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Catalysis of Protein Folding by Cyclophilins from Different Species*

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E. Ralf Schönbrunner‡, Sabine Mayer‡, Maximilian Tropschug§, Gunter Fischer¶, Nobuhiro Takahashi‖, and Franz X. Schmid‡**

From ‡Biochemisches Laboratorium, Universität Bayreuth, D-8580 Bayreuth, Federal Republic of Germany, the §Institut für Physiologische Chemie, Universität München, D-8000 München, Federal Republic of Germany, ¶WB Biochemie, Sektion Biowissenschaften, Martin-Luther-Universität Halle, Domplatz 1, D-O-4020 Halle, Federal Republic of Germany, and ‖TONEN Corporation, Research and Development Laboratory, 1-3-1 Nishi-Tsurugaoka, Ohi-machi, Iruma-gun, Saitama 354, Japan

Cyclophilins are a class of ubiquitous proteins with yet unknown function. They were originally discovered as the major binding proteins for the immunosuppressant cyclosporin A. The only known catalytic function of these proteins *in vitro* is the *cis/trans* isomerization of *X*aa-Pro bonds in oligopeptides. This became clear after the discovery that bovine cyclophilin is identical with porcine prolyl isomerase. This enzyme accelerates slow, proline-limited steps in the refolding of several proteins. Here we demonstrate that the cyclophilins from man, pig, *Neurospora crassa*, *Saccharomyces cerevisiae*, and *Escherichia coli* are all active as prolyl isomerases and as catalysts of protein folding. This evolutionary conservation suggests that catalysis of prolyl peptide bond isomerization may be an important function of the cyclophilins. It could be related with *de novo* protein folding or be involved in regulatory processes. Catalysis of folding is very efficient in the presence of the high cellular concentrations of prolyl isomerase.

Peptidyl-prolyl *cis/trans* isomerases (PPIases)[1] catalyze the slow *cis* ⇌ *trans* isomerization of *X*aa-proline peptide bonds in short synthetic peptides. This enzymatic activity was first detected and purified from porcine kidney by Fischer *et al.* (1984). Proline isomerization is widely accepted to be one of the rate-determining steps in protein folding (Brandts *et al.*, 1975; Schmid and Baldwin, 1978; Schmid *et al.*, 1986). Porcine PPIase accelerates such reactions in the refolding of several small proteins *in vitro* (Lang *et al.*, 1987; Lang and Schmid, 1988; Lin *et al.*, 1988; Fischer and Schmid, 1990). PPIase from porcine kidney is a cytosolic protein of 17-kDa molecular mass. Sequencing surprisingly revealed this protein to be identical with cyclophilin from bovine thymocytes (Takahashi *et al.*, 1989; Fischer *et al.*, 1989). Cyclophilin had been identified originally as the major high affinity binding protein for the immunosuppressive drug cyclosporin A (CsA) (Handschumacher *et al.*, 1984; Harding *et al.*, 1986). Cyclophilins are very widely distributed. They have been found in virtually all organisms and in different cell compartments such as the cytosol, mitochondria, the endoplasmic reticulum, and in the periplasm of *Escherichia coli* (Handschumacher *et al.*, 1984; Danielson *et al.*, 1988; Tropschug *et al.*, 1988; Davis *et al.*, 1989; Lightowlers *et al.*, 1989; Kawamukai *et al.*, 1989; Shieh *et al.*, 1989; Schneuwly *et al.*, 1989; Haendler *et al.*, 1989; Dietmeier and Tropschug, 1990; Liu and Walsh, 1990). The various members of this protein family share a high degree of sequence similarity. Several cyclophilins have been shown to display prolyl isomerase activity when assayed with a synthetic oligopeptide (Davis *et al.*, 1989; Tropschug *et al.*, 1990).[2]

In protein folding, the efficiency of porcine 17-kDa PPIase/cyclophilin as a catalyst depends, among other factors, on the accessibility of the respective proline-containing chain segments for the isomerase (Lang *et al.*, 1987; Kiefhaber *et al.*, 1990b, 1990c). The slow folding reactions of some proteins are not accelerated by this prolyl isomerase even though proline-controlled steps appear to be involved (Lang *et al.*, 1987; Lin *et al.*, 1988). Catalysis of folding is strongly inhibited by CsA (Fischer *et al.*, 1989).

