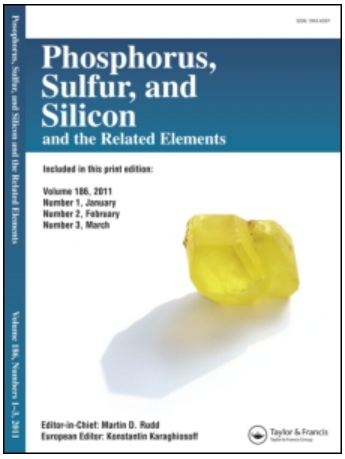

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

STIMULATION BY COPPER(II) OF REACTIVITY OF VARIOUS SULFHYDRYL-CONTAINING COMPOUNDS WITH 2,2-DIPHENYL-1-PICRYLHYDRAZYL

Robert C. Smith[a]; Jeri Z. Gore[a]
[a] Department of Animal and Dairy Sciences, Alabama Agricultural Experiment Station, Auburn University, Alabama

To cite this Article Smith, Robert C. and Gore, Jeri Z.(1991) 'STIMULATION BY COPPER(II) OF REACTIVITY OF VARIOUS SULFHYDRYL-CONTAINING COMPOUNDS WITH 2,2-DIPHENYL-1-PICRYLHYDRAZYL', Phosphorus, Sulfur, and Silicon and the Related Elements, 62: 1, 105 — 110
To link to this Article: DOI: 10.1080/10426509108034465
URL: http://dx.doi.org/10.1080/10426509108034465

Phosphorus, Sulfur, and Silicon, 1991, Vol. 62, pp. 105–110
Reprints available directly from the publisher
Photocopying permitted by license only

STIMULATION BY COPPER(II) OF REACTIVITY OF VARIOUS SULFHYDRYL-CONTAINING COMPOUNDS WITH 2,2-DIPHENYL-1-PICRYLHYDRAZYL

ROBERT C. SMITH† and JERI Z. GORE
Department of Animal and Dairy Sciences, Alabama Agricultural Experiment Station, Auburn University, Alabama 36849-5415

(Received January 8, 1991; in final form April 19, 1991)

The reaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with various sulfhydryl-containing compounds including ergothioneine, glutathione, 1-methyl-2-mercaptoimidazole (methimazole), and 2-mercaptoimidazole was stimulated 7–14 fold by the addition of copper (II) sulfate. The reaction of DPPH with other 2-imidazolethiones substituted at both the 4 and 5 positions was not stimulated by Cu(II). The stimulation may result from the formation of a complex between Cu(II) and the sulfhydryl-containing compounds.

Key words: 2,2-diphenyl-1-picrylhydrazyl; Cu(II); sulfhydryl; 2-imidazolethione; radical; antioxidant.

INTRODUCTION

The stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) reacts with a variety of antioxidants including ascorbic acid, a-tocopherol, uric acid, and sulfhydryl-containing compounds such as cysteine and glutathione.[1–8] DPPH reacts with thiols by abstracting a hydrogen to form diphenylpicrylhydrazine ($DPPH_2$) and a thiol radical (reaction 1).

$$DPPH^{\cdot} + RSH \rightarrow DPPH_2 + RS^{\cdot} \quad (1)$$

The thiol radical either reacts with a second molecule of DPPH to form DPPH-SR (reaction 2).

$$DPPH^{\cdot} + RS^{\cdot} \rightarrow DPPH\text{-}SR \quad (2)$$

or with a second thiol radical to form a sulfide (reaction 3).

$$RS^{\cdot} + RS^{\cdot} \rightarrow RSSR \quad (3)$$

Both types of reactions have been demonstrated.[3]

Methimazole (1-methyl-2-imidazolethione), which inhibits thyroid hormone biosynthesis, reacts with DPPH at a faster rate than other thiols including 6-mercaptopurine, 2-thiouracil, and 6-propylthiouracil.[3] Cysteine reacts with DPPH at over 100 times the rate of glutathione.[7] Other thiols such as 1,5-dimethyl-4-mercaptoimidazole are also more reactive than glutathione with one-electron acceptors.[9,10]

† Author to whom correspondence should be addressed.

