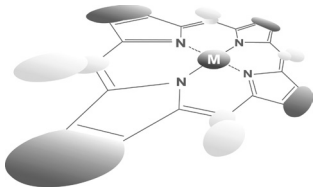
Journal of Porphyrins and Phthalocyanines
J. Porphyrins Phthalocyanines 2018; **22**: 1–7
DOI: 10.1142/S1088424618500840

Published at http://www.worldscinet.com/jpp/



Selective conversion of aldehydes to carboxylic acids by hemoglobin and air

Mahdi Hajimohammadi*[a], Maryam Khalaji Verjani[a], Hoda Ghasemi[a], Nasser Safari[b] and Günther Knör[c◊]

[a]*Faculty of Chemistry, Kharazmi University, G. C., P. O. Box 14911-15719 Tehran, Iran*
[b]*Department of Chemistry, Faculty of Sciences, Shahid Beheshti University, G. C., Evin, 19839-6313 Tehran, Iran*
[c]*Institute of Inorganic Chemistry, Johannes Kepler University Linz (JKU), 4040 Linz, Austria*

ABSTRACT: A new green, environmentally friendly and economically feasible method for the oxygenation of benzaldehyde, cinnamaldehyde, 4-chlorobenzaldehyde and 4-bromobenzaldehyde to the corresponding carboxylic acids using air in the presence of hemoglobin as a water-soluble catalyst in aqueous media at room temperature is illustrated. The resulting products were obtained with (77–100%) conversion and 100% selectivity within a reasonable amount of time. In addition, the first direct characterization of a high-valent iron intermediate ($HbFe^{IV+}$=O) measured using Mass Spectroscopy (MS) and UV-vis spectroscopy proved that the major route for oxidation of aldehydes is ($HbFe^{IV+}$=O) production.

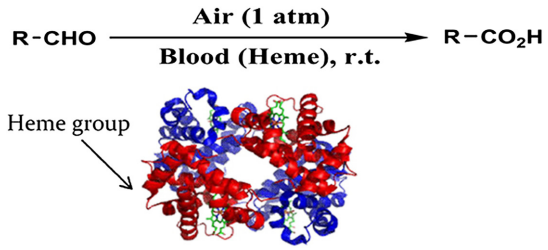
KEYWORDS: aldehydes, carboxylic acids, green chemistry, hemoglobin, high-valent iron intermediate.

INTRODUCTION

The global market for catalyst manufacture exceeds 14 billion dollars, and it can be estimated that catalysts induce an end-user business of over 7,500 billion dollars yearly [1]. Mammalian hemoglobin (Hb) is best known for its oxygen-carrying capacity, which facilitates oxygen delivery from the lungs to respiring tissues [2]. Many studies have found that Hb only has oxygen carrier properties but recently Minning *et al.* have discovered enzymatic activity of a heme-thiol-NO redox triad, and have shown that Ascaris hemoglobin functions to detoxify oxygen [3]. In the United States, the rendering industry collects and processes approximately 54 billion pounds per year of animal by-products and on-farm mortalities. These animal by-products, which include fat trim, meat, viscera, bone, blood and feathers are also collected and processed by the rendering industry. 4226.5 million pounds/year of poultry blood is produced, which in turn broadens the potential to use this waste product as an efficient and fine material catalyst [4]. On the other hand, oxidation is one of the most fundamental reactions in synthetic organic chemistry and a variety of oxidants have been developed. The oxidation of aldehydes to carboxylic acids has been a long-standing interest in synthetic organic chemistry [5]. The popular conventional method is the use of the Jones reagent [6]. This is a stoichiometric reaction using highly acidic conditions, which may not be tolerated by acid-sensitive functionalities in the substrate. Moreover, the generation of Cr-based side products may be viewed as a potential environmental hazard. Other efficient reagents that have been reported in the literature to achieve such transformation include oxone [7] calcium hypochlorite [8] and 2-hydroperoxyhexafluoro-2-propanol [9]. Some interesting methodologies involving metal-mediated transformation of the aldehyde functionality to carboxylic acid have also been recently reported [10]. In our previous studies, an efficient system for porphyrin-sensitized aerobic oxidation of alcohols, aldehydes, and alkenes was developed in the presence of visible light [11]. In continuation of our studies on porphyrins and aerobic oxidation of organic compounds, in this work, we describe the enzymatic

◊ SPP full member in good standing

*Correspondence to: Mahdi Hajimohammadi, tel.: +98-21-888329220-3, fax.: +98-21-88830857, email: hajimohammadi@khu.ac.ir.