The cellular functions of cyclophilins and of their prolyl isomerase activity are still unknown. Here we address the question of whether or not the catalysis of slow, proline-controlled steps in protein folding is a general function of cyclophilins. In our investigation, we employ cyclophilins from pig, man, *Neurospora crassa*, *Saccharomyces cerevisiae*, and from *Escherichia coli*. To evaluate their potential as catalysts of protein folding, we use ribonuclease T1 (RNase T1) as a model system. The refolding of this protein is dominated by the slow isomerizations of two prolyl peptide bonds, both of which are accelerated by porcine PPIase (Fischer *et al.*, 1989; Kiefhaber *et al.*, 1990a, 1990b, 1990c). Our results indicate that all cyclophilins that were investigated do in fact catalyze the *in vitro* refolding of our model protein.

EXPERIMENTAL PROCEDURES

Materials—Recombinant RNase T1 was isolated from *E. coli* as described by Quaas *et al.* (1988). Urea (ultrapure) was from Schwarz/

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** To whom correspondence and reprint requests should be addressed: Biochemisches Laboratorium, Universität Bayreuth, Postfach 101251, D-8580 Bayreuth, Federal Republic of Germany.

[1] The abbreviations used are: PPIase, peptidyl prolyl *cis/trans* isomerase; CsA, cyclosporin A.

[2] Since these proteins were discovered independently by virtue of the high affinity for CsA and the catalysis of *X*aa-Pro peptide bond isomerization, they were given different names, cyclophilin and prolyl isomerase, respectively. Here we use the term "cyclophilin" to indicate that a particular protein belongs to the family of CsA binding proteins, and the term "prolyl isomerase" is used to emphasize the catalytic function of these proteins. Remember that another class of prolyl isomerases was discovered recently that is inhibited by the novel immunosuppressant FK 506 and is not homologous with the cyclophilins (Siekierka *et al.*, 1989; Harding *et al.*, 1989; Tropschug *et al.*, 1990). For the originally discovered prolyl isomerase from porcine kidney (Fischer *et al.*, 1984), we mainly use the term 17-kDa PPIase/cyclophilin to facilitate relation to previous work on this protein.

Mann. Concentrated stock solutions of urea were made fresh daily, in order to minimize cyanate formation. Concentrations of urea were determined by the refractive index (Pace, 1986). CsA was a generous gift from Sandoz AG, Basel. Purification of porcine 17-kDa PPIase/cyclophilin is described by Fischer *et al.* (1989), of *N. crassa* cyclophilin by Tropschug *et al.* (1988). Purification of the cyclophilins from yeast and *E. coli* and of the 19-kDa PPIase from porcine kidney will be described elsewhere. All proteins migrated as single bands on SDS-polyacrylamide gels. Protein concentrations were determined by absorbance at 280 nm. The procedure of Gill and von Hippel (1989) was used to calculate absorption coefficients.

Methods—The prolyl isomerase activity of the different cyclophilins was measured by using the chromogenic peptide succinyl-Ala-Ala-Pro-Phe-4-nitroanilide in a coupled assay with chymotrypsin that was developed by Fischer *et al.* (1984). The assay conditions were as described by Fischer *et al.* (1989). A HP 8652 and a Kontron Uvikon 860 spectrometer, both with thermostatted cells, were used. The temperature was controlled in the cell with a thermistor probe and kept at 10.0 ± 0.1 °C.

Unfolded RNase T1 was produced by a 2-h incubation of 100 μM RNase T1 in 8.0 M urea, 0.1 M Tris/HCl, pH 8.0, at 10 °C. Refolding was started by diluting 25 μl of unfolded protein with 975 μl of refolding buffer (0.1 M Tris/HCl, pH 8.0) in the cell of a Hitachi 4020 fluorescence spectrophotometer. Refolding conditions were 2.5 μM RNase T1 in 0.2 M urea, 0.1 M Tris/HCl, pH 8.0. The temperature in the cell was maintained at 10 ± 0.2 °C. Refolding was monitored by the increase in tryptophan fluorescence at 320 nm (10 nm band width) after excitation at 268 nm (1.5 nm band width). Rapid mixing was accomplished by a magnetic stirring bar placed directly in the cell. The dead time of mixing was about 2 s. Blanks of the refolding solution containing the buffer and the appropriate additive were recorded before the addition of unfolded RNase T1 and subtracted from the kinetic progress curve to account for the contributions of the solvent and the various cyclophilins to the fluorescence signal.

