Two-Layer ONIOM Calculation of Gas-Phase Acidities of Selected ACE Inhibitors

M. SMIEŠKO and M. REMKO

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, SK-832 32 Bratislava
e-mail: remko@fpharm.uniba.sk

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Ab initio density functional theory calculations were used to investigate gas-phase acidities of three selected ACE inhibitors expressed as enthalpy of deprotonation at 298.15 K ($\Delta H_{298\,\mathrm{K}}$). This study took advantage of the recently developed ONIOM method, which allowed to calculate properties of dissociating functional groups of ACE inhibitors with high accuracy with the Becke3LYP method with 6-311+G(d,p) basis set – the High layer, while the other atoms were included in the Low layer, for which the Hartree—Fock method with 3-21G* basis set was used. Structure and reactivity of captopril, perindoprilat, and omapatrilat and their respective ionic forms are discussed.

Angiotensin-converting enzyme inhibitors (ACE inhibitors) are nowadays well-known group of compounds used especially in treatment of essential hypertension, congestive heart failure, and diabetic nephropathy. These compounds interfere in the most important blood pressure regulating system of human body Renin—Angiotensin—Aldosterone System (RAAS) as competitive inhibitors of angiotensin-converting enzyme (ACE). After binding of an inhibitor into the active site of ACE, inactive natural substrate angiotensin I cannot be hydrolyzed to angiotensin II, which is the most potent vasoconstrictor known.

Peak plasma concentrations of inhibitors do not correlate with peak hypotensive effect and similarly hypotensive effect does not always follow plasma concentration of ACE. This is caused by the existence of local tissue form of the enzyme, which has probably major role in the long-term regulation, while inhibition of plasmatic ACE accounts for acute decrease in blood pressure. The concentration of an inhibitor in a particular tissue depends on the physicochemical characteristics of its molecule, *e.g.* molecular size, dissociation constant, lipophilicity, as well as the presence of blood-tissue barriers and the ability of the tissue to transform inactive pro-drugs into active form [1].

Most of the classic ACE inhibitors are typical pro-drugs. Presence of two acidic functional groups causes lowering of the gastrointestinal absorption. In order to achieve better absorption after oral administration, in the dosage form ACE inhibitor is present in esterified form, *i.e.* one of the acidic functional groups is esterified to ethyl ester, *e.g.* perindopril. Thus better lipid solubility is achieved as well. After absorption ester is hydrolyzed by nonspecific hydrolases to respective active form, *e.g.* perindoprilat in the liver, but may also occur in the gastrointestinal tract, extravascular tissue, and kidney. Active form then binds in the active site of ACE. Based on crystallographic data of similar enzymes [2, 3] and on the mutation study [4] the proposed binding mode for ACE inhibitors assumes that both N-terminal and C-terminal functional groups of inhibitor are ionized (Fig. 1).

Practically all clinically used ACE inhibitors contain the C-terminal carboxyl group. Ionized carboxyl group is thought to form an interaction with positively charged lysine or arginine residue in the active site (Fig. 1). Most ACE inhibitors contain another acidic functional group proximal to the N-terminus of the molecule. This functional group is very important because it forms monodentate or bidentate interaction with the bivalent zinc cation present in the active site. The potency of ACE inhibitors appears to be mainly determined by the strength of binding to zinc cation. The first potent ACE inhibitor captopril binds to the zinc cation with deprotonated thiol group. In the course of development of new ACE inhibitors most frequently the zinc-binding functional group was carboxyl group, *e.g.* perindoprilat, enalaprilat, trandolaprilat, but recently designed dual ACE/NEP inhibitors form bidentate interaction to zinc with deprotonated α-sulfanyl moiety, *e.g.* omapatrilat.

Acidity in solution is influenced by the solute—solvent interactions. As a result the acidity trends obtained in aqueous solution might be inverted with respect to those found in the gas phase, when small intramolecular effects, which are usually masked in so-

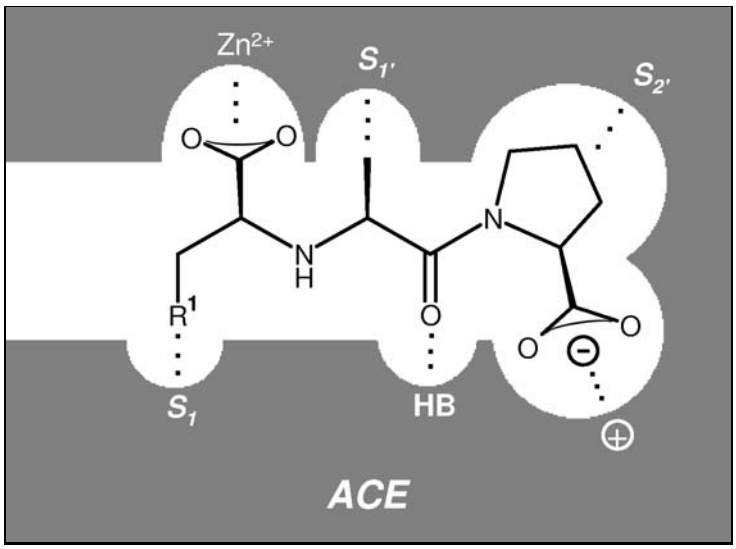


Fig. 1. Proposed binding mode of an ACE inhibitor in the active site of ACE. C-Terminal carboxyl forms interaction with positively charged residue (Lys or Arg) and N-terminal group binds (in this case another carboxyl group) to bivalent zinc ion in the active site.

lution, determine the gas-phase acidity of the drug [5, 6]. Thus, gas-phase acidities are important to understand how substituents affect the reactivity of a given molecule.