Scheme 1. Oxidation of aldehydes to carboxylic acids with heme as an active site of blood

oxidation of aldehydes to carboxylic acids using air (1 atm) in the presence of sheep Hb and water as a solvent (Scheme 1). The reactions proceeded smoothly under these conditions, with 77–100% conversion and 100% selectivity.

EXPERIMENTAL

General procedure for the synthesis of carboxylic acids

In the absence of light, 0.5 mmol liquid aldehyde was added to 0.565 g/dL sheep Hb in pH = 7.0. Air (1 atm) was bubbled throughout the solution for 7–150 h. Plasma was separated from blood by centrifuging at 6000 rpm for 15 mins. In the light reaction, 0.5 mmol benzaldehyde, 0.565 g/dL sheep Hb and air (1 atm) were irradiated under visible light (288 power LED lamps, 1 W, 2.3 V (59660 LUX) for 7 h. After oxidation of the aldehydes, the solvent was removed under vacuum and the residue was separated by column chromatography (silica gel, *n*-hexane/EtOAc, 13:1) to obtain the corresponding carboxylic acids (Table 1). The product structures from entries 1, 8, 9 and 10 in Table 1 were confirmed by the melting points and by ^{1}H NMR spectra. The yield and selectivity of the products were determined by ^{1}H NMR.

***Benzoic acid* (1):** Colorless crystal, mp >120–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.88 (brs, 1H, OH), 8.15 (d, *J* = 7.5 Hz, 2H), 7.47–7.65 (m, 3H) ppm.

***Cinnamic acid* (8):** Colorless crystal, mp >133–135 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 10.80 (brs, 1H, OH), 7.78 (d, *J* = 16.0 Hz, 1H), 7.37–7.59 (m, 5H), 6.44 (d, *J* = 16.0 Hz, 1H) ppm.

4-*chlorobenzoic acid* (9): Colorless crystal, mp > 238–241. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 10.28 (brs, 1H, OH), 8.03 (d, *J* = 8.3 Hz, 2H), 7.51 (d, *J* = 8.3 Hz, 2H) ppm.

4-*Bromobenzoic acid* (10): Colorless crystal, mp > 253–255. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.94 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H) ppm, OH not observed.

The consumption of the starting aldehydes and the formation of the corresponding carboxylic acids monitored by a Bruker AMX 300 MHz spectrometer using TMS as internal standard. Spectral changes were monitored with a Shimadzu UV-2100 spectrophotometer. Also, Mass spectra of Hb were carried out by a Bruker autoflex III smartbeam mass spectrometer.

General procedure for the synthesis of iron(II)-tetrakis-(4-sulfonatophenyl)porphyrin (Fe^{III}TPPS): Fe^{III}TPPS was synthesized according to the literature [12].

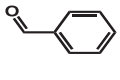
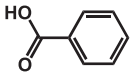
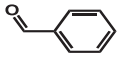
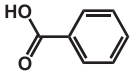
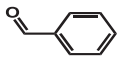
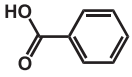
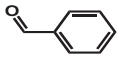
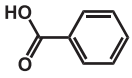
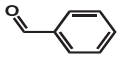
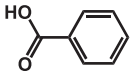
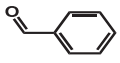
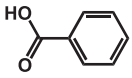
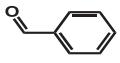
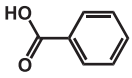
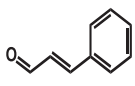
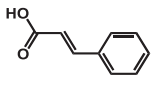
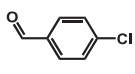
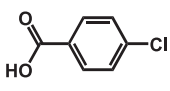
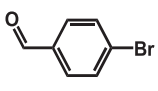
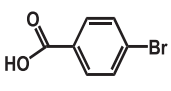
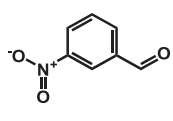
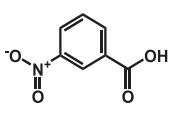
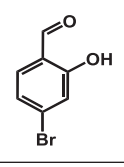
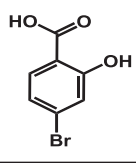
RESULTS AND DISCUSSION

