Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5084–5087

The molecular basis for the selection of captopril *cis* and *trans* conformations by angiotensin I converting enzyme

Andreas G. Tzakos,[a,†] Nawazish Naqvi,[b,†] Konstantinos Comporozos,[c] Roberta Pierattelli,[d] Vassiliki Theodorou,[c] Ahsan Husain[b,*] and Ioannis P. Gerothanassis[c,*]

[a]*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK*
[b]*Centre for Heart Failure Research, University of Alabama, 901, 19th street south, Birmingham, AL 35294, USA*
[c]*Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, Ioannina, GR 45110, Greece*
[d]*Department of Chemistry and CERM, University of Florence, Via Sacconi 6, 50019 Sesto Fiorentino, Italy*

Received 4 June 2006; revised 11 July 2006; accepted 12 July 2006
Available online 2 August 2006

Abstract—Enzyme–inhibitor recognition is considered one of the most fundamental aspects in the area of drug discovery. However, the molecular mechanism of this recognition process (induced fit or prebinding and adaptive selection among multiple conformers) in several cases remains unexplored. In order to shed light toward this step of the recognition process in the case of human angiotensin I converting enzyme (hACE) and its inhibitor captopril, we have established a novel combinatorial approach exploiting solution NMR, flexible docking calculations, mutagenesis, and enzymatic studies. We provide evidence that an equimolar ratio of the *cis* and *trans* states of captopril exists in solution and that the enzyme selects only the *trans* state of the inhibitor that presents architectural and stereoelectronic complementarity with its substrate binding groove.

ACE (EC 3.4.15.1) plays a central role in the metabolism of vasoactive peptides and its gene is a candidate for several cardiovascular diseases.[1] It has two metalloproteinase domains (called N- (ACE_N) and C- (ACE_C)), each containing a canonical Zn^{2+}-binding sequence motif: HExxH (His-Glu-x-x-His),[2] and interdomain co-operation upon inhibitor binding has been reported.[3,4] Inhibitors of ACE are effective and widely used drugs for the therapy of hypertension, heart disease, diabetic neuropathy, and atherosclerosis.[5–7] Rationalization of the substrate domain differentiation[8] and atomic level pharmacophore refinement of nine inhibitors for both the N- and C-domains of ACE has been recently reported by us.[9]

Captopril, 1-[2(*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, is the first angiotensin-converting enzyme inhibitor for treatment of hypertension and heart failure. The incorporation of the active-analog approach, which allows the stepwise identification of the substrate pharmacophoric pattern associated with the recognition by a specific receptor,[10] was the basis for discovery efforts that produced the large family of the ACE inhibitors.[11] Like other proline-containing peptides, captopril normally exists as an equilibrium mixture of *cis* and *trans* isomers, with respect to the proline amide bond (Fig. 1A). The crystal structures of the ACE enzyme solved recently in complex with captopril,[12–14] as well as the X-ray structure of the inhibitor in its unbound state,[15] comprise only the *trans* isomer of the inhibitor. However, a key question still exists concerning the molecular basis for the selection of the inhibitor isomerization states during the recognition processes.

The ^{1}H NMR spectra of captopril as a function of pH and solvent (H_2O, D_2O, and DMSO-d_6) have been recorded at 600 MHz and the *cis*/*trans* equilibrium constant of captopril has been measured by the relative integrals of the α- and δ-proton resonances of the two isomerization states of the proline moiety (Fig. 1A). These signals are well resolved and allowed us to determine accurately the equilibrium constants for the *cis*-to-*trans* interconversion at different temperatures and to calculate the thermodynamic parameters enthalpy

Keywords: Angiotensin-converting enzyme; Captopril; Isomerization; Mutagenesis; NMR.
* Corresponding authors. E-mail addresses: ahusain@physiology.uab.edu; igeroth@cc.uoi.gr
† AGT and NN were equally contributed.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.034