The objective of this study was to investigate the dissociation of functional groups of three typical members of large ACE inhibitor family. *Ab initio* density functional theory methods were used to characterize gas-phase geometries and acidities of these compounds expressed as the enthalpy and Gibbs energy of deprotonation at 298.15 K ($\Delta H_{298\,\mathrm{K}}$ and $\Delta G_{298\,\mathrm{K}}$).

COMPUTATIONAL DETAILS

The starting geometries of ACE inhibitors captopril, perindoprilat, and omapatrilat were modeled based on previous conformational studies [3, 7—9] and published crystallographic data [2, 10—12]. Starting geometries for anions were prepared by deleting the appropriate hydrogen atom(s). For every neutral inhibitor or its anions multiple conformations were calculated and examined, but only most stable conformations are discussed and used for calculation of thermodynamic parameters.

The geometries were completely optimized with the Gaussian 98 program [13], using the two-layer ONIOM method [14—16]. The ONIOM approach allows studying essential part(s) of the molecular systems in more detail, *i.e.* with high level of theory, while the other atoms are studied at computationally acceptable level. The model system and real molecule used for the two-layer ONIOM calculations are shown in Formula 1. The real systems consist of the entire inhibitor molecules and their respective anions. The model systems are acetic acid, thiomethanol, and (*S*)-α-sulfanylpropionamide. The two levels of theory used for energy calculations were density functional theory (DFT) [17] at the Becke3LYP level [18—20] in conjunction with a polarized triple split valence 6-311+G(d,p) basis set for the High layer and the Hartree—Fock level [21] with double zeta 3-21G* basis set for the Low layer.

The integrated energy for the two-layer ONIOM approach is defined as

$$\begin{aligned} &E(\mathrm{ONIOM2}) = \\ &= E(\mathrm{High,\ Model}) + E(\mathrm{Low,\ Real}) - E(\mathrm{Low,\ Model}) \\ &= E(\mathrm{High,\ Model}) + \Delta E(\mathrm{Low,\ Real} \leftarrow \mathrm{Model}) \qquad (1) \end{aligned}$$

The gas-phase acidity $\Delta E(\mathrm{A})$ was defined as the energy of deprotonation ΔE for the equation

$$\mathrm{AH(g)} \rightarrow \mathrm{A^-(g)} + \mathrm{H^+(g)} \qquad (A)$$

The energy of deprotonation ΔE, at $T = 0$ K, was computed using the equation

$$\Delta E = E(\mathrm{A^-}) - E(\mathrm{AH}) \qquad (2)$$

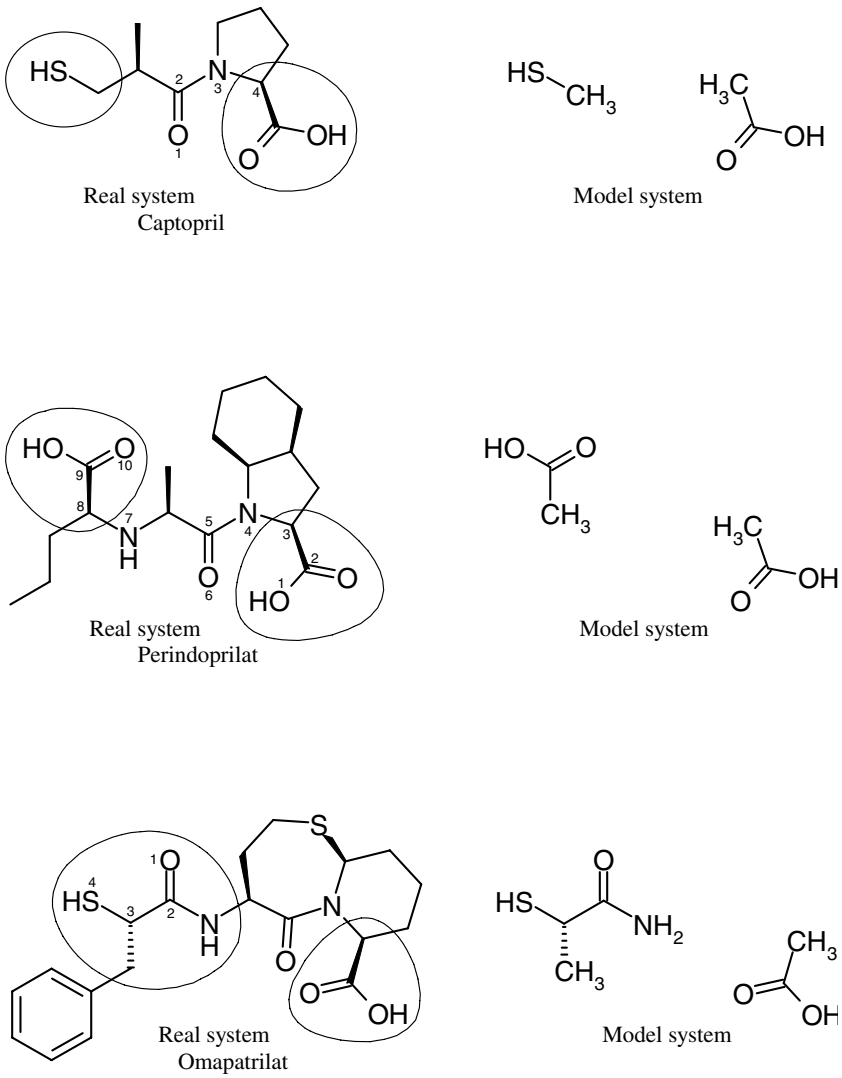
where E stands for the total energies of the stable conformations of the acid and its anion. The values of ONIOM extrapolated energies (E_{ONIOM}) were used.