To check the generality of this method, oxidation of different aldehydes was accomplished by air in the presence of sheep Hb and water solvent (Table 1). The reactions were performed in a tube without any particular precautions. Under these conditions, aldehydes (Table 1, entries 1–10) were oxidized to the corresponding acids with 77–100% yield. The results of the oxidation reactions in the absence of blood plasma were as good as those of the reactions in the presence of blood plasma (Table 1, entry 2). Also, the yield of benzaldehyde oxidation in the presence of human blood was as good as those of the reaction in the presence of sheep blood (Table 1, entry 3). On the other hand, the reaction didn't provide efficient yield in the presence of light, which is the key factor in photooxygenation reactions [13] (Table 1, entry 4). In addition, in the presence of N^{3-}, which is a well-known singlet oxygen scavenger [14], benzaldehyde was completely converted to benzoic acid (Table 1, entry 5). These results confirmed that Hb did not act as a metalloporphyrin to produce singlet oxygen. It is important to note that oxidation of the substrate does not continue in the absence of blood or when bubbling of air is interrupted (Table 1, entries 6 and 7). Therefore, the presence of blood and O_2 are essential for the conversion of benzaldehyde to benzoic acid.

In the presence of aldehydes with nitrogen or oxygen atoms on the benzyl substituent, the oxidation process of aldehydes was blocked (Table 1, entries 11 and 12). This might be due to strict hindrance of Hb, and it revealed that the oxidation of different aldehydes was selected by Hb. Our suggestion for Hbs selectivity is based on its ring size which is related directly to the size of the aldehyde to approach the complicated Hb structure. Throughout aldehyde oxidation, the modified Hb concentration was also studied, and high conversion was observed in 0.565 g/dl sheep Hb (Table 2, entries 1–4). The rate of benzaldehyde oxidation in neutral conditions was similar to that in acidic and alkaline pH (Table 2, entry 2, 5 and 6).

According to the literature, with a change in the oxidation number of iron in none-heme enzymes from +2 or +3 to +4, one blue shift at the Soret band of hemoglobin occurs and the intensity of Q band is decreased [15]. The existence of an axial ligand was confirmed by UV-vis spectra in the oxidation of 4-bromobenzaldehyde

Table 1. Oxidation of aldehydes in the presence of Hb[a]

Entry	Aldehyde	Acid	Time (h)	Yield %	TON	TOF
1			7	>99	1142	163.14
2[b]			7	>99	1142	163.14
3[c]			7	>99	822	117.5
4[d]			7	90	1027	147
5[e]			7	>99	1142	163.14
6[f]			7	Trace	—	—
7[g]			7	Trace	—	—
8			15	91	1039	69.26
9			150	77	879	5.8
10			150	>99	1142	7.61
11			150	Trace	—	—
12			150	Trace	—	—

[a] 5×10^{-4} mol liquid aldehyde, air (1 atm), 0.565 g/dL sheep Hb in the dark reaction condition, [b] 0.565 g/dl sheep Hb in the absence of blood plasma, [c] 0.785 g/dl man Hb, [d] irradiated with 288 power LED lamps, 1 W, 2.3 V (59660 LUX), [e] in the presence of N^{3-} as a singlet oxygen scavenger, [f] in the presence of continues nitrogen bubbling, [g] without sheep Hb.

(Fig. 1). In this figure, the red and purple insets show the spectra of solutions at the beginning of the oxidation reaction with sheep and human blood, respectively. The Soret band of the heme group at 418 nm disappeared and the band at 385 nm increased, suggesting the formation of the oxidant active species ($HbFe^{IV+\bullet}=O$) in the reaction medium after air bubbling. The mass spectrometric method was applied for the identification of $HbFe^{IV+\bullet}=O$ (Scheme 2). Mass spectroscopy of the green-colored intermediate (oxygenated Hb after reaction with 4-bromobenzaldehyde), gave a peak at m/z = 630.5, a value which correlated with molecular mass of Fe=O protoporphyrin IX^+ ion as an active site of hemoglobin [16].

