Inorg. Chem. **2008**, *47*, 6569−6571

Heme Peroxidase-Catalyzed Iodination of Human Angiotensins and the Effect of Iodination on Angiotensin Converting Enzyme Activity

Bhaskar J. Bhuyan and Govindasamy Mugesh*

Department of Inorganic & Physical Chemistry, Indian Institute of Science, Bangalore 560 012, India

Received March 3, 2008

The heme peroxidase-catalyzed iodination of human angiotensins I and II is described. It is observed that lactoperoxidase (LPO) can effectively and selectively iodinate the tyrosyl residues in angiotensin peptides. The thiourea/thiouracil-based peroxidase inhibitors effectively inhibit the iodination reactions, indicating that iodination is an enzymatic reaction and the mechanism of iodination is similar to that of peroxidase-catalyzed iodination of thyroglobulin. This study also shows that the monoiodo Ang I is a better substrate for the angiotensin converting enzyme than the native peptide.

Angiotensin II (Ang II), an octapeptide and the active pressor agent in the renin−angiotensin system,[1] is produced in vivo from the decapeptide angiotensin I (Ang I) by cleavage of the C-terminal dipeptide His-Leu. The conversion of Ang I to Ang II is catalyzed by the zinc-containing angiotensin converting enzyme (ACE).[2] It is known that radioactive monoiodinated Ang II (^{125}I-Ang II) can be used to characterize Ang II receptors in the central nervous system, vasculature, adrenal, and other tissues.[3] It has been demonstrated that specific, high-affinity I-Ang sites are present in bovine aortic endothelial cells and canine coronary artery endothelium.[4]

Speth et al. have shown that monoiodo-[Ile^5]Ang II is more active than the natural peptide [Ile^5]Ang II in increasing the blood pressure.[5] They have also shown that the monoiodo-Ang II is a potent, full agonist analogue of Ang II and a more potent pressor agent than the native Ang II.[6] This leads to an assumption that Ang II and related peptides would increase the blood pressure upon iodination. However, very little is known about the site of iodination in angiotensin peptides and the effect of such iodination on the ACE activity. Although heme peroxidases such as lactoperoxidase (LPO) can halogenate many amino acid residues in proteins and peptides,[7] the peroxidase-catalyzed halogenation of angiotensins and the effect of peroxidase inhibitors on the halogenation reactions have not been studied in detail. In this paper, we describe that LPO-catalyzed iodination of Ang I and Ang II produces the corresponding mono- and diiodo peptides. We also describe, for the first time, that monoiodo-Ang I is a better substrate for ACE than the native peptide.

Iodination of Ang I and Ang II was studied by using LPO/H_2O_2/I^-, and the initial rates for the reactions were determined by a high-performance liquid chromatography (HPLC) method. The isolation and characterization of the products confirm that iodination takes place at the tyrosyl residues. Iodination of Ang I and Ang II by the LPO system initially produces the corresponding monoiodo peptides, which undergo further iodination to produce the diiodo compounds in the presence of excess H_2O_2 and I^-. However, only the formation of monoiodo-Ang I and -Ang II was observed during the initial period even at higher concentrations of H_2O_2 and I^-. The diiodo peptides were detected only when ∼80% of Ang I and Ang II have been converted to the corresponding monoiodo species. This indicates that monoiodo-Ang I and -Ang II would have more physiological significance than the diiodinated peptides.

A detailed kinetic study on LPO-catalyzed monoiodination suggests that the catalytic efficiency (η) for the iodination

* To whom correspondence should be addressed. E-mail: mugesh@ipc.iisc.ernet.in.

(1) (a) Skeggs, L. T.; Doven, F. E.; Levins, M.; Lentz, K.; Kahn, J. R. The biochemistry of renin−angiotensin system. In *The Renin-Angiotensin System*; Johnson, J. A., Anderson, R. R. Eds.; Plenum Press: New York, 1980; pp 1−27. (b) Matsusaka, T.; Ichikawa, I. *Annu. Rev. Physiol.* **1997**, *59*, 395–412.