The enthalpy of deprotonation, $\Delta H_{298\,\mathrm{K}}$, was computed using the equations

$$\Delta H_{298\,\mathrm{K}} = \Delta E_{298\,\mathrm{K}} + \Delta(pV) \qquad (3)$$

$$\Delta E_{298\,\mathrm{K}} = [E_{298\,\mathrm{K}}(\mathrm{A^-}) + 3/2RT] - E_{298\,\mathrm{K}}(\mathrm{AH}) \qquad (4)$$

where $E_{298\,\mathrm{K}}$ stands for the total energies of the stable conformations of the acids and their anions (including the thermal energy correction at $T = 298.15$ K). In eqn (*3*) we substituted $\Delta(pV) = RT$ (one mol of gas



Formula 1. Real and model systems and atom numbering for every inhibitor studied.

is obtained in the reaction (*A*)). The gas-phase Gibbs energy, $\Delta G_{298\,\mathrm{K}}$, of the proton abstraction reaction may be calculated from

$$\Delta G_{298\,\mathrm{K}} = \Delta H_{298\,\mathrm{K}} - T\Delta S_{298\,\mathrm{K}} \qquad (5)$$

The enthalpy of deprotonation was calculated using eqn (*3*). The entropy contribution is given by

$$-T\Delta S_{298\,\mathrm{K}} = -T[S(\mathrm{A}^-) + S(\mathrm{H}^+) - S(\mathrm{AH})] \qquad (6)$$

For $T = 298.15$ K at the standard pressure (101 325 Pa), the second term $TS(\mathrm{H}^+) = 32.5$ kJ mol^{-1} [22]. Thus,

$$\Delta G_{298\,\mathrm{K}} = \Delta H_{298\,\mathrm{K}} - T[S(\mathrm{A}^-) - S(\mathrm{AH})] - 32.5 \qquad (7)$$