Interestingly, the degradation of blood Hb was dependent on aldehyde oxidation and in the presence of non-oxidable aldehydes, hemoglobin was also not degraded (Fig. 2a). Also in the presence of water-soluble ferrous porphyrin ($Fe^{III}TPPS$) as a model of Hb, conversion of benzaldehyde to benzoic acid (Table 2, entry 7) and degradation of $Fe^{III}TPPS$ (Fig. 2b) were similar to benzaldehyde oxidation in the presence of Hb.

Table 2. Effect of hemoglobin concentration, pH and catalyst on benzaldehyde oxidation[a]

Entry	Yield of benzoic acid %
1	73
2[b]	>99
3[c]	>99
4[d]	>99
5[e]	>99
6[f]	>99
7[g]	>99

[a] 5×10^{-4} mol benzaldehyde, air (1 atm), 0.2825 g/dl sheep Hb, [b]0.565 g/dl sheep Hb, [c]1.13 g/dl sheep Hb, [d]2.26 g/dl sheep Hb. [e]0.565 g/dl sheep Hb in acetate buffer (0.2 M, pH = 4.0). [f]0.565 g/dl sheep Hb in the carbonate buffer (0.2 M, pH = 10.0). [g]0.565 g/dl Fe^{III}TPPS as a model of Hb was applied.

It seems that in the process of conversion of benzaldehyde to benzoic acid by Hb and air one reactive oxygen species (ROS) such as H_2O_2 or $^{\bullet}OH$ was generated. The iodometric method, a very sensitive assay for H_2O_2 determination [17], did not show H_2O_2 generation in the oxidation ambient. In order to investigate the generation of hydroxyl radical as one of the most reactive ROS, α-tocopherol (vitamin E) was applied in the benzaldehyde oxidation process. In the presence of α-tocopherol as a well-known $^{\bullet}OH$ scavenger [18], the oxidation reaction was stopped and Hb was not degraded (Fig. 3).

Our proposal mechanism is based on iron changeable oxide capacity that started by Hb autooxidation, in which H_2O is the sixth ligand coordinated in Hb (Scheme 2). This ligand leaves its position by oxygen bubbling and consequently produces the main species $HbFe^{+3}$–O–OH to generate the reactive species, which should be the iron (IV) oxo Hb radical cation ($HbFe^{IV+\bullet}=O$) observed by the UV-vis method. Recent calculations also support this formulation [19]. Finally, similar to the mechanism

