Copper(II)-Catalyzed Amide Isomerization: Evidence for N-Coordination

Christopher Cox, Dana Ferraris, Narasimha N. Murthy, and Thomas Lectka*

Department of Chemistry, Johns Hopkins University 3400 N. Charles Street, Baltimore, Maryland 21218

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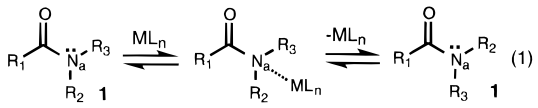
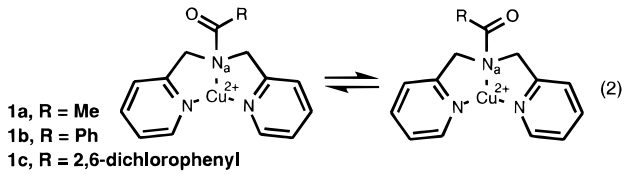
Amide isomerization (AI) plays an important role in protein folding, where it can be the rate-determining step with proline-containing polypeptides and proteins.[1] Peptidyl prolyl isomerases (PPIases), including cyclophilin and FKBP, are "rotamase" enzymes that catalyze protein folding *in vitro* and *in vivo* through the isomerization of proline residues.[2] However, PPIases are believed to be unable to catalyze the folding of some proteins with sterically inaccessible prolines.[3] Thus the development of small synthetic catalysts for protein folding that have better access to sterically encumbered sites would complement the activity of PPIases. As a first step in our plan to develop synthetic rotamases for peptide folding, we show in this communication that Cu(II) ions in small amounts can efficiently catalyze isomerization of amides that contain metal binding sites designed to favor N-coordination on steric and entropic grounds. Most importantly, we also found that the *side chain in substituted prolines* serves as a crucial binding site for Cu(II) to catalyze AI. In addition, we present the first spectroscopic and crystallographic proof of Cu(II)−N_a coordination[4] in tertiary amides (imides).

The effect of metal ions on rotational barriers in amides has not been well studied; however, published data suggest that Lewis acids in general raise the barrier,[5] with the sole exception being a report that a large excess of Ag^+ modestly lowers the barrier in *N*,*N*-dimethylacetamide.[6] This inhibition may occur through coordination of the Lewis acid to oxygen, which reinforces the double-bond character of the C−N bond. On the other hand, coordination of the metal to N_a should disrupt amide resonance and catalyze AI (eq 1).



We reasoned that a less oxophilic late transition metal salt such as Cu(II) would be more likely to bind N_a.[7] A way to enhance the effect is to include other metal binding sites so that favorable chelate rings could form that involve N_a.[8]

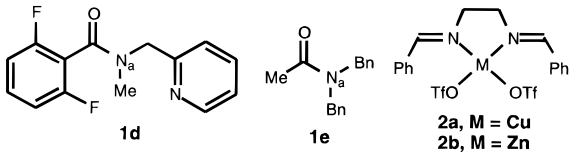


Ligand **1a**[9] provides an ideal test system to observe metal−N_a coordination in a tertiary amide. Treatment of **1a** with Cu(II) should produce a tridentate complex containing two five-membered rings involving N_a (eq 2). The amide rotation barrier (ΔG_{act}) in free **1a** was 19.0 kcal/mol (entry a, Table 1), as measured by the ^{1}H saturation transfer (ST) NMR method,[10,11] but when we treated **1a** with 5 mol % $Cu(OTf)_2$ in $CDCl_3$ at 20 °C, the rotational barrier dropped to 16.3 kcal/mol (entry b). In the presence of only 2 mol % $Cu(OTf)_2$, ΔG_{act} for phenyl-substituted amide **1b** was lowered by 1.0 kcal/mol (entries c, d). The effect was largest in amide **1c**, where ΔG_{act} was lowered by 6 kcal/mol (entries e, f), representing a >23 000-fold rate enhancement.[12] In general, we found that the potential for energy lowering is greatest in amides with high rotational barriers such as **1c**.