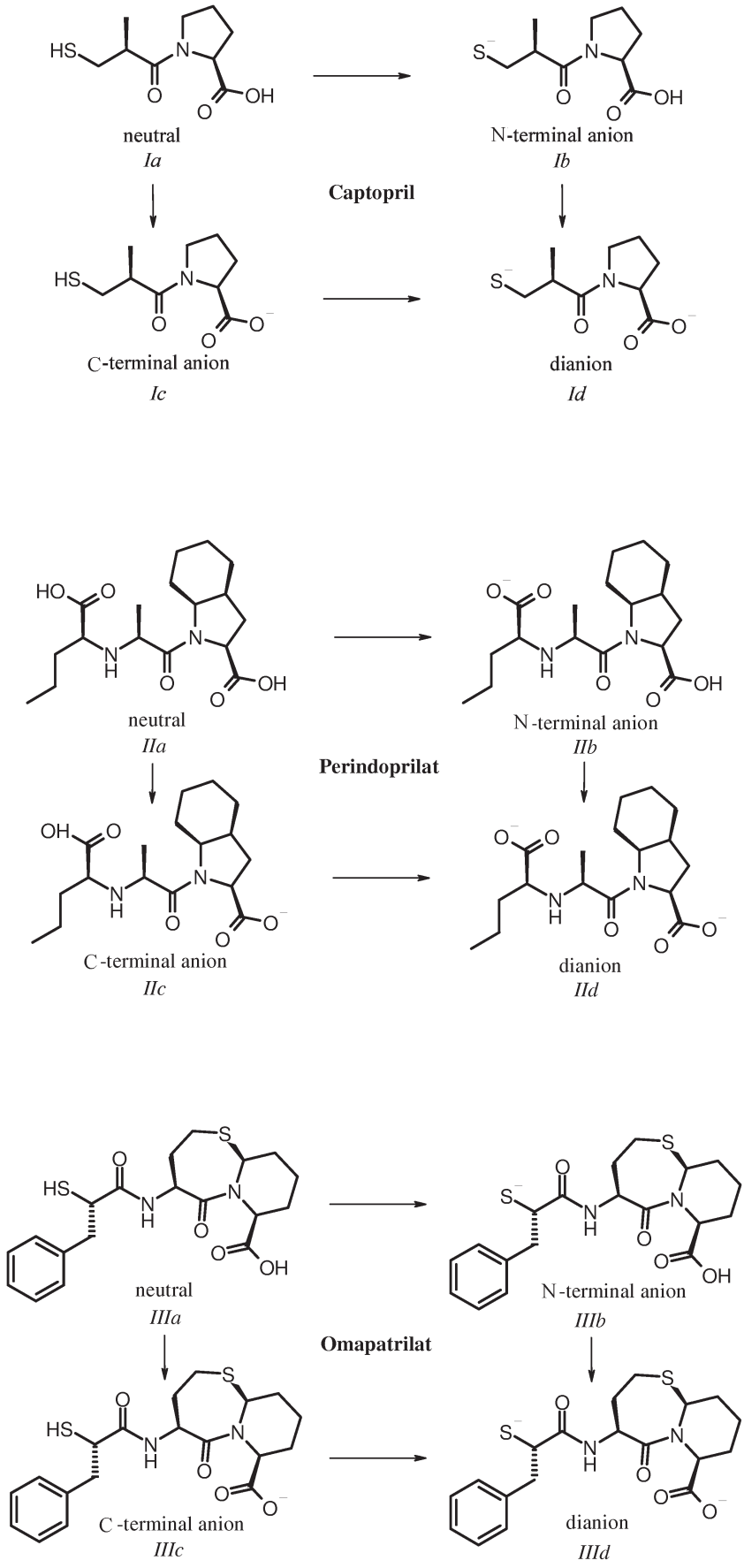
Notice that there is an inverse relationship between the magnitude of ΔG and the strength of the acid. The more positive the value of the ΔG, the weaker the acid. It has been shown that the integrated MO approach, ONIOM, provides an ideal method for accurate calculations for large systems. For such molecules accurate calculations are often too expensive and out of reach [23—25]. Recent calculations with the ONIOM2 method have indicated that the B3LYP/DZP:HF/3-21G level of treatment can provide data in excellent agreement with the results computed at the full B3LYP/DZP level at a fraction of computational cost [26].

The detailed scheme of dissociation pathways of studied ACE inhibitors is in Scheme 1, where neutral molecules as well as ionic species are described.

RESULTS AND DISCUSSION

Conformational Changes

Lowest-energy conformations are visualized in Fig. 2 in stick representation. For clarity hydrogen atoms, except those of dissociating functional groups, are not displayed. At optimized geometries harmonic



Scheme 1. The dissociation pathways for ACE inhibitors studied.