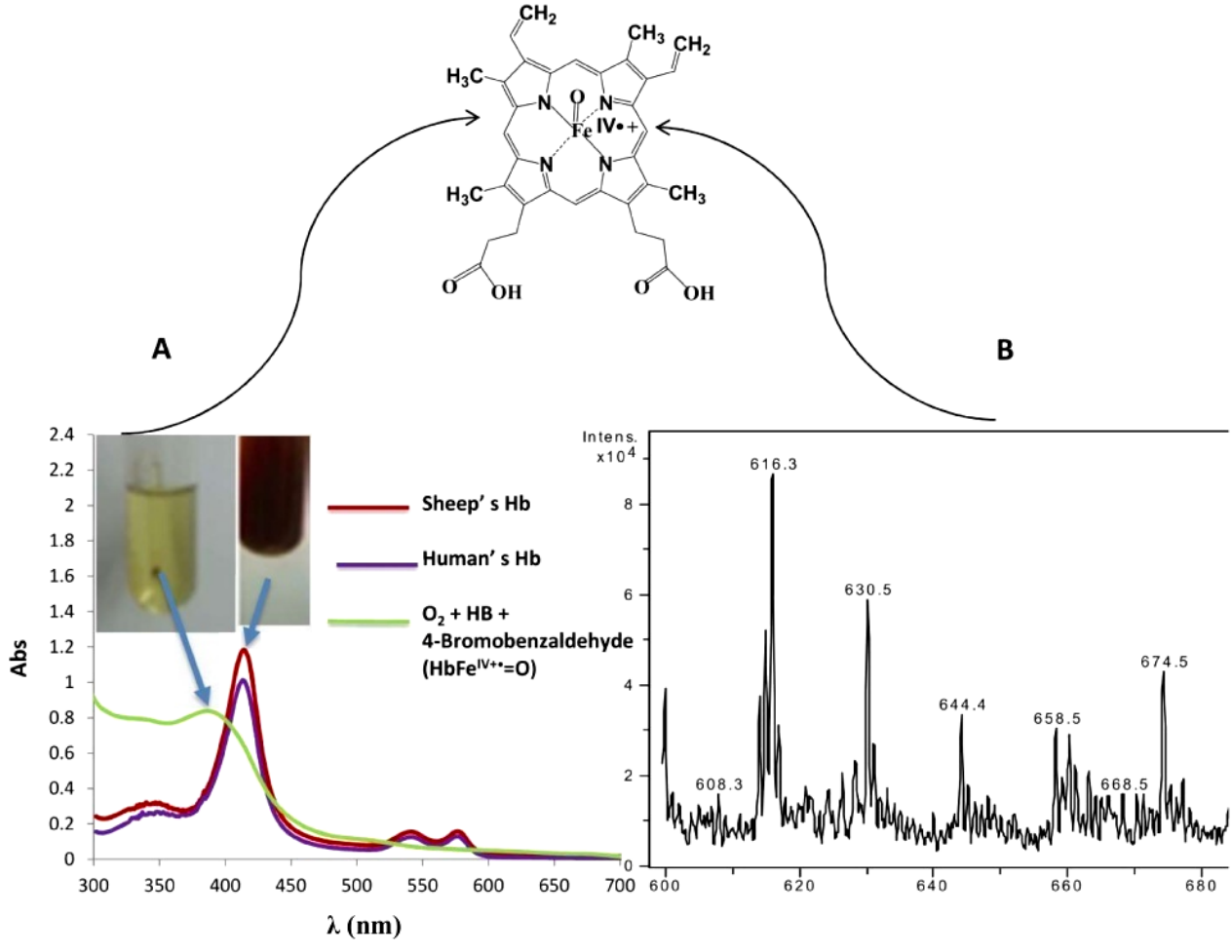


Fig. 1. Spectral changes (a) and mass spectra (b) of blood solution during the reaction of 4-bromobenzaldehyde (5×10^{-4} mol) and air (1 atm) in the absence of light