RESULTS

PPIase Activities of the Various Cyclophilins—For our comparative study, we employed cyclophilins from man, pig (17-kDa PPIase and 19-kDa PPIase), fungi (*N. crassa* and yeast), and *E. coli*. All of these proteins are active as prolyl isomerases when assayed with the synthetic peptide succinyl-Ala-Ala-Pro-Phe-4-nitroanilide, which is cleaved by chymotrypsin only when the Ala-Pro bond is in the *trans* conformation (Fischer *et al.*, 1984). The k_{cat}/K_m values for this peptide of the different cyclophilins are summarized in Table I. They were derived from the analysis of kinetic progress curves under conditions where the peptide concentration was much smaller than the K_m value (which is estimated to be ≥0.5 mM

TABLE I

Prolyl isomerase activity of cyclophilins from different sources

The prolyl isomerase activities of the individual cyclophilins were determined by using the coupled assay with chymotrypsin as described by Fischer *et al.* (1984). Succinyl-Ala-Ala-Pro-Phe-4-nitroanilide was used as a substrate. The assays were carried out in 0.1 M Tris/HCl, pH 8.0, at 10 °C in the presence of 75 μM assay peptide and 75 μM chymotrypsin. Under these conditions, the substrate concentration is much smaller than K_m, and the observed isomerization kinetics were strictly first order. The k_{cat}/K_m values were derived from the slopes of plots as shown in Fig. 1 for cyclophilin from *N. crassa*, assuming $k_{cat}/K_m = (k - k_0)/[\text{PPIase}]$, where k and k_0 are the first order rate constants of isomerization in the presence and absence of PPIase activity, respectively. The concentrations of the individual PPIases were determined by absorbance at 280 nm.

Source of protein	$10^{-6} \times k_{cat}/K_m$
	$M^{-1}\ s^{-1}$
Porcine kidney (17-kDa PPIase)	5.9
Human recombinant	5.1
S. cerevisiae, cytosol	3.3
N. crassa, cytosol	2.8
E. coli, cytosol	1.9

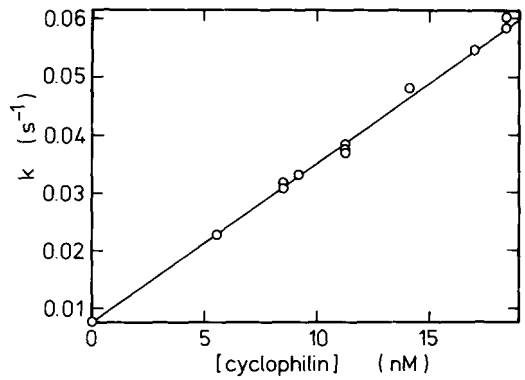

FIG. 1. **Prolyl isomerase activity of cyclophilin from *N. crassa*.** The rate constant of isomerization of the assay peptide Suc-Ala-Ala-Pro-Phe-4-nitroanilide is shown as a function of cyclophilin concentration. Measurements were carried out in 0.1 M Tris/HCl, pH 8.0, at 10 °C in the presence of 75 μM assay peptide and 75 μM chymotrypsin. The isomerization was monitored by the absorbance increase at 390 nm. The slope of the line is equivalent to k_{cat}/K_m and is given in Table I.