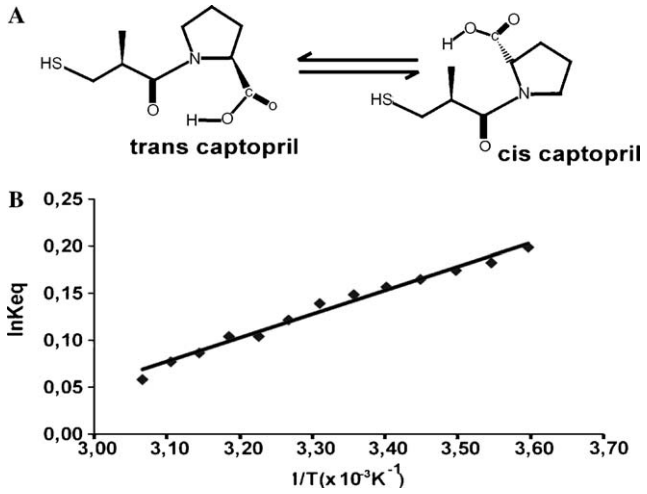

Figure 1. (A) *Trans* and *cis* isomers of captopril. (B) Representative Van't Hoff plot in D_2O.

($\Delta H°$), entropy ($\Delta S°$), and Gibbs free energy ($\Delta G°$) of the equilibrium (Table 1) on the basis of the Van't Hoff equation (Fig. 1B).

Remarkably, a similar ratio for the *cis*-to-*trans* isomerization states is observed at neutral pH. The $\Delta G°$ is statistically the same in H_2O, D_2O, and DMSO-d_6 at acidic pH and almost the same in H_2O and D_2O at neutral pH. Since $\Delta G°$ in DMSO-d_6 solution is almost the same as in H_2O or D_2O, it seems reasonable to assume that the existence of exchangeable protons on the solvent does not affect the *cis*/*trans* equilibrium. The *trans* isomer in D_2O solution is enthalpically and entropically favored ($\Delta H° = -3.63 \pm 0.28$ kJ mol^{-1} and $\Delta S° = 1.75 \pm 0.23$ J K^{-1}), relative to the *cis* isomer at acidic pH, whereas it is enthalpically favored but entropically disfavored ($\Delta H° = -1.87$ kJ mol^{-1} and $\Delta S° = -3.76 \pm 0.31$ J K^{-1}), relative to the *cis* isomer at neutral pH. The decreasing enthalpic benefit of the *trans* isomer at pH 6.8, compared to that at pH 2.15, should be attributed to the repulsive interaction of the dipole of the peptide bond with the negative charge of the carboxylate group. The increasing entropy contribution, which results in nearly equal populations of the *cis* (46%) and *trans* (54%) isomers at physiological pH, should, similarly, be attributed to the repulsive interaction between the dipole of the peptide bond with the negative charge of the carboxylate group, which results in a reduction in the conformational space of the interacting groups and the associated solvent molecules of water.These data together with the X-ray structure of the ACE–captopril complex (where only the *trans* isomer is present) suggest, in a crude approximation, that in the first step of recognition ~50% of the inhibitor is readily available for its direct selection by the enzyme.

In order to rationalize this aspect in detail, we performed flexible docking calculations[9,16] of both isomers (*trans* and *cis*) of captopril to the C-domain of hACE. The first finding is that for both isomers, the carbonyl oxygen of the peptide bond is locked by two strong hydrogen bonds with residues H1089 and H929 of the enzyme (Fig. 2). This is in accordance with our previous mutagenesis studies, where mutation of H1089 abolishes binding of captopril to the enzyme.[17] The accommodation of the *trans* captopril carboxy-terminal proline moiety by the enzyme S2′ subsite is mediated through ionic interactions and hydrogen bonds with K1087, Q857, and Y1096 (Fig. 2A).