(2) (a) Natesh, R.; Schwager, S. L. U.; Sturrock, E. D.; Acharya, K. R. *Nature* **2003**, *421*, 551–554. (b) Sturrock, E. D.; Natesh, R.; Rooyen, J. M. V.; Acharya, K. R. *Cell. Mol. Life Sci.* **2004**, *61*, 2677–2686, and references cited therein.

(3) (a) Kurcbart, H.; Coll, A.; Vancheri, L.; Rosa, U. *Biochim. Biophys. Acta* **1971**, *230*, 160–169. (b) Tsomides, T. J.; Eisen, H. N. *Anal. Biochem.* **1993**, *210*, 129–135. (c) Loot, A. E.; Buiten, A. V.; Roks, A. J. M.; Henning, R. H. *J. Pharmacol. Toxicol. Methods* **2005**, *51*, 51–55.

(4) (a) Tallant, E. A.; Lu, X.; Weiss, R. B.; Chappell, M. C.; Ferrario, C. M. *Hypertension* **1997**, *29*, 388–393. (b) Ferrario, C. M.; Chappell, M. C.; Tallant, E. A.; Brosnihan, K. B.; Diz, D. I. *Hypertension* **1997**, *30*, 535–541. (c) Handa, R. K. *J. Am. Soc. Nephrol.* **2000**, *11*, 1377–1386.

(5) Speth, R. C.; Pajka, S.; Taylor, S. M.; Hussain, A. *Fed. Proc.* **1985**, *44*, 1240.

(6) Husain, A.; Pajka, S. F.; Taylor, S. M.; Speth, R. C. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 71–77.

(7) (a) Morrison, M.; Schonbaum, G. R. *Annu. Rev. Biochem.* **1976**, *45*, 861–888. (b) Taurog, A.; Dorris, M. L.; Doerge, D. R. *Arch. Biochem. Biophys.* **1996**, *330*, 24–32. (c) Roy, G.; Mugesh, G. *J. Am. Chem. Soc.* **2005**, *127*, 15207–15217.

10.1021/ic800395k CCC: $40.75
Published on Web 07/04/2008

of Ang I is comparable with that of Ang II, indicating that the tyrosyl residues are the favored targets for iodination in both Ang I and Ang II. This is in agreement with the report of Huber et al. that free tyrosine can be iodinated at the same rate as some tyrosine-containing peptides.[8] Although other amino acids such as histidine can be iodinated,[7a,9] we have observed that LPO does not catalyze the iodination of the histidine residue present in Ang I and Ang II. This is probably due to the low intrinsic reactivity of the imidazole group present in histidine as compared with the phenolic group in tyrosine.

Iodination of Ang was not observed in the absence of LPO, H_2O_2, or I^-, indicating that iodination is clearly an enzymatic reaction. Obinger et al. have shown that the peroxidase-catalyzed reactions are complicated and the human peroxidase may perform a halogenation cycle and/or peroxidase cycle or they may act as poor (pseudo)catalases depending on the availability of the substrate.[10] Furthermore, the crystal structure of LPO indicates that the access channel to the distal heme cavity is relatively narrow and the inner surface of the channel contains hydrophobic residues.[11] In contrast to free tyrosine and small peptides, access of large peptides such as Ang I and Ang II to the active site of LPO would be difficult. Therefore, iodination of Ang I and Ang II may take place at the surface of the enzyme and not at the distal heme cavity. This is in agreement with the report of Huber et al. that the reaction of I^- with H_2O_2 at the active site of LPO produces a highly reactive intermediate, which diffuses from the enzyme and performs iodination outside the active site cavity.[8]