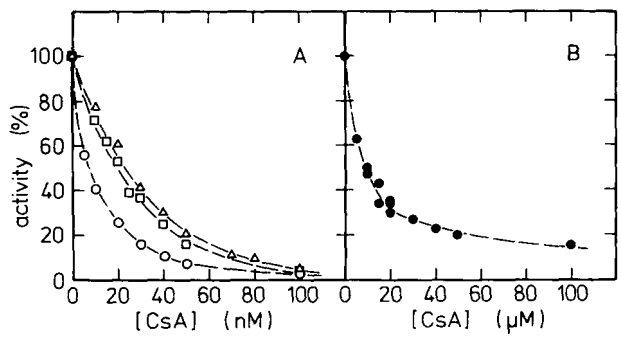

FIG. 2. **Inhibition by CsA of the prolyl isomerase activity of cyclophilin (*A*) from porcine kidney (17-kDa PPIase) (○), *N. crassa* (△), *S. cerevisiae* (□), and (*B*) from *E. coli* (●).** The decrease in relative activity with increasing cyclophilin concentration is shown. The concentrations of PPIases were 4.3 nM (pig), 8.9 nM (*N. crassa*), 7.6 nM (*S. cerevisiae*), and 16 nM (*E. coli*). At these concentrations and in the absence of inhibitor, the isomerization rate of the assay peptide was increased 4.3-fold (pig, *N. crassa*, and *S. cerevisiae*) or 5-fold (*E. coli*), relative to the uncatalyzed isomerization ($k_0 = 7.7 \times 10^{-3}\ s^{-1}$). Note the 1000-fold difference in the CsA concentration scale between *panels A* and *B*.

cis-peptide for the porcine 17-kDa PPIase).[3] Data for the porcine 19-kDa PPIase are not included in Table I, since the absorption coefficient of this PPIase could not be determined yet, due to the small amount of protein available. In all cases, the reactions obeyed first order kinetics, and the rate constant of the catalyzed reaction increased linearly with isomerase concentration, as shown in Fig. 1 for cyclophilin from *N. crassa*. The catalytic efficiencies of the various prolyl isomerases are very high, and the variance between the species is small. All eukaryotic PPIases are strongly inhibited by CsA, as shown for the proteins from pig, yeast, and *N. crassa* in Fig. 2*A*. Inhibition of cyclophilin from *E. coli* by CsA is roughly 1000-fold weaker (Fig. 2*B*).

Catalysis of Protein Folding by the PPIases—To test the ability of the various cyclophilins to catalyze slow protein folding reactions, we used RNase T1 as a model system. Its slow folding kinetics (Kiefhaber *et al.*, 1990a, 1990b, 1990c) are determined by the isomerization of two prolines, Pro^{39} and Pro^{55}, both of which are *cis* in the native protein (Heinemann and Saenger, 1982). Catalysis by porcine 17-kDa

[3] R. Schönbrunner and G. Fischer, unpublished results.