Table 1. Rotational Barriers for Amides **1** (see Eq 1)[a]

entry	amide	solvent	ΔG_{act}	metal salt	mol %	T (°C)
a	**1a**	$CDCl_3$	19.0 ± 0.2			40
b	**1a**	$CDCl_3$	16.3 ± 0.1	$Cu(OTf)_2$	5	−23
c	**1b**	THF-d_8	15.6 ± 0.2			5
d	**1b**	THF-d_8	14.6 ± 0.2	$Cu(OTf)_2$	2	0
e	**1c**	DMSO-d_6	23.4 ± 0.2[b]			125
f	**1c**	$CDCl_3$	17.4 ± 0.1	$Cu(OTf)_2$	5	50
g	**1d**	THF	20.0 ± 0.2[c]			50
h	**1d**	THF	18.9 ± 0.2[c]	$Cu(OTf)_2$	10	50
i	**1e**	$CDCl_3$	17.5 ± 0.1			50
j	**1e**	$CDCl_3$	17.7 ± 0.2	$Cu(OTf)_2$	5	50

[a] Determined by the ^{1}H NMR saturation transfer method. [b] Barrier not determinable in $CDCl_3$ by saturation transfer. [c] Determined by ^{19}F saturation transfer.



Substoichiometric amounts of metal confirm that Cu(II) undergoes fast exchange and is a true catalyst. As expected, there is no measurable catalysis in an amide lacking an additional binding site at the Cu(II) concentrations employed (**1e**, entries i, j). The barrier in bidentate amide **1d** decreases modestly by 1.1 kcal (10 mol % $Cu(OTf)_2$), as measured by ^{19}F ST where the paramagnetic effects of Cu(II) are mitigated by the large chemical shift range of the ^{19}F nucleus and its remote position.[13]

We also found that preformed complexes can catalyze AI, a fact that has importance for the design of synthetic rotamases. Unfortunately, measuring catalyzed AI when amides **1** are treated with $Cu(OTf)_2$−bis(imine) complex **2a** is impossible due

(1) Schmid, F. X. In *Protein Folding*; Creighton, T. E., Ed.; W. H. Freeman: New York, 1992; pp 197−241.

(2) For two overviews, see: (a) Schmid, F. X.; Mayr, L. M.; Mücke, M.; Schönbrunner, E. R. *Adv. Protein Chem.* **1993**, *44*, 25. (b) Schreiber, S. L. *Science* **1991**, *251*, 283.

(3) Kördel, J.; Drakenberg, T; Forsen, S.; Thulin, E. *FEBS Lett.* **1990**, *263*, 27.

(4) N_a denotes the amide nitrogen.

(5) Fussenegger, R.; Rode, B. M. *Chem. Phys. Lett.* **1976**, *44*, 95.

(6) Waghorne, W. E.; Ward, A. J. I.; Clune, T. G.; Cox, B. G. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 1131.

(7) Other transition metals were screened, including Ag(I), Ni(II), and Zn(II), but Cu(II) was found to be the most effective.

(8) Precedents for metal-N_a coordination in tertiary amides are virtually nonexistent; N_a-coordination involving undeprotonated amides in peptides has been observed in a crystal structure of a Cu(II)−urea complex (Maslak, P.; Sczepanski, J. J.; Parvez, M. *J. Am. Chem. Soc.* **1991**, *113*, 1062) and in an Ir(II)−8-amidoquinoline complex (Lee, J. C.; Müller; Pregosin, P.; Yap, G. P. A.; Rheingold, A. L.; Crabtree, R. H. *Inorg. Chem.* **1995**, *34*, 6295).

(9) All new compounds were synthesized by standard literature methods and gave satisfactory analyses (IR, NMR, MS, elemental).

(10) Forsén, S.; Hoffman, R. A. *Acta Chem. Scand.* **1963**, *17*, 1787. For a review of the theory of saturation transfer, see: Sanders, J. K. M.; Mersh, J. D. *Prog. Nucl. Magn. Reson. Spectrosc.* **1983**, *15*, 353. For development and applications to rotation of amides, see: Perrin, C. L.; Thoburn, J. D.; Kresge, J. *J. Am. Chem. Soc.* **1992**, *114*, 8800. Details of our experimental protocol are given in the supporting information.

(11) The methylene protons of **1a**−**1c** and **1e** were sampled in quadruplicate runs. The quantities of Cu(II) catalyst employed were limited by paramagnetic broadening at high concentrations. With some exceptions, generally a range of 4−10 mol % Cu(II) gave the best balance of catalysis and reproducibility.

(12) Due to the high rotational barrier in **1c**, it was measured in DMSO-d_6. In general we found that the solvent effect on AI ($CDCl_3$ to DMSO-d_6) is negligible for free amides **1**.

(13) Although other NMR active nuclei have been used in ST experiments, this study represents the first use of ^{19}F to our knowledge.