In the case of the *cis* form of the inhibitor its pyrrolidine ring is loosely stabilized by the S2′ subsite via weak hydrophobic interactions with the Y1096 and the Y1099 rings, for which it has limited access due to the bulky hydroxyl group of Y1096 (Fig. 2C); however, the 'locking' interactions provided mainly by K1087 and Y1096 are present in the *trans* isomer and are absent in the *cis* form. This less favorable accommodation of the *cis* isomer of captopril by the enzyme active site is in accordance to the estimated free energies of binding (~ −5 kcal/mol for *cis* and ~ −7 kcal/mol for *trans*) and thus, it is in agreement with the conformational state of the inhibitor as can be seen by the X-ray structure of the complex.[13,14]

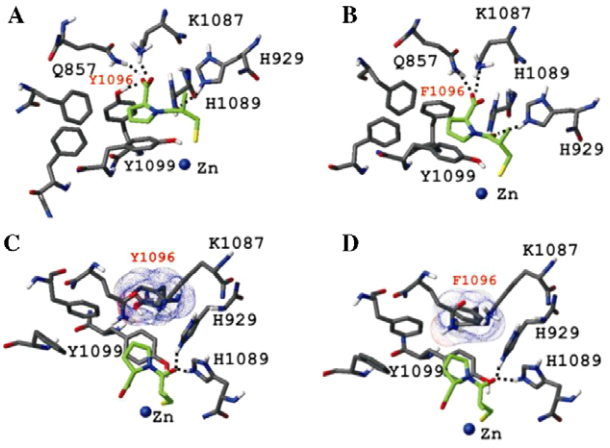

Figure 2. The binding of captopril *trans* conformation to the hACE (A) and Y1096F mutant (B). The binding of captopril *cis* conformation to the hACE (C) and Y1096F mutant (D).

Table 1. Apparent enthalpy ($\Delta H°$), entropy ($\Delta S°$), and free energy ($\Delta G°$) changes for the conversion of the *cis* to the *trans* isomer of captopril at 298 K

Solvent	pH	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$T\Delta S°$ (kJ mol^{-1})	$\Delta G°$ (kJmol^{-1})	R^2
D_2O	6.80	−1.87 ± 0.09	−3.76 ± 0.31	−1.12 ± 0.09	−0.75 ± 0.09	0.97
90% H_2O 10% D_2O	6.80	−2.04 ± 0.11	−5.63 ± 0.37	−1.68 ± 0.11	−0.36 ± 0.11	0.97
D_2O	2.15	−3.63 ± 0.28	1.76 ± 0.93	0.52 ± 0.28	−4.15 ± 0.28	0.93
90% H_2O 10% D_2O	2.15	−3.87 ± 0.47	0.47 ± 0.64	0.14 ± 0.19	−4.01 ± 0.47	0.98
DMSO-d_6	2.15	−4.25 ± 0.13	0.13 ± 0.40	0.04 ± 0.12	−4.29 ± 0.13	0.99

A grid flexible docking-calculation search has been performed, by mutating iteratively the enzyme carboxylate docking partners of captopril (since the rest of the molecule shares the same architectural topology), allowing to identify a case in which both isomers could have equal probability to be accommodated by the enzyme. Specifically, the Y1096F mutant resulted in comparable free energies of binding for both states of the inhibitor (~ -6.2 kcal/mol for *cis* and ~ -6.7 kcal/mol for *trans*). This can be rationalized by the fact that the carboxylate group of the inhibitor could be selectively 'locked' in the *trans* conformation through ionic interactions with the side chain of K1087 (Fig. 2B), whilst the polar to hydrophobic alteration accomplished in the Y1096F mutant, as also the elimination of the bulky hydroxyl group of Y1096, open access to a strong hydrophobic packing of the pyrrolidine ring of the inhibitor, in the *cis* form, with the side chains of F1096 and Y1099 (Fig. 2D). Interestingly, a similar intra-molecular aromatic (Tyr)–proline interaction has been recently suggested to stabilize the *cis* conformation of the Tyr–Pro amide bond,[18] whereas in the current case the aromatic residue is provided by the enzyme (Y1099).