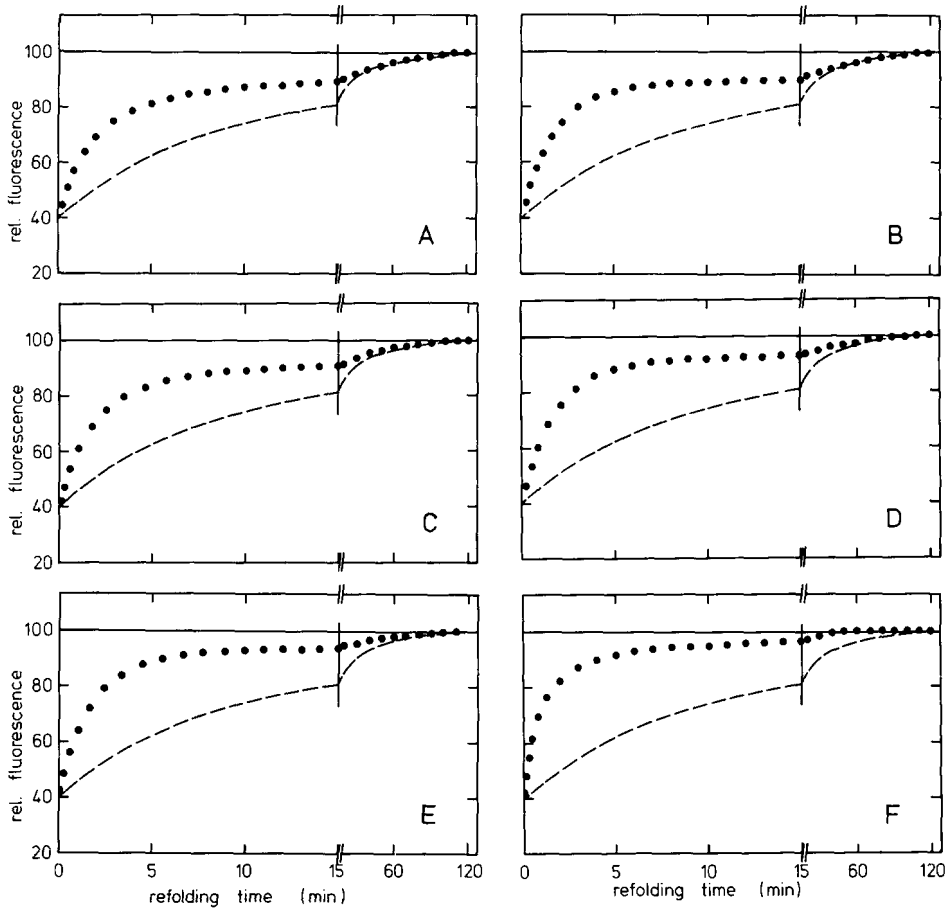

FIG. 3. **Catalysis of the slow refolding reactions of RNase T1 by the different cyclophilins.** The increase in tryptophan fluorescence during refolding is shown as a function of the refolding time. The time course of uncatalyzed refolding in the absence of prolyl isomerase activity is given as a *broken line* in every panel to facilitate comparison. The following PPIases were used. *A*, 65 nM porcine 17-kDa PPIase; *B*, porcine 19-kDa PPIase; *C*, 75 nM human recombinant cyclophilin; *D*, 115 nM yeast cyclophilin; *E*, 135 nM *N. crassa* cyclophilin; *F*, 200 nM *E. coli* cyclophilin. A reliable molar concentration of the porcine 19-kDa PPIase cannot be given yet, since the small amount available is not sufficient to determine its absorption coefficient. The concentrations of the various prolyl isomerases were selected such that the PPIase activities present in the refolding solutions would lead to a 50-fold increase in the isomerization rate of the assay peptide in all cases. Refolding was initiated by a 40-fold dilution of unfolded RNase T1 (kept in 8.0 M urea, 0.1 M Tris/HCl, pH 8.0) to final concentrations of 2.5 μM RNase T1 in 0.2 M urea, 0.1 M Tris/HCl, pH 8.0, at 10 °C in the presence of the various PPIases.

PPIase/cyclophilin has been demonstrated previously (Fischer *et al.*, 1988; Kiefhaber *et al.*, 1990b). The slow refolding kinetics of RNase T1 in the absence and presence of the various cyclophilins are compared in Fig. 3. Identical amounts of PPIase activity (equivalent to a 50-fold acceleration of the isomerization of the assay peptide) were used in all experiments. The results in Fig. 3 clearly demonstrate that catalysis of slow protein folding is a highly conserved property of all investigated cyclophilins. The efficiency of catalysis is very similar for the different proteins. In general, the early time period of slow refolding, *i.e.* the "intermediate phase,"[4] is catalyzed very well. It is largely determined by the *trans* → *cis* isomerization on the Ser^{54}-Pro^{55} peptide bond (Kiefhaber *et al.*, 1990c). The "very slow" terminal phase of folding is presumably limited in rate by Tyr^{38}-Pro^{39} isomerization (Kiefhaber *et al.*, 1990c). Catalysis of this reaction is poor in the experiments where eukaryotic cyclophilins were employed (Fig. 3, *A–E*). In contrast, cyclophilin from *E. coli* (Fig. 3*F*) catalyzes this folding reaction well. A 2-fold increase in the rate of the very slow phases of folding requires the presence of 0.5 μM porcine 17-kDa PPIase, but only 0.15 μM *E. coli* cyclophilin. There is good evidence that the *trans* → *cis* isomerization of Tyr^{38}-Pro^{39} occurs in an already largely folded intermediate, where steric hindrance retards isomerization (Kiefhaber *et al.*, 1990b). This may be the reason why catalysis of this reaction by the eukaryotic cyclophilins is poor. The steric requirements of the enzyme from *E. coli* appear to be different. A quantitative kinetic analysis of the curves shown in Fig. 3 points to further minor differences in catalysis. Refolding in the absence of PPIases and in the presence of the cyclophilin from *N. crassa* and of porcine 19-kDa PPIase can be represented as the sum of two kinetic phases. In the presence of cyclophilins from *E. coli*, yeast, man, and pig (17-kDa PPIase), the intermediate phase appears to be heterogeneous, and the entire slow refolding kinetics are best represented as a sum of three exponential terms (*cf.* also Kiefhaber *et al.*, 1990b). In all cases except the