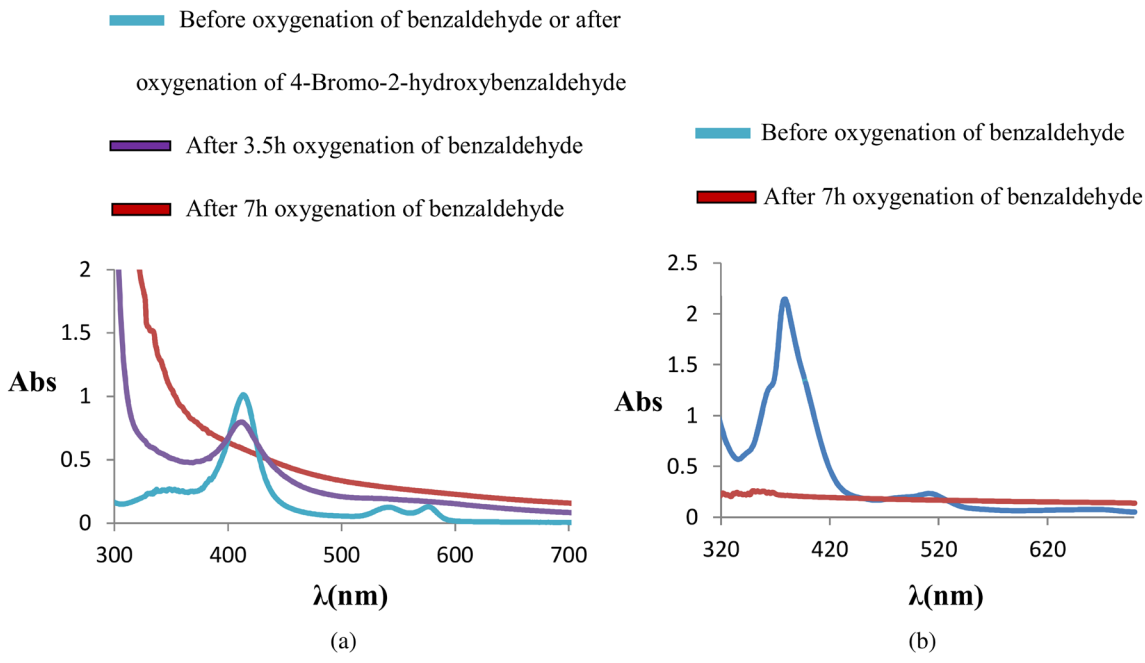


Fig. 2. UV-vis spectra of Hb degradation during the oxygenation of oxidable aldehyde (benzaldehyde) and non-oxidable aldehyde (4-bromo-2- hydroxybenzaldehyde)

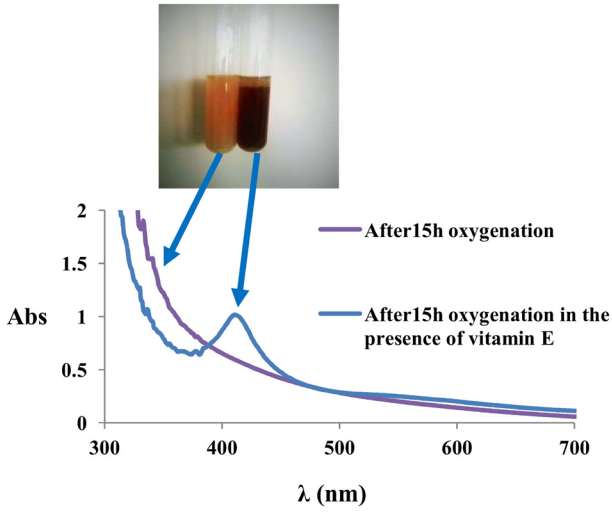
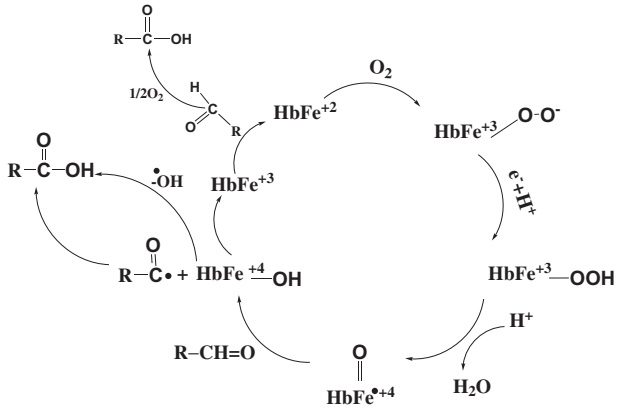


Fig. 3. Benzaldehyde oxidation process in the presence of vitamin E (orange tube) and in the absence of vitamin E (dark red)



Scheme 2. Proposed mechanism for aldehyde oxidation by hemoglobin in aqueous media

of substrate oxidation by cytochrome P450 enzymes [20], $HbFe^{IV+\bullet}=O$ species abstracts a hydrogen atom from a carbon molecule of the aldehyde and produces $HbFe^{IV}$–OH species and a carbon radical. The Fe^{IV}–OH species, which can also be observed as a complex of Fe^{+3} with a hydroxyl radical, then undergoes a recombination step in which the hydroxyl radical equivalent and carbon radical combine to produce the acid product. Finally, aldehyde acts as an electron donor and regenerates $HbFe^{+2}$ from $HbFe^{+3}$ [21].

CONCLUSION

In this study, we found evidence that Hb can display an oxidoreductase-like enzymatic function in addition to its oxygen carrier properties. These combined features could turn a food processing "waste material" of animal origin into an environmentally friendly and renewable catalyst for the oxidation of aldehydes as an important component in industry, instead of applying costly or hazardous oxidizing agents. On the other hand, it could be speculated that the inevitable autoxidation of Hb to the ferric form methemoglobin not only should be considered as a protein inactivation process. Instead, with potential implications for medicine and pharmacology,

such a reactivity might represent an additional native function of Hb-metabolites related to the intracellular degradation processes of organic compounds in the liver and other extra-hepatic tissues caused by hemoprotein catalysts of the cytochrome P450 enzyme family.