The rate of iodination in Ang I and Ang II increases linearly with the concentration of LPO in the range of 2−12 nM (Figure S10, Supporting Information). In contrast, an increase in the iodide concentration above ~30 μM leads to an inhibition of the iodination of both Ang I and Ang II (Figure 1). This is due to the oxidation of I^- to I_2 by the LPO/H_2O_2 system, as was previously postulated for thyroid peroxidase (TPO) and LPO.[12] The reaction of I_2 with I^- produces I_3^-, which is probably responsible for the inhibition of LPO. It has been shown that the partial transformation of I_2 to I_3^- occurs spontaneously in the presence of excess I^-.[13] The crystal structure of a triiodide-bound heme peroxidase from *Arthromyces ramosus* indicates that I_3^- binds to the heme at the distal side.[14] In the case of peroxide variation, there was no significant inhibition at high H_2O_2 concentrations (Figure S12, Supporting Information). It is known that

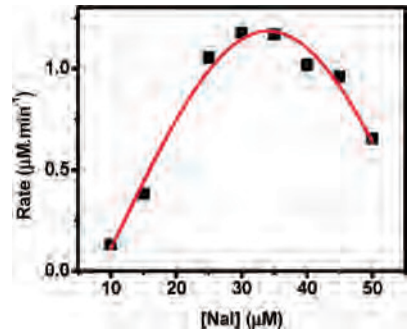

Figure 1. Effect of the iodide concentration on LPO-catalyzed iodination of Ang II. The concentrations of Ang II, hydrogen peroxide, and LPO were 20 μM, 40 μM, and 10 nM, respectively. All of the experiments were carried out in a sodium phosphate buffer (50 mM), pH 7.1 at 25 °C.

Table 1. Kinetic Parameters for Iodination of Ang I and Ang II by the LPO/H_2O_2/I^- System[a]

	V_{max} (μM·min^{-1})	K_M (mM)	k_{cat} (min^{-1})	η ($\times 10^6$ M^{-1}·min^{-1})
Ang I	6.61 ±0.30	0.35 ±0.02	661.4 ±30.1	1.92 ±0.02
Ang II	2.70 ±0.26	0.09 ±0.01	269.5 ±26.2	2.92 ±0.10

[a] Assay condition: The experiments were carried out in a phosphate buffer (50 mM, pH 7.1) at 25 °C. LPO: 10 nM. Ang I: 20 μM. Ang II: 20 μM. H_2O_2: 40 μM. Iodide: 20 μM.

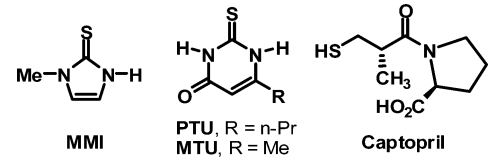

Figure 2. Chemical structures of MMI, PTU, MTU, and captopril.

a large excess of H_2O_2 can convert TPO and LPO to less active or inactive forms.[15]

Obinger et al. have shown that the reaction of the LPO Compound I with I^- is extremely fast.[16] Dorris et al. have shown that peroxidase inhibitors such as methimazole (MMI) and 5-*n*-propyl-2-thiouracil (PTU) inhibit the TPO- and LPO-catalyzed reactions by reacting with Compound I.[12] Therefore, we studied the effect of MMI, PTU, and a related compound, 5-methyl-2-thiouracil (MTU; Figure 2), on iodination of angiotensins catalyzed by LPO. The initial rates for the conversion of Ang II to monoiodo-Ang II were determined by a HPLC method. Because the formation of diiodo-Ang II was also observed in the reaction, the initial 5−10% of the conversion was followed where the formation of the diiodo compound was not observed.