[4] The two major phases of the refolding of RNase T1 are labeled intermediate (faster phase) and very slow (slower phase), to take account of the fact that, in addition to the major, slow phases, there is a small, fast, folding reaction (3.5%) of the unfolded molecules with correct proline isomers. Under the given refolding conditions, time constants of $\tau_1 = 3000$ s and $\tau_2 = 500$ s are observed for the very slow and the intermediate phase, respectively (Kiefhaber *et al.*, 1990a, 1990b, 1990c).

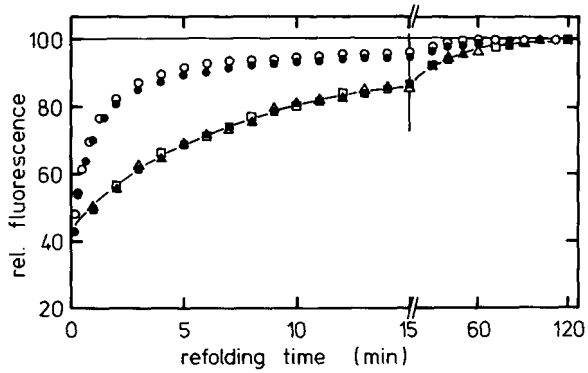

FIG. 4. **Inhibition by CsA of the catalytic effect of PPIases on the slow refolding of RNase T1.** The increase in RNase T1 fluorescence at 320 nm is shown as a function of refolding time (——) in the absence of PPIases, but in the presence of 1 μM CsA; in the presence of 1 μM CsA and cyclophilins from *E. coli* (●), *N. crassa* (▲), *S. cerevisiae* (□), man (■), and the 19-kDa PPIase from pig (△). ○, refolding in the presence of *E. coli* cyclophilin without CsA. The same PPIase concentrations were used as in the experiments of Fig. 3.

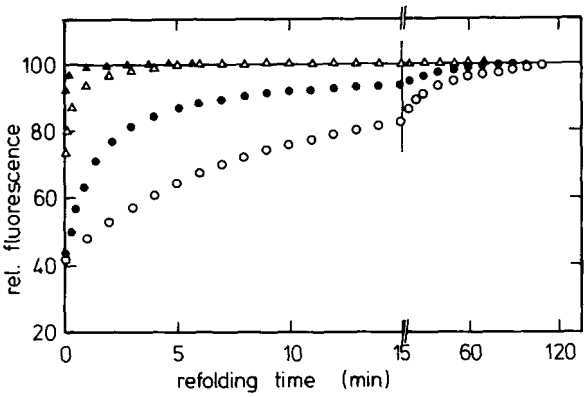

FIG. 5. **Catalysis of slow refolding of RNase T1 in the presence of increasing concentrations of cyclophilin from *E. coli*.** The increase in protein fluorescence at 320 nm is shown as a function of the time of refolding in 0.2 M urea, 0.1 M Tris/HCl, pH 8.0, at 10 °C in the presence of the following concentrations of *E. coli* PPIase. ○, 0 μM; ●, 0.1 μM; △, 2.0 μM; ▲, 29 μM. Unfolding and refolding was carried out as described in Fig. 3.

protein from *E. coli*, catalysis of RNase T1 refolding is completely inhibited in the presence of 1 μM CsA, as shown in Fig. 4. *E. coli* cyclophilin is only marginally inhibited by 1 μM CsA, as expected from the weak binding of CsA to this protein (Fig. 2*B*).