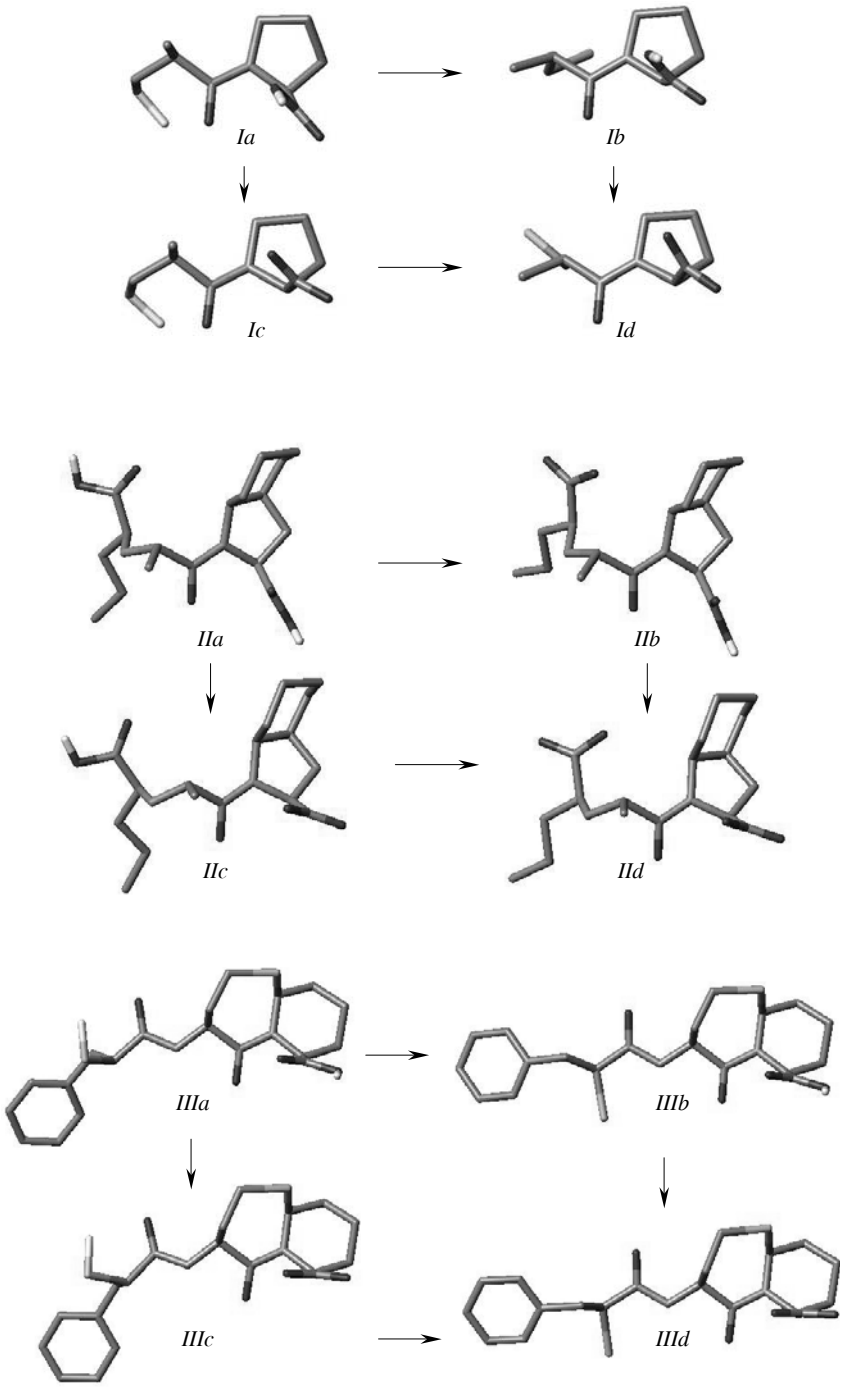


Fig. 2. Optimized structures of studied ACE inhibitors. Arrows show dissociation pathways corresponding to Scheme 1.

vibrational frequency analysis was performed. No imaginary frequencies were found, *i.e.* the reported structures represent gas-phase minima.

The molecule of captopril undergoes major conformational changes during the process of deprotonation, especially in the surfanylacyl fragment. In the gas phase both *Ia* and *Ic* are stabilized by intramolecular hydrogen bond between thiol hydrogen and carbonyl oxygen ($r_{H\cdots O}$ = 0.261 nm, $\alpha_{S\text{-}H\cdots O}$ = 114.6° in *Ia*; $r_{H\cdots O}$ = 0.249 nm, $\alpha_{S\text{-}H\cdots O}$ = 118.7° in *Ic*). After deprotonation of the thiol group, the repulsion between negatively charged carbonyl oxygen and sulfur atoms causes the rearrangement of the sulfanylacyl fragment and the distortion of planarity of the peptide bond of *Ib* ($\phi_{O1\text{-}C2\text{-}N3\text{-}C4}$ = 10.1°). The deprotonation of C-terminal carboxyl causes only smaller conformational changes as it is connected to the rigid five-membered ring system. The dihedral angle of the peptide bond is slightly distorted from ideal planarity ($\phi_{O1\text{-}C2\text{-}N3\text{-}C4}$ = −4.6° in *Ia vs.* $\phi_{O1\text{-}C2\text{-}N3\text{-}C4}$ = −25.3° in *Ic*), when the negative charge on C-terminal carboxyl causes the repulsion from the carbonyl oxygen. The conforma-

Table 1. Gas-Phase Energies $E_{298\,K}$ of Studied ACE Inhibitors at $T = 298.15$ K

Molecule	$E_{298\,K}$/hartree		
	Captopril	Perindoprilat	Omapatrilat
Neutral	−1 026.271 222	−1 141.753 404	−1 934.564 803
C-Terminal anion	−1 025.748 485	−1 141.226 208	−1 934.048 811
N-Terminal anion	−1 025.722 417	−1 141.236 061	−1 934.028 164
Dianion	−1 025.107 928	−1 140.622 409	−1 933.438 052

Table 2. Gas-Phase Acidities $\Delta H_{298\,K}$ and Gibbs Energies $\Delta G_{298\,K}$ of Studied ACE Inhibitors at $T = 298.15$ K

Dissociation pathway	Captopril		Perindoprilat		Omapatrilat	
	$\Delta H_{298\,K}$	$\Delta G_{298\,K}$	$\Delta H_{298\,K}$	$\Delta G_{298\,K}$	$\Delta H_{298\,K}$	$\Delta G_{298\,K}$
	kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}
Neutral → C-term. anion	1 378.6	1 352.4	1 390.4	1 361.1	1 360.9	1 330.4
Neutral → N-term. anion	1 447.1	1 415.9	1 364.5	1 332.3	1 415.1	1 383.6
C-Term. anion → dianion	1 688.0	1 654.2	1 591.5	1 558.9	1 609.7	1 577.1
N-Term. anion → dianion	1 619.5	1 590.7	1 617.3	1 587.7	1 555.5	1 523.9

tion of *Id* represents the most favourable spatial arrangement with respect to the repulsion of negatively charged groups and atoms. In *Id* the dihedral angle of peptide bond was found almost planar ($\phi_{O1\text{-}C2\text{-}N3\text{-}C4} = -1.7°$), probably because the presence of negative charges at opposite ends of the molecule equalizes mutual repulsion of both C-terminal carboxyl and deprotonated thiol group from carbonyl oxygen.