Acknowledgments

The authors gratefully acknowledge the financial support of Kharazmi University (Grant Number: D/2001).

REFERENCES

1. Bartholomew C and Farrauto R. *Fundamentals of Industrial Catalytic Processes,* Wiley: New Jersey, 2006.
2. Nagababu E and Rifkind J. *Antioxid. Redox Signaling* 2004; **6**: 967–978.
3. Minning DM, Gow AJ, Bonaventura J, Braun R, Dewhirst M, Goldberg DE and Stamler JS. *Nature* 1999; **401**: 497–502.
4. (a) Blay PA. *Food Fears: From Industrial to Sustainable Food Systems,* Routledge: New York, 2008; (b) The State of Food and Agriculture, 2002. Executive Summary; Expert Consultation and Workshop on Protein Sources for the Animal Feed Industry. Food and Agriculture Organization in association with the International Feed Industry Federation. Bangkok, Thailand, April 29–May 2.
5. (a) Hollingworth GJ, Katritzky AR, Meth-Cohn O, Rees CW and Pattenden G. In *Comprehensive Organic Functional Group Transformations*, Elsevier Science: Oxford, 1995; **5**: 23; (b) Larock RC. In *Comprehensive Organic Transformations: A Guide to Functional Group Preparations, 2nd ed*.; Wiley-VCH: New York, 1999; 1653; (c) Smith MB and March J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th ed*.; Wiley-Interscience: New York, 2001; 917–919; (d) Sheldon RA and Van Bekkum H. *Fine Chemicals Through Heterogeneous Catalysis,* Wiley-VCH:Weinheim, 2001.
6. (a) Bowden K, Heilbron IM, Jones ERH and Weedon BCL. *J. Chem. Soc*. **1946**: 39–45; (b) Heilbron I, Jones ERH and Sondheimer F. *J . Chem. Soc*. **1949**: 604–607; (c) Bladon P, Fabian JM, Henbest HB, Koch HP and Wood GW. *J. Chem. Soc*. **1951**: 2402–2411; (d) Curtis RG, Heilbron I, Jones ERH and Woods GF. *J. Chem. Soc*. 1953; 457–464; (e) Bowers A, Halsal TGl, Jones ERH and Lemin AJ. *J. Chem. Soc*. **1953**; 2548; (f) Djerassi C, Engle RR and Bowers A. *J. Org. Chem*. 1956; **21**: 1547–1548.
7. Benjamin RT, Sivakumar M, Hollist GO and Borhan B. *J. Org. Lett*. 2003; **5**:1031–1034.
8. Nwaukwa SO and Keehn PM. *Tetrahedron Lett*. 1982; **23**: 3131–3134.
9. Ganem B, Heggs RP, Biloski AJ and Schwartz DR. *Tetrahedron Lett*. 1980; **21**: 685–688.
10. (a) Yamada T, Rhode O, Takai T and Mukaiyama T. *Chem. Lett*. **1991**; 5–8; (b) Bhatia B, Punniyamurthy T and Iqbal J. *J. Org. Chem.* 1993; **58**: 5518–5523; (c) Heaney HJ. *Top. Curr. Chem*. 1993; **164**: 1–125; (d) Kharata AN, Pendleton P, Badalyan A, Abedini M and Amini M. *J. Mol. Catal. A* 2001; **175**: 277–283; (e) Biella S, Prati L and Rossi M. *J. Mol. Catal. A* 2003; **197**: 207–212; (f) Grill JM, Ogle JW and Miller SA. *J. Org. Chem.* 2006; **71**: 9291–9296; (g) Joseph JK, Jain SL and Sain JB. *Catal. Commun*. 2007; **8**: 83–87. (h) Lim M, Yoon CM, An G and Rhee H. *Tetrahedron Lett*. 2007; **48**: 3835–3839; (i) Zhou XT, Ji HB, Yuan QL, Xu JC, Pei LX and Wang LF. *J. Chin. Chem. Lett.* 2007; **18**: 926–928; (j) SlobodaRozner D, Neimann K and Neumann R. *J. Mol. Catal. A*. 2007; **262**: 109–113; (k) Mukhopadhyay C and Datta A. *Catal. Commun*. 2008; **9**: 2588–2592; (l) Uyanik M and Ishihara K. *J. Chem. Commun*. 2009; **16**: 2086–2099.
11. (a) Hajimohammadi M, Safari N, Mofakham H and Deyhimi F. *Green Chem*. 2011; **13**: 991–997; (b) Hajimohammadi M, Schwarzinger C, Knör G. *RSC. Adv*. 2012; **2**: 3257–3260; (c) Hajimohammadi M, Mofakham H, Safari N and Manesh AM. *J. Porphyrins Phthalocyanines* 2012; **16**: 93–100. (d) Mehrabi Kalajahi SS, Hajimohammadi M and Safari N. *Reac. Kinet., Mech. Catal*. 2014; **113**: 629–640. (e) Hajimohammadi M, Safari N, Mofakham H and Shaabani A. *Tetrahedron Lett*. 2010; **51**: 4061–4065.
12. Huszank R and Otto H. *Chem. Commun*. 2005; **2**: 224–226.
13. DeRosa M. *Coord. Chem. Rev.* 2002; **233**: 351–371.
14. Harbour JR and Issler SL. *J. Am. Chem. Soc*. 1982; **104**: 903–905.
15. (a) Costas M, Mehn MP, Jensen MP and Que L. *J. Chem. Rev.* 2004; **104**: 939–986. (b) Decker A and Solomon EI. *Curr. Opin. Chem. Biol*. 2005; **9**: 152–163. (c) Price JC, Barr EW, Tirupati B, Bollinger JM and Krebs C. *J. Biochemistry* 2003; **42**: 7497–7508. (d) Proshlyakov DA, Henshaw TF, Monterosso GR, Ryle MJ and Hausinger RP. *J. Am. Chem. Soc*. 2004; **126**: 1022–1023. (e) Riggs-Gelasco PJ, Price JC, Guyer RB, Brehm JH, Barr EW, Bollinger JM and Krebs C. *J. Am. Chem. Soc.* 2004; **126**: 8108–8109. (f) Amineh A and Safari N. *J. Porphyrins Phthalocyanines* 2010; **14**: 335–342. (g) Soldi C, Lamb KN, Squitieri RA, González-López M, Di Maso MJ and Shaw JT. *J. Am. Chem. Soc*. 2014; **136**: 13542–13545.
16. Gledhill M. *J. Anal. Chem*. 2014; **841**: 33–43.
17. Antolovich M, Prenzler P, Patsalides E, McDonald S and Robards K. *Analyst* 2002; **127**: 183–189.
18. Sies H. *Exp. Physiol*. 1997; **82**: 291–295.