In order to validate these findings, we mutated the hACE C-domain residues Y1096 and K1087 to Phe and Ala, respectively,[19] and detailed kinetic studies have been performed on these mutants. Indeed, Figure 3 shows that the Y1096F and K1087A mutations produced a decrease in captopril binding affinity ($1/K_i$), indicating experimentally their importance for captopril docking. However, we noticed an interesting phenomenon: both mutants behave completely differently in their inhibitor profile and, most importantly, data with captopril inhibition for Y1096F best fit using a two-site model, whereas data for wild-type hACE C-domain and the K1087A mutant fit on one-site model (Table 2).

Given these considerations, we could suggest that the observed equimolar ratio of the two states (*cis*, *trans*) of the inhibitor in solution is related to the two states observed in our kinetic studies for the Y1096F mutant that could accommodate both of them with the same probability in accordance with the docking calculations. Thus, since both states of the inhibitor could be accommodated by the modified enzyme, either the *cis* or the *trans* form of the inhibitor should be engineered and restrained achieving a more potent drug with complementary architecture to the native enzyme binding groove.

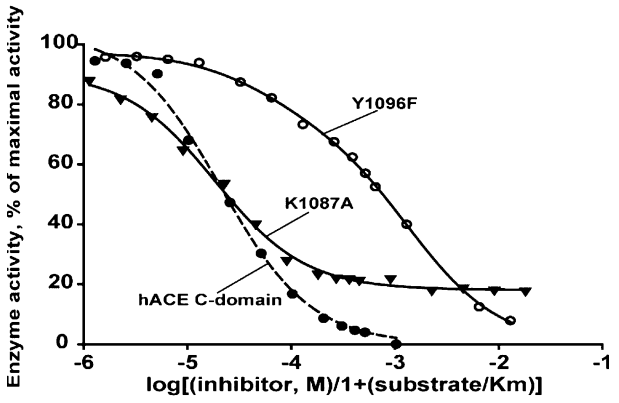

Figure 3. Inhibition of wild-type *h*ACE C-domain, Y1096F and K1087A mutants of *h*ACE C-domain by the ACE inhibitor captopril.

Table 2. Determination of kinetic constants for the hACE and its Y1096F and K1087A mutants

Enzyme	$\log EC_{50}$ value-1	$\log EC_{50}$ value-2
hACE C-domain	−4.6735	—
Y1096F	−2.8469	−4.2347
K1087A	−4.7319	—

In conclusion, the combination of pharmacophore-hypotheses and protein-structure based virtual screening is successful in developing lead compounds. However, as the present study revealed, many uncertainties remain about the details of the ligand–receptor interactions, possibly restraining the fine-tuning of the lead compound to a more potent drug. Specifically, our NMR studies on the ACE inhibitor captopril in solution were able to map the presence of equal population of the *cis* and *trans* conformational states at physiological pH. A flexible docking grid-search allowed the identification of a point mutation (Y1096F) of the hACE-C enzyme that could preferentially incorporate both states of the inhibitor with the same probability. This in silico finding has been independently validated through mutagenesis and enzymatic studies. The results of this combinatorial approach through NMR, flexible docking, mutagenesis, and enzymatic studies pinpoint the importance of the multiple conformational states of the inhibitor that should be taken into account in the frame of the drug design process so as to maximize the inhibitory affinity for the enzyme and minimize the side-effects of the interaction. The next step in this line of research will be the utilization of our approach toward the investigation of the current system in a drug-refinement effort.[9]

Acknowledgments

Dr. L. Elantak, Dr. N. Locker, and Dr. A. Troganis are gratefully acknowledged for suggestions and comments on the manuscript. AGT acknowledges an award from the Institute 'Leonidas Zervas'. This research was funded by the program 'Heraklitos' of the Operational Program for Education and Initial Vocational Training of the Hellenic Ministry of Education under the 3rd Community Support Framework and the European Social Fund. GRST PENED-99 Program (to A.G.T., I.P.G.), and EC Access to Research Infrastructures Action of the Improving Human Potential Programme (Contract No. HPRI-CT-2001-00147). A.G.T. and K.C. acknowledge support from the EC Marie Curie Programme (No. HPMT-CT-2000-00137).