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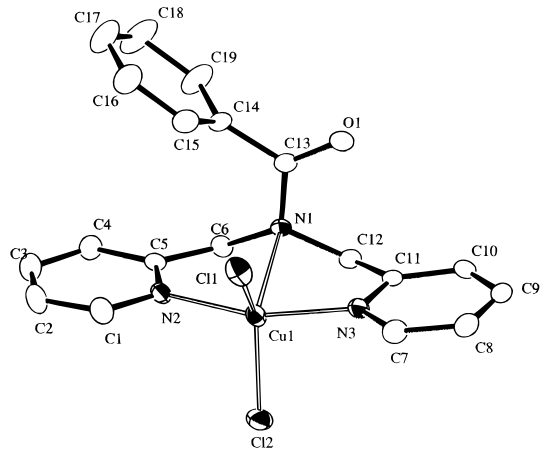
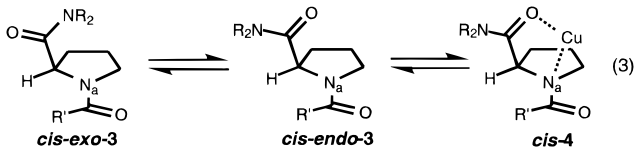


Figure 1. Crystal structure of **1c**·$CuCl_2$ (25% ellipsoids). Selected bond distances (Å): Cu(1)−N(1) 2.49; Cu(1)−N(2) 2.04; Cu(1)−N(3) 2.02; Cu(1)−Cl(1) 2.27; Cu(1)−Cl(2) 2.28. Selected bond angles (deg): N(1)−Cu(1)−Cl(2) 106.0; N(1)−Cu(1)−Cl(1) 114.6; N(1)−Cu(1)−N(3) 79.0; N(1)−Cu(1)−N(2) 78.2; N(2)−Cu(1)−Cl(1) 96.6; N(2)−Cu(1)−Cl(2) 92.3; N(2)−Cu(1)−N(3) 157.0; Cl(1)−Cu(1)−Cl(2) 139.3. Selected dihedral angle (deg): C(6)−N(1)−C1(3)−C1(2) −146.0.

to paramagnetic broadening in the ^{1}H NMR spectrum of the sample; however, the Zn(II) complex **2b** (25 mol %) lowers the barrier of amide **1a** by 2.5 kcal in $CDCl_3$.

Additional evidence for catalysis by N-coordination comes from IR and ESR spectroscopy. One-to-one solutions of **1a**−**1d** and $Cu(OTf)_2$ in CH_2Cl_2 revealed shifts of the amide carbonyl stretch to higher frequency (15−40 cm^{-1}), consistent with a strengthened C═O bond. Additionally, a notable difference in superhyperfine splitting in the ESR spectrum of **1b**·$Cu(OTf)_2$ at −110 °C in CH_2Cl_2 solution was observed when $^{15}N_a$ was substituted for $^{14}N_a$, a result indicative of direct Cu−N_a bonding.[14]

The most compelling evidence for catalysis by N-coordination is the X-ray structure of a crystalline **1b**·$CuCl_2$ complex that reveals clear N_a-coordination (Cu−N_a distance = 2.49 Å; Figure 1).[15] In the crystal Cu(II) is approximately trigonal bipyramidal; N_a (N(1)) is notably pyramidalized due to metal coordination (dihedral angle C(6)−N(1)−C(13)−C(12) = −146.0°). To our knowledge, this structure represents the first example of a metal coordinated to N_a of a tertiary amide and suggested to us that Cu(II) could also bind to the ring N_a of prolyl peptides.



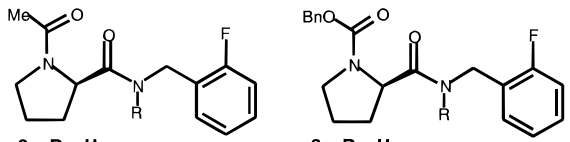
(3)

With a view toward catalyzing the folding of polypeptides in solution, we screened a number of substituted prolines **3** as more realistic models for catalysis. Because of its cyclic structure and its conformation, proline in peptides contains a natural binding site for metals involving N_a (eq 3). Due to $A_{1,3}$-strain,[16] the proline unit should prefer to dispose its C_α-substituent pseudoaxially, and when the C_α-carbonyl is *endo*[17] it is poised to form a five-membered metal chelate containing the ring N_a for a tertiary amide (*cis*-**4**). Treatment of prolyl amide **3a** (Table 2, entries k, l) with 5 mol % $Cu(OTf)_2$ in THF lowered ΔG_{act} from 17.8 kcal/mol to 16.8 kcal/mol for the *trans*-to-*cis* isomerization,[18] as monitored by ^{19}F ST. Energy lowering was enhanced in prolyl carbamate **3c** (1.3 kcal/mol, entries o, p), which contains a more electron-rich N_a. Under the same conditions, the barrier in prolyl amide **3b** dropped by 2.0 kcal/mol.[19] We observed the largest energy lowering (4.3 kcal/mol) in proline **3d** (entries q, r).[20] Significantly, in both *N*-acetylpyrrolidine and *N*-Cbz-pyrrolidine, no energy lowering occurred by ^{1}H ST under standard conditions with 5 mol % $Cu(OTf)_2$. Taken together, these observations are consistent with the ability of the side chain functional group to bind the metal and catalyze AI.