The molecule of perindoprilat undergoes only minor conformational changes. Here again due to deprotonation of C-terminal group and repulsion from carbonyl oxygen, the dihedral angle of the peptide bond is slightly distorted from ideal planarity ($\phi_{O6\text{-}C5\text{-}N4\text{-}C3} = -3.7°$ in *IIa vs.* $\phi_{O6\text{-}C5\text{-}N4\text{-}C3} = -24.1°$ in *IIc* and $\phi_{O6\text{-}C5\text{-}N4\text{-}C3} = -19.2°$ in *IId*) and the carboxyl group alters its position by axial rotation ($\phi_{O1\text{-}C2\text{-}C3\text{-}N4} = 151.9°$ in *IIa*; $\phi_{O1\text{-}C2\text{-}C3\text{-}N4} = -172.9°$ in *IIc*, $\phi_{O1\text{-}C2\text{-}C3\text{-}N4} = -173.2°$ in *IId*). Minor conformational changes are also observed at the position of N-terminal carboxyl, which after deprotonation rotates around the axis C8—C9 ($\phi_{N7\text{-}C8\text{-}C9\text{-}O10} = 78.8°$ in *IIa vs.* $\phi_{N7\text{-}C8\text{-}C9\text{-}O10} = 56.4°$ in *IIb*, $\phi_{N7\text{-}C8\text{-}C9\text{-}O10} = 64.1°$ in *IId*).

Due to the presence of constrained bicyclic system in the C-terminal part of molecule, omapatrilat significantly changes its shape after deprotonation of N-terminal α-sulfanylacyl group only. In the gas phase *IIIa* and *IIIc* are stabilized by intramolecular hydrogen bond between α-thiol hydrogen and neighbouring carbonyl oxygen ($r_{H\cdots O} = 0.268$ nm, $\alpha_{S\text{-}H\cdots O} = 103.1°$ in *IIIa*; $r_{H\cdots O} = 0.242$ nm, $\alpha_{S\text{-}H\cdots O} = 109.7°$ in *IIIc*). After deprotonation of the thiol group, the repulsion between negatively charged carbonyl oxygen and sulfur atoms causes the rotation of the whole sulfanylacyl fragment to the *trans* arrangement around the bond C2—C3 ($\phi_{O1\text{-}C2\text{-}C3\text{-}S4} = -179.3°$ in *IIIb* and $\phi_{O1\text{-}C2\text{-}C3\text{-}S4} = -161.2°$ in *IIId*) and thus negatively charged sulfur neighbours the hydrogen atom of amide group. However, X-ray crystallography proved that α-sulfanylacyl group forms bidentate interaction with the zinc cation in the active site – carbonyl oxygen and sulfur are in the slightly distorted *cis* arrangement so that they can bind to zinc cation. This raises a question whether omapatrilat enters the ACE active site with protonated α-sulfanylacyl group, *i.e.* by H-bonding stabilized *cis* conformation similar to that of bound inhibitor in the active site or whether omapatrilat rearranges the conformation at this part of *IIId* from *trans* to *cis* upon entering to the active site.

Gas-Phase Deprotonation Enthalpies and Gibbs Energies

The ONIOM calculated gas-phase energies at 298.15 K ($E_{298\,K}$) of neutral inhibitors and their respective ionic species are summarized in Table 1. The calculated gas-phase acidities at 298.15 K (deprotonation enthalpies $\Delta H_{298\,K}$ and Gibbs energies $\Delta G_{298\,K}$) for both the first and the second ionization are summarized in Table 2.

The only published experimental data concerning dissociation of ACE inhibitors are pK_a values of captopril, lisinopril, and enalapril. The pK_a values of nonpro-drug inhibitor captopril are 3.7 for C-terminal carboxyl and 9.8 for the thiol group (apparently, S^- is protonated at pH = 7) [27]. Lisinopril, another nonpro-drug inhibitor, contains two acidic and two basic functional groups. The pK_a values are assigned as follows: N-terminal carboxyl p$K_a = 2.5$ and C-terminal carboxyl p$K_a = 4.0$. The pK_a value for

enalapril (N-terminal ethyl ester of enalaprilat) is 3.0 for C-terminal carboxyl [28, 29].