The rate constants of the reaction between *n*-hexyl mercaptan and DPPH in cyclohexane were almost twice the rate constants in benzene.[11] Since benzene forms a weak complex with DPPH, it was suggested that solvation of the DPPH accounted for some of the lowering of the rate constant. The rate constants for the reaction of substituted phenols with DPPH varied over a 29,100-fold range.[12] Both polar and steric influences accounted for the variation in rates. The reactivity of 2-imidazolethiones with DPPH was dependent on the substituents at the 4 and 5 positions of the imidazole ring.[13] A 2-imidazolethione substituted with a methyl group at position 5 and a (methoxyphenyl) methanone group at position 4 reacted with DPPH at a rate that was 10–20% that of 2-imidazolethiones substituted with a methyl group at position 5 and either a phenylmethanone, ethanone, or carboxamide group at position 4. During the course of these investigations, it was observed that the rate of reaction of some of the 2-imidazolethiones with DPPH was stimulated if Cu(II) was present. This paper is a report of these observations.

RESULTS AND DISCUSSION

A variety of compounds with a free sulfhydryl group have previously been shown to react with DPPH to form $DPPH_2$ and result in a decrease in absorbance at 517 nm[3,13]; it is assumed that each molecule of DPPH reacts with only one thiol. The rate of disappearance of DPPH measured spectrophotometrically is used to follow the rate of the reaction. The rates of reaction between DPPH and the sulfhydryl compounds used in the present study varied from 1.9 nmoles/min for glutathione to 105.5 nmoles/min for 5-methyl-2-thioxoimidazole-4-N,N-dimethylcarboxamide (Table I). The large variation in rates of reaction of these sulfhydryl-containing

TABLE I

Effect of Cu(II) on the reaction of sulfhydryl compounds with 2,2-diphenyl-1-picrylhydrazyl[1]

Compound added[1]	nmoles DPPH reacted/min/ml[2]	
	Before Cu(II) addition	After Cu(II) addition
None	< 0.07	< 0.07
Glutathione	1.9±0.6	14.2± 1.6
Methimazole	7.8±1.3	110.7±11.3
2-Mercaptoimidazole	12.9±4.6	186.4±30.0
Ergothioneine	32.3±8.0	244.5±45.7
(5-Methyl-2-thioxoimidazole-4-yl) (4-methoxyphenyl)-methanone	13.4±3.6	11.6± 1.9
5-Methyl-2-thioxoimidazole-4-[4-(4-methylthio)phenyl]-methanone	71.3±3.3	66.9± 6.6
5-Methyl-2-thioxoimidazole-4-carboxamide	72.5±9.7	63.7± 7.3
5-Methyl-2-thioxoimidazole-4-N,N-dimethylcarboxamide	105.5±8.0	112.8±10.9

1 The concentration of the sulfhydryl compounds and the DPPH was 100 μM and the concentration of Cu(II) was 10 μM.

2 Each value is the mean ± standard deviation for three experiments.

compounds with DPPH may result from differences in their nucleophilic and hence their redox properties.[10] When Cu(II) was added to DPPH and either ergothioneine, glutathione, methimidazole, or 2-mercaptomidazole, the rate of reaction of DPPH with the sulfhydryl compound was increased from 7 to 14 fold (Table I). The increase in reactivity of DPPH with these compounds in the presence of Cu(II) was dependent on the concentration of Cu(II). The reaction was not stimulated by ferric(III) chloride or ferrous (II) sulfate. Cu(II) did not affect the reactivity of DPPH with other 2-imidazolethiones substituted at both the 4 and 5 positions.

Representative results for ergothioneine and glutathione are shown in Figure 1. The time scale is in seconds for ergothionine and in minutes for glutathione. When 10 μM Cu(II) was added to either compound in the presence of DPPH, the rate of the reaction increased about 7-fold. The increased rate was observed whether the Cu(II) was preincubated with either the DPPH or the sulfhydryl-containing compound before the experiment was initiated.

