrium constant varied with medium in which the peptide was dissolved and the presence of metal salt and water content also affected the equilibrium. Particularly anhydrous TFE or THF and LiCl salt were their choice since in those solvent/salt combinations the equilibrium mixture contained 40~60% of *cis*-prolyl isomer of some peptides at 0℃.

The isomer distributions at equilibrium of seven different peptide substrates varying Xaa in Suc-Ala-Xaa-Pro-Phe-*p*NA in anhydrous TFE which contained 0.47 M LiCl are shown in Table 1. The *cis-trans* equilibrium constants were determined to be from 0.24 (Gly) to 0.85 (Ala), which corresponds to 19.4% to 45.9% of cis population, respectively, under experimental conditions. In data not shown, the populations of *cis*-isomer in TFE or dimethylsulfoxide (DMSO) free of LiCl were found to be less than 10%. This clearly reflects the effect of LiCl on the solubility and stability of a peptide in an organic solvent (Seebach *et al.*, 1989). For uncertain reasons, LiCl did not raise the percentage of *cis*-isomer in THF solution as much as in TFE solution (not shown).

Activity of porcine spleen PPIase with these substrates were measured and k_{cat}/K_m values were compared as the measure of substrate specificity of PPIase. At concentrations of [S] much lower than the K_m value, k_{cat}/K_m for PPIase catalyzed reaction can be determined from the observed first order rate constant, nonenzymatic rate constant and concentration of enzyme, according to Eq. (6)

$$k_{cat}/K_m=(k_{obs}-k_{ne})/[E] \quad (6)$$

k_{cat}/K_m for porcine spleen PPIase catalyzed *cis-trans* isomerization of 7 different synthetic peptides are listed along with their first order rate constant for uncatalyzed reactions in Table 2. At 25℃ nonenzymatic thermal isomerization rate constants range from 2.94± 0.22 s^{-1} (Glu) to 6.11± 0.51 s^{-1} (Leu). It is interesting that the least reactive subsrate for PPIase isomerizes spontaneously most slowly and the most reactive substrate reacts also nonenzymatically with the highest rate. But with any other peptides such correlation was not found. The second order rate constants for PPIase catalyzed reactions are, overall, higher than k_{cat}/K_m values reported previously for the hFKBP (Harrison and Stein, 1990b) and the greater sensitivity for substrate change. As in hFKBP, porcine spleen PPIase shows preference for hydrophobic amino acid side chains at the P1 position. On the basis of the specificity data, these substrates can be classified into 3 groups; the high specificity groups (Leu and Ile) with hydrophobic side chains, very low specificity groups (Glu and Gly) and intermediate specificity groups (His, Lys and Ala). Ile peptide was always less reactive than Leu peptide which may indicate that the enzyme active site hydrophobic pocket may accommodate C_γ-substitution better than C_β-substitution. The present data is not strong enough to explain the mechanism. However, if we assume that the partial twisted amide mechanism (Park *et al.*, 1992;

Fischer *et al.*, 1993) is working in this enzyme, the bulky C_β-substitution may provide steric hindrance in the transition state. The large difference in reactivity of Glu and Lys or His suggests that the possible stabilization of the transition state is due to interactions between the positive groups of amino acid side chain and the negative peptide carbonyl oxygen in the resonance structure.

In summary, PPIase purified from porcine spleen catalyzed prolyl peptide *cis-trans* isomerization reaction, and it can be assayed without interference from the initial burst phase reaction rate in 2% TFE and 9.4 mM LiCl by the coupled enzyme method at 25℃. Porcine spleen PPIase was strongly inhibited by FK506 but not by CsA identifying it as one of the FKBP, whose molecular weight is ~14,000 on the basis of SDS-PAGE. True enzyme concentration and inhibition constant were determined by active site titration with the tight binding inhibitor FK506. Applying the TFE/LiCl solvent system in enzyme catalysis , the population of *cis* isomer of all tested Suc-Ala-Xaa-Pro-Phe-pNA peptides can be increased with varying degrees of success. The standard substrate (Xaa=Ala) exhibited the highest ratio of *cis* to *trans* (0.85). In the study of substrate specificity of porcine spleen PPIase using 7 different synthetic tetrapeptides, the activity ratio of the most reactive to the least reactive peptides is 2636 fold, and the reactivity of the intermediate group peptides are two orders of magnitude higher than the least ones. Such substrate specificity information would be useful for the rational design of inhibitors involved in both protein folding processes and immunoresponses.

Acknowledgement

This study was supported by research grant (1995) from Kyung Hee University. We wish to thank Mrs. E. Lee at Denver Swedish Hospital for the generous gifts of CsA and FK506.

References

Brandts, J. F., Halvorson, H. R. and Brennan, M. (1975) *Biochemistry* **14**, 4953.

Fischer, G. (1994) *Angew. Chem.* **106**, 1479.

Fischer, G., Bang, H., Berger, E. and Schelenberger, A. (1984) *Biochim. Biophys. Acta* **791**, 87.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. and Schmidt, F. X. (1989) *Nature* **337**, 476.

Fischer, G., Wöllner, S., Schönbrunner, R. and Scherer, G. (1994) Proceedings of 5th Akabori Conference, German-Japanese-Symposium on Peptide Chemistry, Dresden, pp. 142-145.

Friedman, I., Trahey, M. and Weissmann, I. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6815.

Harding, M. W., Galat, A., Uehling, D. E. and Schreiber, S. L. (1989) *Nature* **341**, 758.

Harrison, R. K. and Stein, R. L. (1990a) *Biochemistry* **29**, 1684.

Harrison, R. K. and Stein, R. L. (1990b) *Biochemistry* **29**, 3813.

Johnson, J. L. and Toft, D. O. (1994) *J. Biol. Chem.* **209**, 24989.

Kern, D., Kern, G., Scherer, G., Fischer, G. and Drakenberg, T. (1995) *Biochemistry* **34**, 13594.

Kofron, J. L., Kuzmic, P., Kishore, V., Colon, B. E. and Rich, D. H. (1991) *Biochemistry* **30**, 6127.

Lang, K. and Schmidt, F. X. (1988) *Nature* **331**, 453.

Lee, C., Hoffmann, K. and Zocher, R. (1992) *Biochem. Biophys. Res. Commun.* **182**, 1282.

Nadeau, K., Das, A. and Walsh, C. T. (1993) *J. Biol. Chem.* **246**, 1479.

Park, S. T., Aldape, R. A., Futer, O., DeCenzo, M. T. and Livingston, D. J. (1992) *J. Biol. Chem.* **267**, 3316.

Schönbrunner, E. R., Mayer, S., Tropschug, M., Fischer, G., Takahashi, N. and Schmidt, F. X. (1991) *J. Biol. Chem.* **266**, 3630.

Schmidt, F. X., Mayer, L., Mücke, M. and Schönbrunner, R. (1993) *Adv. Protein Chem.* **44**, 25.

Seebach, D., Thaler, A. and Beck, A. K. (1989) *Helv. Chim. Acta* **72**, 857.

Siekierka, J. J., Hung, S. H. Y., Poa, M., Lin, C. S. and Sigal, N. S. (1989) *Nature* **341**, 755.

Takahashi, N., Hayano, T. and Suzuki, M. (1989) *Nature* **337**, 473.

Tropschug, M., Wachter, E., Mayer, S., Schönbrunner, E. R. and Schmidt, F. X. (1990) *Nature* **346**, 674.

Williams, J. W. and Morrison, J. F. (1979) *Methods Enzymol.* **63**, 437.