Theoretical pK_a values calculated with software ACD/pK_a DB V3.5 [30] are as follows: pK_a = 2.2 ± 0.1 for N-terminal and pK_a = 3.8 ± 0.4 for C-terminal carboxyl of lisinopril; pK_a = 3.7 ± 0.4 for C-terminal carboxyl of perindopril, pK_a = 3.8 ± 0.4 for C-terminal carboxyl of enalapril.

Assuming that perindoprilat and lisinopril are very similar molecules (diacids, common pharmacophore, similar calculated pK_a values) and that there is an inverse relationship between the magnitude of ΔG and the strength of the acid, then the results of this study are consistent with the experimental data. In the case of perindoprilat both the $\Delta H_{298\,K}$ and $\Delta G_{298\,K}$ of deprotonation of N-terminal carboxyl group are by 25.9 kJ mol^{-1} and 28.8 kJ mol^{-1} lower than those of C-terminal carboxyl indicating that N-terminal carboxyl is more acidic than C-terminal carboxyl. This is in agreement with experimental and theoretical data for lisinopril, when N-terminal carboxyl is more acidic (exp. pK_a = 2.5, theor. pK_a = 2.2) than C-terminal carboxyl (exp. pK_a = 4.0, theor. pK_a = 3.8). That also explains why in all pro-drug ACE inhibitors N-terminal carboxyl is "shielded" with ethyl group – respective ethyl ester represents less-dissociating and more lipophilic pro-drug form of inhibitor, which is then better absorbed in the gastrointestinal tract. Increased acidity of N-terminal carboxyl is caused by proximity of secondary amino group.

In the case of captopril the results show that gas-phase $\Delta H_{298\,K}$ and $\Delta G_{298\,K}$ of deprotonation for C-terminal carboxyl are by 11.8 kJ mol^{-1} and 8.7 kJ mol^{-1} lower than those of perindoprilat. This means that C-terminal carboxyl of captopril is only slightly more acidic than the respective group of perindoprilat. This is in agreement with the experiment as published experimental pK_a value for C-terminal carboxyl is 3.7 for captopril and 4.0 for lisinopril, the pK_a of which could be applicable to perindoprilat (theor. pK_a = 3.7 ± 0.4). The gas-phase $\Delta H_{298\,K}$ and $\Delta G_{298\,K}$ of deprotonation for thiol group are by 68.5 kJ mol^{-1} and 63.5 kJ mol^{-1} higher than those of C-terminal carboxyl, which indicates that pK_a value for thiol group is much higher than pK_a value for carboxyl. This is in agreement with the published pK_a value for thiol group of captopril (pK_a = 9.8).

Neither experimental nor theoretical pK_a values were published for omapatrilat. The gas-phase $\Delta H_{298\,K}$ and $\Delta G_{298\,K}$ of deprotonation for C-terminal carboxyl of omapatrilat are by 17.7 kJ mol^{-1} and 22.0 kJ mol^{-1} lower than those of captopril. This means that C-terminal carboxyl of omapatrilat is even more acidic than the respective group of captopril (pK_a = 3.7). This finding is in agreement with the published theoretical pK_a = 3.3 ± 0.4 for cilazapril, an ACE inhibitor that has similar constrained C-terminal bicyclic system as omapatrilat and its pK_a might be similar to pK_a for C-terminal carboxyl of omapatrilat. The gas-phase $\Delta H_{298\,K}$ and $\Delta G_{298\,K}$ of deprotonation for N-terminal α-sulfanylacyl group of omapatrilat are by 32.0 kJ mol^{-1} and 32.3 kJ mol^{-1} lower than those of captopril. This functional group is more easily deprotonated than thiol group of captopril, probably because neighbouring amide hydrogen compensates the negative charge on sulfur atom.

CONCLUSION

The molecules of captopril and omapatrilat undergo significant conformational changes during the deprotonation, especially in the sulfanylacyl fragment. In the neutral molecule of both inhibitors thiol group forms intramolecular hydrogen bond with carbonyl oxygen, but deprotonation of thiol group causes strong repulsion between carbonyl oxygen and sulfur atom, which results in conformational changes. Perindoprilat undergoes only minor conformational changes upon the deprotonation.

The general trends in gas-phase enthalpies and Gibbs energies of deprotonation of three ACE inhibitors studied correlate with their experimental and theoretical pK_a values. The results of this study confirmed the rationale of shielding of the most acidic N-terminal carboxyl of diacid ACE inhibitors by hydrolyzable ethyl ester. The most easily dissociating acidic functional group of three ACE inhibitors studied is C-terminal carboxyl of omapatrilat with gas-phase deprotonation $\Delta G_{298\,K}$ = 1330.4 kJ mol^{-1}. The least acidic functional group is thiol group of captopril with gas-phase deprotonation $\Delta G_{298\,K}$ = 1654.2 kJ mol^{-1}.

In this study the two-layer ONIOM(B3LYP/6-311+G(d,p):HF/3-21G*) method has been proven to produce very good results with fair computational costs and thus it is suitable for studying large systems, which can be divided into logical subsystems.