The inhibitory plots show that MMI strongly inhibits iodination with an IC_{50} value of 2.74 ± 0.35 μM. The thiouracil-based antithyroid agents PTU and MTU also show strong inhibition with IC_{50} values of 8.85 ± 0.40 and 5.76 ± 0.51 μM, respectively. Interestingly, the antihypertensive drug and ACE inhibitor, captopril, inhibits iodination and the IC_{50} value of 9.51 ± 0.35 μM observed for this compound is found to be comparable with that of PTU (Figure 3). The common feature observed in MMI, PTU, and MTU is that

(8) Huber, R. E.; Edwards, L. A.; Carne, T. J. *J. Biol. Chem.* **1989**, *264*, 1381–1386.
(9) (a) Wolff, J.; Covelli, I. *Eur. J. Biochem.* **1969**, *9*, 371–377. (b) Krohn, K. A.; Knight, L. C.; Harwig, J. F.; Welch, M. J. *Biochim. Biophys. Acta* **1977**, *490*, 497–505.
(10) Furtmüller, P. G.; Zederbauer, M.; Jantschko, W.; Helm, J.; Bogner, M.; Jakopitsch, C.; Obinger, C. *Arch. Biochem. Biophys.* **2006**, *445*, 199–213.
(11) Singh, A. K.; Singh, N.; Sharma, S.; Singh, S. B.; Kaur, P.; Bhushan, A.; Srinivasan, A.; Singh, T. P. *J. Mol. Biol.* **2008**, *376*, 1060–1075.
(12) Magnusson, R. P.; Taurog, A.; Dorris, M. L. *J. Biol. Chem.* **1984**, *259*, 197–205.
(13) (a) Huwiler, M.; Kohler, H. *Eur. J. Biochem.* **1984**, *141*, 69–74. (b) Sun, W.; Dunford, H. B. *Biochemistry* **1993**, *32*, 1324–1331.
(14) Fukuyama, K.; Kunishima, N.; Amada, F.; Kubota, T.; Matsubara, H. *J. Biol. Chem.* **1995**, *270*, 21884–21892.
(15) Ohtaki, S.; Nakagawa, H.; Kimura, S.; Yamazaki, I. *J. Biol. Chem.* **1981**, *256*, 805–810.
(16) Furtmüller, P. G.; Jantschko, W.; Regelsberger, G.; Jakopitsch, C.; Arnhold, J.; Obinger, C. *Biochemistry* **2002**, *41*, 11895–11900.

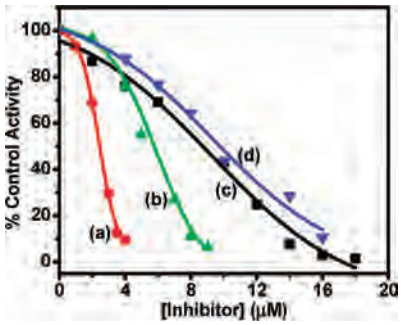

Figure 3. Inhibition of LPO-catalyzed iodination of Ang II by (a) MMI, (b) MTU, (c) PTU, and (d) captopril. The experiments were carried out in a sodium phosphate buffer (50 mM), pH 7.1 at 25 °C. The concentrations of Ang II, sodium iodide, hydrogen peroxide, and LPO were 20 μM, 20 μM, 40 μM, and 6.5 nM, respectively.

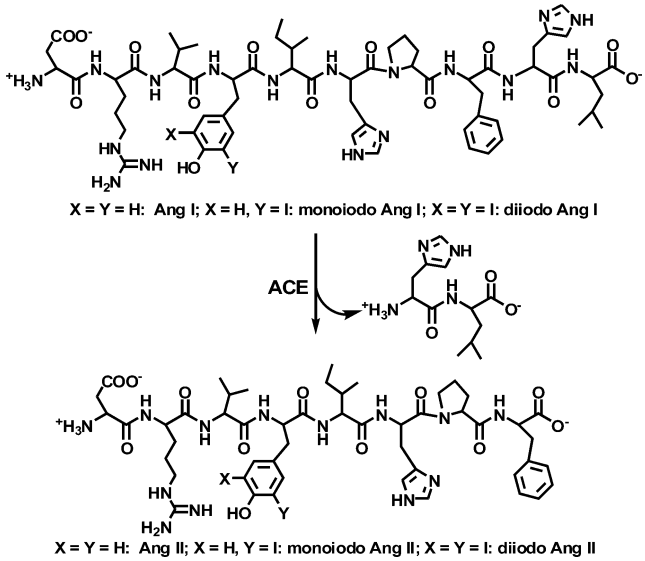

Figure 4. Hydrolysis of the terminal His-Leu dipeptide in Ang I, monoiodo-Ang I, and diiodo-Ang I by ACE. The experiments were carried out in HEPES (50 mM), pH 8.2 at 25 °C. The concentrations of Ang I, sodium chloride, and ACE were 30 μM, 200 μM, and 1 mU, respectively.