The addition of Cu(II) to 2-imidazolethiones results in the formation of Cu(II) complexes.[14,15] The addition of Cu(II) to ergothioneine decreased the absorbance at 256 nm presumably due to the formation of a Cu(II) complex (Figure 2). The addition of Cu(II) at a molar concentration equal to half that of ergothioneine

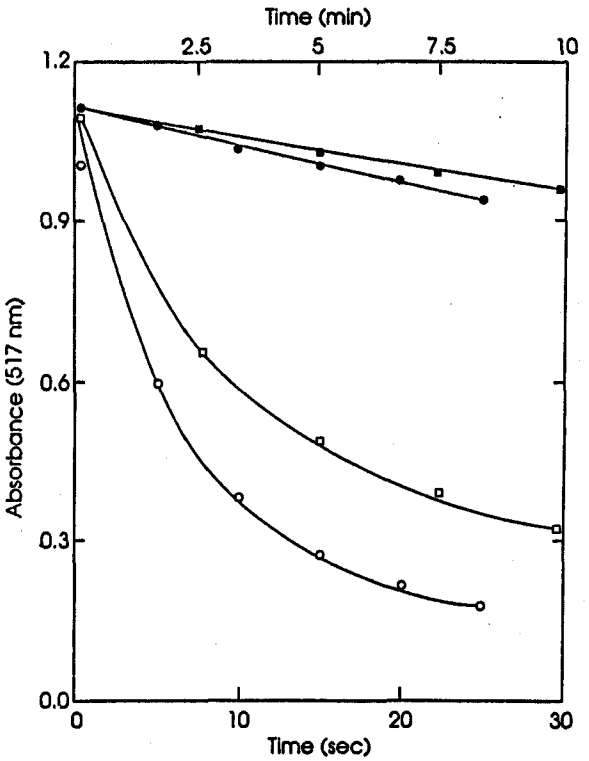

FIGURE 1 Effect of Cu(II) on the reactivity of ergothioneine and glutathione with DPPH. The reaction mixtures contained 100 μM glutathione (■), 100 μM glutathione plus 10 μM Cu(II) (□), 100 μM ergothioneine (●), and 100 μM ergothioneine plus 10 μM Cu(II) (○). Note the time scale at the top of the figure is in minutes for the glutathione reactions and the time scale at the bottom is in seconds for the ergothioneine reactions.

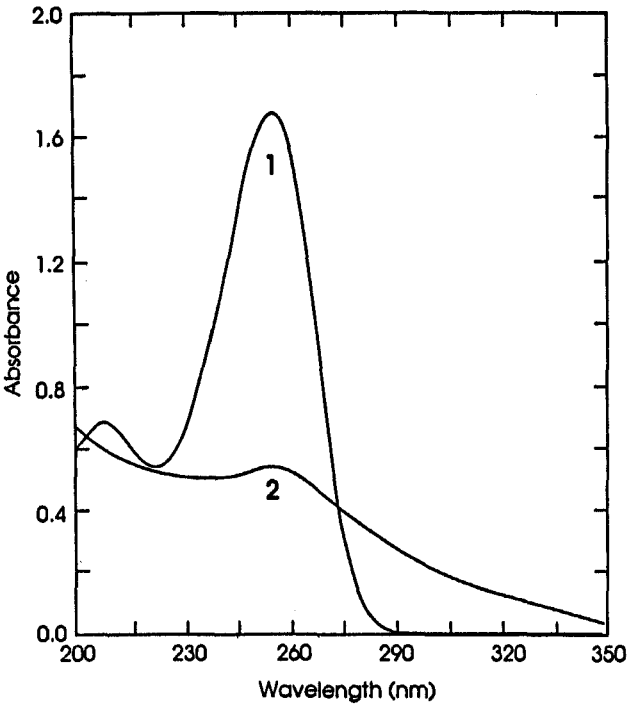

FIGURE 2 Ultraviolet absorption spectrum of 100 μM ergothioneine with no addition (curve 1) and with 50 μM Cu(II) (curve 2). Cu(II) at the same concentration was used in the reference cell for curve 2.

resulted in the maximum decrease in absorbance, suggesting that each Cu(II) was complexed to two ergothioneines. This interaction could have resulted in the formation of intermediates which were more reactive with DPPH than the parent compound.