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REFERENCES

1. Opie, L. H., *Angiotensin Converting Enzyme Inhibitors*, 2nd Edition. Wiley—Liss, New York, 1994.
2. Gaucher, J. F., Selkti, M., Tiraboschi, G., Prangé, T., Roques, B. P., Tomas, A., and Fournié-Zaluski, M. C., *Biochemistry 38*, 12569 (1999).
3. Bohacek, R., De Lombaert, S., McMartin, C., Priestle, J., and Grutter, M., *J. Am. Chem. Soc. 118,* 8231 (1996).
4. Fernandez, M., Liu, X., Wouters, M. A., Heyberger, S., and Husain, A., *J. Biol. Chem. 276,* 4998 (2001).
5. Aue, D. H., Webb, H. M., and Bowers, M. T., *J. Am. Chem. Soc. 98*, 318 (1976).
6. Tunon, I., Silla, E., and Pascual-Ahuir, J. L., *J. Am. Chem. Soc. 115*, 2226 (1993).

7. Andrews, P. R., Carson, J. M., Caselli, A., Spark, M. J., and Woods, R., *J. Med. Chem. 28*, 393 (1985).
8. Mayer, D., Naylor, Ch. B., Motoc, I., and Marshall, G. R., *J. Comput.-Aided Mol. Des. 1*, 3 (1987).
9. Smieško, M. and Remko, M., *Chem. Pap. 56,* 138 (2002).
10. Hausin, R. J. and Codding, P. W., *J. Med. Chem. 33*, 1940 (1990).
11. Hausin, R. J. and Codding, P. W., *J. Med. Chem. 34*, 511 (1991).
12. Pascard, C., Guilhem, J., Vincent, M., Rémond, G., Portevin, B., and Laubie, M., *J. Med. Chem. 34*, 663 (1991).
13. *Gaussian 98* (Revision A.7), Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Zakrzewski, V. G., Montgomery, Jr., J. A., Stratmann, R. E., Burant, J. C., Dapprich, S., Millam, J. M., Daniels, A. D., Kudin, K. N., Strain, M. C., Farkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, J., Petersson, G. A., Ayala, P. Y., Cui, Q., Morokuma, K., Malick, D. K., Rabuck, A. D., Raghavachari, K., Foresman, J. B., Cioslowski, J., Ortiz, J. V., Baboul, A. G., Stefanov, B. B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Gomperts, R., Martin, R. L., Fox, D. J., Keith, T., Al-Laham, M. A., Peng, C. Y., Nanayakkara, A., Gonzalez, C., Challacombe, M., Gill, P. M. W., Johnson, B., Chen, W., Wong, M. W., Andres, J. L., Gonzalez, C., Head-Gordon, M., Replogle, E. S., and Pople, J. A., Gaussian, Pittsburgh, PA, 1998.
14. Svensson, M., Humbel, S., Froese, R. D. J., Matsubara, T., Sieber S., and Morokuma, K., *J. Phys. Chem. 100,* 19357 (1996).
15. Humbel, S., Sieber S., and Morokuma, K., *J. Chem. Phys. 105,* 1959 (1996).
16. Froese, R. D. J. and Morokuma, K., in *Encyclopedia of Computational Chemistry.* (Schleyer, P. v. R., Editor.) P. 1224. Wiley, New York, 1998.
17. Parr, R. G. and Yang, W., *Density-Functional Theory of Atoms and Molecules*. Oxford University Press, Oxford, 1989.
18. Becke, A. D., *Phys. Rev. A38,* 3098 (1988).
19. Becke, A. D., *J. Chem. Phys. 98,* 3098 (1993).
20. Lee, C., Yang W., and Parr, R. G., *Phys. Rev. B37*, 785 (1988).
21. Hehre, W. J., Radom, L., Schleyer, P. v. R., and Pople, J. A., *Ab initio Molecular Orbital Theory.* Wiley, New York, 1986.
22. Alberty, R. A. and Silbey, R. J., *Physical Chemistry*, 2nd Edition. P. 878, Wiley, New York, 1997.
23. Remko, M., Lyne, P. D., and Richards, W. G., *Phys. Chem. Chem. Phys. 2*, 2511 (2000).
24. Remko, M., Walsh, O. A., and Richards, W. G., *J. Phys. Chem. A105*, 6926 (2001).
25. Remko, M., *J. Phys. Chem. A106*, 5005 (2002).
26. Roggero, I., Civalleri, B., and Ugliengo, P., *Chem. Phys. Lett. 341*, 625 (2001).
27. http://www.micromedex.com/products/demos/webready/Professional/Usp_DI/data_001.html
28. Qin, X. Z., Ip, D. P., and Tsai, E. W., *J. Chromatogr. 626*, 251 (1992).
29. Qin, X. Z., Nguyen, D. S. T., and Ip, D. P., *J. Liq. Chromatogr. 16*, 3713 (1993).
30. ACD/pKa DB V3.5 for Windows, Advanced Chemistry Development, Toronto, Canada.