19. (a) Groves JT, Haushalter RC, Nakamura M, Nemo TE and Evans BJ. *J. Am. Chem. Soc*. 1981; **103**: 2884–2886. (b) Groves JT and Watanabe Y. *J. Am. Chem. Soc.*, 1988; **110**: 8443–8452; (c) Gross Z and Nimri S. *Inorg. Chem.* 1994; **33**: 1333–1341; (d) Pan Z, Zhang R and Newcomb M. *J. Inorg. Biochem*. 2006; **100**: 524–532; (e) Eldik R. *J. Coord. Chem. Rev*., 2007; **251**: 1649–1662; (f) Ah-Rim H, Yu JJ, Yaeun K, Lee YJ, Mi SS and Wonwoo N. *J. Chem. Commun*. 2008; **9**: 1076–1078.

20. (a) Groves JT, McClusky GA, White RE and Coon M. *Biochem. Biophys. Res. Commun*. 1978; **81**: 154–160. (b) Foster AB. *J. Adv. Drug Res*. 1985; **14**: 1–40; (c) Hjelmeland LM, Aronow L and Trudell JR. *Biochem. Biophys. Res. Commun*. 1977; **76**: 541–549.

21. Li H, Kundu TK and Zweier JL. *J. Biol. Inorg. Chem.* 2009; **284**: 33850–33858.