A general feature of the participation of transition metal ions in free radical reactions is that they convert poorly reactive species into more reactive ones.[16] In the present study, it was shown that Cu(II) stimulates the reaction of some sulfhydryl-containing compounds including ergothioneine and glutathione with the stable free radical DPPH. It has been proposed that ergothioneine, which is present in high concentrations in muscle of some animals, functions as an intracellular interceptor of hydrogen peroxide, radicals, and electrophilic organic molecules.[17] Glutathione, the most abundant low molecular weight thiol in mammalian cells, protects against oxidative stress by both direct reaction with radicals and by serving as a substrate for glutathione peroxidase to reduce toxic peroxides.[18] Cu(II) also stimulates the generation of hydroxyl radicals ($OH^{\cdot}$) by the buffers 4-(2-hydroxyethyl)1-piperazinethanesulfonic acid) (Hepes) and 1,4-piperazine bis(ethanesulfonic acid) (Pipes), and histidine, which chelates Cu(II).[19] It was suggested that this stimulation was a result of formation of a Cu(II) complex with these compounds that promoted $OH^{\cdot}$ formation. Cu(II) also catalyzes DNA strand scission in the presence of 5-alkylresorcinols[20] or hydrogen peroxide.[21,22] With the 5-alkylresorcinols, it was suggested that the benzene nucleus may first be oxygenated at C–4.

This derivative could then chelate Cu(II) to produce a complex that in the presence of oxygen would initiate degradation of DNA. With the hydrogen peroxide, it was suggested that the Cu(II), while bound to a region containing guanosines, was reduced to Cu(I) which reacted with hydrogen peroxide to generate $OH^{\cdot}$. The $OH^{\cdot}$ produced could then react with guanosine in this region of the DNA.[21] Copper-peroxide complexes rather than hydroxyl radical have also been proposed as the main active species causing DNA damage.[22]

DPPH reacts with ferrous ions in equimolar proportions to form $DPPH_2$ quantitatively.[23] This reaction rate was stimulated by halide ions and carboxylic acids.[24] DPPH formed a 1:1 complex with Cu(I) in which the Cu(I) appeared to be coordinated through the picryl ring.[25] Cu(II) formed the identical complex with $DPPH_2$.[25] The mechanism for stimulation of DPPH reactivity with 2-imidazolethiones by Cu(II) could be through complexing of Cu(II) by either or both of the reactants to produce a more reactive intermediate. Previous studies have shown that Cu(II) binds to both DPPH and 2-imidazolethiones.[14,15,25] The binding of copper to sulfhydryl-containing antioxidants could lead to the formation of more reactive complexes.

EXPERIMENTAL

Reactivity with DPPH.

The assessment of the reactivity of the sulfhydryl-containing compounds with DPPH in the presence and absence of copper(II) sulfate was carried out by a method similar to that previously used.[6] The sulfhydryl compounds and Cu(II) were dissolved in deionized water at a concentration of 1.0 mM. The water was purified with a Barnstead NANOpure II system to reduce contamination by metals. The DPPH was dissolved in 95% ethanol at a concentration of 0.11 mM. 10 μl of 1 mM Cu(II) was added to 0.9 ml DPPH, the sample mixed, and the cuvette placed in a model 25 Beckman spectrophotometer. The absorbance of each sample was monitored continuously at 517 nm. After 5 min of preincubation, 0.1 ml of the sulfhydryl compounds (1 mM) was added to the cuvette, the sample mixed, and the absorbance at 517 nm read continuously. The decrease in absorbance over the first 10 to 30 sec was used to calculate an initial rate using an extinction coefficient for DPPH of 14.450 M^{-1} cm^{-1}.[26] In a few experiments, the sulfhydryl compounds were added to the DPPH to initiate the reaction and the Cu(II) added 1 min later.

Chemicals.