Dependence on PPIase Concentration of Folding Catalysis—Cyclophilin from *E. coli* catalyzes all slow phases of RNase T1 refolding with good efficiency (*cf.* Fig. 3*F*). Accordingly, we used this protein to investigate the dependence on PPIase concentration of the catalysis of folding. The refolding of 2.5 μM RNase T1 was measured in the presence of 0 to 29 μM cyclophilin (Fig. 5). At the highest concentration, catalysis is extremely effective: the major, faster phase of folding is complete within the dead time of mixing (2 s) and the time constant of the very slow phase is reduced from $\tau_1 = 3000$ s (in the absence of PPIase) to $\tau_1 = 10$ s in the presence of 29 μM PPIase. This is equivalent to a 300-fold acceleration of folding at a cyclophilin concentration that is similar to the concentration found in the cell. The presence of 0.1 μM PPIase leads to a 6-fold increase in the rate of the intermediate folding phase. Catalysis is independent of RNase T1 concentration.[5] These results demonstrate that PPIases act as true catalysts of protein folding. The catalytic efficiency of cyclophilin from *E. coli* is apparently very high. Under the conditions used in the experiments of Figs. 3 and 4, the saturation of the PPIases with the substrate RNase T1 is presumably very low, since the "substrate" concentration of refolding chains is small (2.5 μM), and the affinity of the PPIases, at least for the assay peptide, is known to be low.

DISCUSSION

Prolyl Isomerase Activity of Cyclophilins Is Conserved—The cyclophilins employed in this study originated from different species, ranging from mammals to bacteria. All were active as prolyl isomerases when assayed with a synthetic peptide. The relative activities are very similar: the k_{cat}/K_m values are high for all cyclophilins and they vary by no more than a factor of 3. This suggests that the prolyl isomerase activity may indeed be related to the biological function of this class of proteins which was originally discovered independently by binding tests for CsA (Handschumacher *et al.*, 1984) and PPIase activity assays (Fischer *et al.*, 1984). Our k_{cat}/K_m values (Table I) agree well with the value of $k_{cat}/K_m = 3.2 \times 10^6$ M^{-1}/cm^{-1}, obtained by Harrison and Stein (1990) for bovine cyclophilin.[6]

Cyclophilins Catalyze Slow Steps in Protein Folding—All cyclophilins tested in this study catalyzed the slow proline-controlled refolding reactions of RNase T1. The accessibilities of the two prolyl peptide bonds (Tyr^{38}-Pro^{39} and Ser^{54}-Pro^{55}) are presumably different in refolding RNase T1 molecules. Ser^{54}-Pro^{55} is exposed to solvent even in the native protein. Therefore, it should be well accessible for PPIases during refolding. Tyr^{38}-Pro^{39} is buried in native RNase T1 (Heinemann and Saenger, 1982) and probably also in folding intermediates. Catalysis of RNase T1 folding by the eukaryotic cyclophilins follows a common pattern. Acceleration of the intermediate phase of folding (dominated by Pro^{55} isomerization) is very efficient, whereas catalysis of the very slow phase (limited in rate by Pro^{39} isomerization) is poor. This suggests that, in addition to the catalytic properties of these cyclophilins, the steric requirements of their active sites are the same with regard to the accessibility to the prolyl peptide bonds in the refolding protein chains. At present, we cannot evaluate the contributions of the different sequences flanking Pro^{39} and Pro^{55} to the observed differences in catalytic efficiency. However, regarding the very low sequence specificity of bovine cyclophilin (Harrison and Stein, 1990), the sequential context of the prolines in refolding chains might be of secondary importance.

Cyclophilin from *E. coli* deviates significantly from this pattern. It catalyzes the very slow folding reaction of RNase T1 much better than the eukaryotic cyclophilins. Apparently, cyclophilin from *E. coli* is able to bind and catalyze prolyl isomerization in sterically more restricted regions of a protein chain or has different steric requirements in general. Another interesting property of cyclophilin from *E. coli* is its low affinity for CsA. We do not know whether the relatively poor binding of CsA and the increased activity toward shielded prolyl peptide bonds in folding proteins are related.

Recently, another immunosuppressant, termed FK 506, has

[5] S. R. Schönbrunner, unpublished results.