Table 2. $Cu(OTf)_2$-Catalyzed Proline Isomerization (Eq 3)[a]

entry	amide	solvent	ΔG_{act}[b]	metal salt[c]	*T* (°C)
k	**3a**	THF	17.8 ± 0.2		30
l	**3a**	THF	16.8 ± 0.2	$Cu(OTf)_2$	30
m	**3b**	THF	19.1 ± 0.1		40
n	**3b**	THF	17.1 ± 0.2	$Cu(OTf)_2$	40
o	**3c**	THF	16.1 ± 0.2		−5
p	**3c**	THF	14.8 ± 0.3	$Cu(OTf)_2$	−20
q	**3d**	THF	18.1 ± 0.2		25
r	**3d**	THF	13.8 ± 0.1	$Cu(OTf)_2$	−20

[a] Determined by ^{19}F saturation transfer. [b] Activation barriers (ΔG_{act}) are reported for the *trans*-to-*cis* isomerization. [c] 5 mol % $Cu(OTf)_2$ used in the catalyzed cases.



Polyproline is a remarkably structured "switch" polypeptide that reversibly interconverts between *all-cis* (right-handed helix) and *all-trans* (left-handed helix) forms depending on solvent polarity.[21] Polyproline has been studied as a model for the folding of the collagen triple helix, which is the only documented case of PPIase-catalyzed folding *in vivo*.[22] We dissolved *trans*-polyproline in $CDCl_3$ and monitored its conversion to *cis* by ^{1}H NMR. In the presence of $Cu(OTf)_2$ (10 wt % Cu(II) relative to polyproline), we found that the rate of conversion of *trans* to *cis* increases by a factor of 10 ((1.4 ± 0.3) kcal lowering of ΔG_{act}) at 23 °C. This result provides a firm basis for our continuing investigations on the catalysis of folding of more complex polypeptides and proteins in the presence of Cu(II) (and other metal ions), which we will report in due course.

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Supporting Information Available: Experimental details of saturation transfer, NMR, and ESR measurements, ESR spectra of **1a**·$Cu(OTf)_2$, and X-ray report for **1b**·$CuCl_2$ (20 pages). Ordering information is given on any current masthead page.

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(14) Basosi, R.; Antholine, W. E.; Hyde, J. S. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1993; pp 103−150.

(15) Green crystals of **1b**·$CuCl_2$ were grown by slow diffusion of ether into a CH_2Cl_2 solution. Crystal data for **1b**·$CuCl_2$: a = 14.360(4) Å, b = 8.368(2) Å, c = 17.073(3) Å, α = 90.00, β = 108.72 (1)°, γ = 90.00, space group = $P2_{1/a}$.

(16) Johnson, F. *Chem. Rev.* **1968**, *68*, 375.

(17) A crystal structure of *t*-Boc-Pro-Pro-OH reveals psuedoaxially disposed, *endo* α-substituents: Thomas, L. M.; Ramasubbu, N.; Bhandary, K. K. *Int. J. Pep. Protein Res.* **1994**, *44*, 207.

(18) At the metal concentrations screened, there was no perturbation of the *cis*/*trans* equilibrium constants for the substituted prolines, consistent with Cu(II)'s role as a true catalyst.

(19) Proton NOE data for proline **3b** are indicative of an *endo* carbonyl, whereas for **3a** ab initio calculations (3-21G basis) indicate that an *exo* carbonyl is the favored conformation, which may account for the greater barrier lowering in **3b**.

(20) In contrast, no catalysis was observed in the rotation of the side chain amido group of **3c**, a fact consistent with our model.

(21) Steinberg, I. Z; Harrington, W. F.; Berger, A.; Sela, M.; Katchalski, E. *J. Am. Chem. Soc.* **1960**, *82*, 5263. Torchia, D. A.; Bovey, F. A. *Macromolecules* **1971**, *4*, 246.

(22) Steinmann, B.; Bruckner, P.; Supertifurga, A. *J. Biol. Chem.* **1991**, *266*, 1299.