Most of the sulfhydryl-containing compounds and DPPH were obtained from Sigma Chemical Co., St. Louis, MO. The 2-imidazolethiones substituted at positions 4 and 5 were a gift from Merrell-Dow Pharmaceuticals, Cincinnati, OH. The copper sulfate was from Baker and Adamson, New York, NY.

ACKNOWLEDGEMENTS

This research was supported by Hatch Project ALA00713 and State funds of the Alabama Agricultural Experiment Station. Alabama Agricultural Experiment Station Journal Series No. 4-912966P.

REFERENCES

1. M. S. Blois, *Nature*, **181,** 1199 (1958).
2. J. Glavind, *Acta Chem. Scand.*, **17,** 1635 (1963).
3. B. H. Berg, *Acta Pharm. Suec.*, **8,** 443 (1971).
4. P. W. Banda, A. E. Sherry and M. S. Blois, *Anal. Lett.*, **7,** 41 (1974).
5. M. Pesh-Imam and R. O. Recknagel, *Toxicol. Appl. Pharmacol.*, **42,** 455 (1977).
6. R. C. Smith, *Bioorg. Chem.*, **11,** 436 (1982).

7. R. C. Smith and J. H. Hargis, *Microchem. J.*, **31,** 18 (1985).
8. R. C. Smith, J. Reeves, M. L. McKee and H. Daron, *Free Rad. Biol. Med.*, **3,** 251 (1987).
9. J. Tsuchiya, T. Yamada, E. Niki and Y. Kamiya, *Bull. Chem. Soc. Jpn.*, **58,** 326 (1985).
10. T. P. Holler and P. B. Hopkins, *J. Am. Chem. Soc.*, **110,** 4837 (1988).
11. K. E. Russell, *J. Phys. Chem.*, **58,** 437 (1954).
12. J. S. Hogg, D. H. Lohmann and K. E. Russell, *Can. J. Chem.*, **39,** 1588 (1961).
13. R. C. Smith, J. C. Reeves, R. C. Dage and R. A. Schnettler, *Biochem. Pharmacol.*, **36,** 1457 (1987).
14. J. R. Creighton, D. J. Gardiner, A. C. Gorvin, C. Gutteridge, A. R. W. Jackson, E. S. Raper and P. M. A. Sherwood, *Inorg. Chim. Acta*, **103,** 195 (1985).
15. E. S. Raper, J. R. Creighton, D. Robson, J. D. Wilson, W. Clegg and A. Milne, *Inorg. Chim. Acta* **143,** 95 (1988).
16. B. Halliwell, FASEB J., **1,** 358 (1987).
17. P. E. Hartman, in Methods in Enzymology, Vol. 186, L. Packer and A. N. Glazer, Eds. (Academic Press, New York, 1990), pp. 310–318.
18. G. T. Saez, W. H. Bannister and J. V. Bannister, in Glutathione: Metabolism and Physiological Function. J. Vina, Ed. (CRC Press, Boca Raton, Florida, 1990), Chap. 23, pp. 237–254.
19. J. A. Simpson, K. H. Cheeseman, S. E. Smith and R. T. Dean, *Biochem. J.*, **254,** 519 (1988).
20. R. T. Scannell, J. R. Barr, V. S. Murty, K. S. Reddy and S. M. Hecht, *J. Am. Chem. Soc.*, **110,** 3650 (1988).
21. J.-L. Sagripanti and K. H. Kraemer, *J. Biol. Chem.*, **264,** 1729 (1989).
22. K. Yamamoto and S. Kawanishi, *J. Biol. Chem.*, **264,** 15435 (1989).
23. C. E. H. Bawn and D. Verdin, *Trans. Faraday Soc.*, **56,** 519 (1960).
24. V. V. Voevodskii, R. I. Zusman, G. I. Shamovskaya and Y. N. Molin, Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk 127 (1968). (*Chem. Abstr.*, **70,** 41190a (1969).
25. F. Leh and J. K. S. Wan, *Bull. Chem. Soc. Jap.*, **45,** 308 (1972).
26. R. H. Poirier, E. J. Kahler and F. Benington, *J. Org. Chem.*, **17,** 1437 (1952).