Supplementary data

Computational methods, details of the enzymatic studies, and thermodynamic parameters determined by NMR. Supplementary data associated with this article

can be found in the online version at doi:10.1016/j.bmcl.2006.07.034.

References and notes

1. Soubrier, F.; Wei, L.; Hubert, C.; Clauser, E.; Alhenc-Gelas, F.; Corvol, P. *J. Hypertens.* **1993**, *11*, 599.
2. Soubrier, F.; Alhenc-Gelas, F.; Hubert, C.; Allegrini, J.; John, M.; Tregear, G.; Corvol, P. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9386.
3. Skirgello, O. E.; Binevski, P. V.; Pozdnev, V. F.; Kost, O. A. *Biochem. J.* **2005**, *391*, 641.
4. Binevski, P. V.; Sizova, E. A.; Pozdnev, V. F.; Kost, O. A. *FEBS Lett.* **2003**, *550*, 84.
5. Acharya, K. R.; Sturrock, E. D.; Riordan, J. F.; Ehlers, M. R. *Nat. Rev. Drug Discov.* **2003**, *2*, 891.
6. Menard, J.; Patchett, A. A. *Adv. Protein Chem.* **2001**, *56*, 13.
7. Candido, R.; Jandeleit-Dahm, K. A.; Cao, Z.; Nesteroff, S. P.; Burns, W. C.; Twigg, S. M.; Dilley, R. J.; Cooper, M. E.; Allen, T. J. *Circulation* **2002**, *106*, 246.
8. Tzakos, A. G.; Galanis, A. S.; Spyroulias, G. A.; Cordopatis, P.; Manessi-Zoupa, E.; Gerothanassis, I. P. *Protein Eng.* **2003**, *16*, 993.
9. Tzakos, A. G.; Gerothanassis, I. P. *Chembiochem* **2005**, *6*, 1089.
10. (a) Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Comput. Aided Mol. Des.* **1987**, *1*, 153; (b) Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, R. G. *J. Comput. Aided Mol. Des.* **1987**, *1*, 3.
11. Kuster, D. J.; Marshall, G. R. *J. Comput. Aided Mol. Des.* **2005**, *19*, 609.
12. Natesh, R.; Schwager, S. L.; Sturrock, E. D.; Acharya, K. R. *Nature* **2003**, *421*, 551.
13. Natesh, R.; Schwager, S. L.; Evans, H. R.; Sturrock, E. D.; Acharya, K. R. *Biochemistry* **2004**, *43*, 8718.
14. Kim, H. M.; Shin, D. R.; Yoo, O. J.; Lee, H.; Lee, J. O. *FEBS Lett.* **2003**, *538*, 65.
15. Fujinaga, M.; James, M. *Acta Crystallogr. B* **1980**, *36*, 3196.
16. Goodsell, D. S.; Morris, G. M.; Olson, A. J. *J. Mol. Recognit.* **1996**, *9*, 1.
17. Fernandez, M.; Liu, X.; Wouters, M. A.; Heyberger, S.; Husain, A. *J. Biol. Chem.* **2001**, *276*, 4998.
18. Taylor, C. M.; Hardre, R.; Edwards, P. J.; Park, J. H. *Org. Lett.* **2003**, *5*, 4413.
19. Naqvi, N.; Liu, K.; Graham, R. M.; Husain, A. *J. Biol. Chem.* **2005**, *280*, 6669.