[6] Significantly higher k_{cat}/K_m values (1.2–1.7 × 10^7 M^{-1} s^{-1}) were reported by Liu *et al.* (1990) for human cyclophilin and several mutants of the human protein. Unfortunately, their analysis appears to be incorrect. A reevaluation of the kinetic data given for wild type protein in Fig. 3*A* of Liu *et al.* (1990) for 0 and 27 nM cyclophilin results in a k_{cat}/K_m value of 3 × 10^6 M^{-1} s^{-1}, which is in agreement with the values reported by others.

been discovered (Sawada *et al.*, 1987), which is not related structurally with CsA. FK 506 binds with high affinity to FK 506 binding proteins (Harding *et al.*, 1989; Siekierka *et al.*, 1989). The FK 506 binding proteins are not similar to the cyclophilins in sequence. However, they are active as prolyl isomerases, and, most intriguingly, they also catalyze slow steps in protein folding (Tropschug *et al.*, 1990). PPIases now consist of at least two structurally unrelated, but apparently functionally related, families of proteins, the cyclophilins and FK-506 binding proteins.

Catalysis of Folding Can Be Very Efficient—Cyclophilins are true catalysts of protein folding. Catalytic amounts (0.1 μM) of prolyl isomerase activity from *E. coli* are sufficient for a 6-fold acceleration of the intermediate phase of refolding of 2.5 μM RNase T1. The cellular concentrations of cyclophilins are still an order of magnitude higher, typically in the 20 μM range (Harding *et al.*, 1986).[7] Under such conditions, *in vitro* refolding of RNase T1 is almost complete within the dead time of mixing at 10 °C (*cf.* Fig. 5).

Largely Folded Protein Chains Can Be Targets of Prolyl Isomerases—Virtually all of the secondary structure and part of the tertiary structure are formed rapidly during folding of RNase T1, well before incorrect prolyl bonds reisomerize (Kiefhaber *et al.*, 1990b). Consequently, these isomerizations do not occur in an extended polypeptide chain, but rather at the stage of ordered, presumably almost native-like folding intermediates. The good catalysis of these slow steps demonstrates that the action of PPIases is not restricted to structureless peptides or protein chain segments, but that *X*aa-Pro bonds in ordered proteins serve as good substrates as well. We do not know at present whether the cellular targets of the prolyl isomerase activity of cyclophilins are indeed proteins, and, if so, whether these are folding intermediates and/or native proteins with alternative proline conformations. A possible function of PPIases in the cell could be the effective catalysis of critical slow steps on the productive folding pathway. Thus, the accumulation of folding intermediates that are sensitive to side reactions, such as aggregation, could be avoided. Premature aggregation of partially folded proteins is a major complication in protein folding (Zettlmeissl *et al.*, 1979). Proteins that can suppress aggregation of newly formed polypeptide chains, such as GroEL (or cpn60) from *E. coli* occur in high concentrations in the cell (Goluobinoff *et al.*, 1989). Whether prolyl isomerases can interact with GroEL, is not known.

Protein Folding and Immunosuppression—The presumed function of cyclophilins in mediating the immunosuppressive effect of CsA is still not understood at the molecular level. There is good evidence now that CsA affects the transcriptional control of the interleukin 2 gene in T cells (Krönke *et al.*, 1984). Apparently, the correct maturation of nuclear transcriptional factors is impaired in the presence of CsA (Emmel *et al.*, 1989; Randak *et al.*, 1990). This could be explained by an inhibitory effect of CsA on the catalysis of their folding in the cell. Delayed folding of regulatory proteins that are in rapid turnover could lead to a strong decrease of their cellular concentrations and thus impair their function.

Role of Cyclophilins in Protein Folding—The cyclophilins share a number of properties that could indicate a role for protein folding in the cell. (i) They are ubiquitous and occur in all cell compartments where folding reactions take place. (ii) Their intracellular concentrations are high, to warrant sufficient availability. (iii) Their sequence specificity appears to be low (Harrison and Stein, 1990), thus different *X*aa-Pro sequences could be recognized. (iv) The catalytic efficiency is very high. This may be important, since isomerization occurs in the absence of PPIases at a significant rate as well. (v) The catalysis of proline-limited folding reactions is highly conserved for cyclophilins of different phylogenetic origin. This is only circumstantial evidence for a role of cyclophilin-like prolyl isomerases in cellular protein folding. Alternatively, a possible function of proline-containing sequences as molecular switches and prolyl isomerases as effectors in cellular signalling could be considered as well (Schmid *et al.*, 1991).

[7] N. Takahashi, unpublished results.

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