the heterocyclic moiety increases the electron density around the sulfur atom. Captopril also exists in its dissociated (thiolate) form rather than the thiol form at physiological pH. The lower IC_{50} value for MMI as compared with that of PTU is due to the difference in the nature of the thione moiety. It is known that the C═S bond in MMI is more polarized than that of PTU.[7c] These observations indicate that the sulfur compounds inhibit iodination reactions by binding to the distal heme side.

After establishing the site of iodination in Ang I and Ang II, we have studied the catalytic effect of ACE on the iodinated peptides (Figure 4) to understand the effect of iodination on the biological activity of angiotensins. The hydrolytic cleavage of the C-terminal dipeptide in mono- and diiodo-Ang I by ACE was followed by a reverse-phase HPLC, and the products were isolated and characterized. These studies reveal that the iodinated Ang I peptides are completely hydrolyzed by ACE and the rate of hydrolysis for monoiodo-Ang I is much higher than that of Ang I and the diiodinated Ang I. The t_{50} value (time required for a 50% conversion) for hydrolysis of monoiodo-Ang I (338.3 ± 4.2 min) is almost 2 times lower than that of Ang I (609.0 ± 3.0 min) and diiodo-Ang I (673.3 ± 21.2 min). These observations clearly indicate that monoiodo-Ang I is a better substrate for ACE than the native Ang I. On the other hand, hydrolysis of Ang I by ACE is unaffected upon diiodination of the tyrosyl residue.

Because ACE-catalyzed hydrolysis of monoiodo-Ang I is much faster than that of Ang I, monoiodo-Ang I is expected to be hydrolyzed more selectively even in the presence of Ang I. To test this hypothesis, the conversion of Ang I to Ang II was followed by a reverse-phase HPLC in the presence of various amounts of monoiodo-Ang I. These studies show that the activity of ACE on Ang I gradually decreases with an increase in the concentration of monoiodo-Ang I. The IC_{50} value (28.29 μM) determined for the inhibition of Ang II formation indicates that monoiodo-Ang I can compete successfully with Ang I for the ACE active site. This is probably due to a stronger binding of the iodinated peptide at the active site of ACE as compared with the native peptide. The interaction of the tyrosyl residue in Ang I at the active site of ACE may play important roles in substrate binding, and therefore, any chemical modification at this amino acid residue would alter the substrate binding and catalytic activity.

In summary, we have shown that LPO-catalyzed iodination of human Ang I and Ang II leads to the formation of mono- and diiodo-Ang I and -Ang II. The commonly used thiourea/thiouracil-based peroxidase inhibitors and the antihypertensive drug captopril effectively inhibit iodination reactions, indicating that the mechanism of iodination of Ang I and Ang II by LPO is identical with that of thyroglobulin by TPO. This study also shows that monoiodo-Ang I is a better substrate for the ACE than the native peptide. Because monoiodo-Ang II is a more potent pressor agent than the native hormone,[6] both direct iodination of Ang II and the efficient hydrolysis of monoiodo-Ang I by ACE could lead to an increase in the blood pressure.

Acknowledgment. This study was supported by the Department of Science and Technology (DST), New Delhi, India. G.M. acknowledges the DST for the award of Ramanna and Swarnajayanti fellowships, and B.J.B. thanks the Council of Scientific and Industrial Research for a research fellowship.

Supporting Information Available: Experimental details, HPLC chromatograms, mass spectra, NMR data, inhibition plots, and other kinetics data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC800395K