ChemPubSoc Europe

DOI: 10.1002/chem.201402833

CHEMISTRY A European Journal Full Paper

Hydrogen Bonds

Detoxifying Polyhalogenated Catechols through a Copper-Chelating Agent by Forming Stable and Redox-Inactive Hydrogen-Bonded Complexes with an Unusual Perpendicular Structure

Yan Li,[a] Chun-Hua Huang,[a] Yu-Xiang Liu,[a] Li Mao,[a] and Ben-Zhan Zhu*[a, b]

Abstract: The use of selective metal chelating agents with preference for binding of a specific metal ion to investigate its biological role is becoming increasingly common. We found recently that a well-known copper-specific chelator 2,9-dimethyl-1,10-phenanthroline (2,9-Me_2OP) could completely inhibit the synergistic toxicity induced by tetrachlorocatechol (TCC) and sodium azide (NaN_3). However, its underlying molecular mechanism is still not clear. Here, we show that the protection by 2,9-Me_2OP is not due to its classic copper-chelating property, but rather due to formation of a multiple hydrogen-bonded complex between 2,9-Me_2OP and TCC, featuring an unusual perpendicular arrangement of the two binding partners. The two methyl groups at the 2,9 positions in 2,9-Me_2OP were found to be critical to stabilize the 2,9-Me_2OP/TCC complex due to steric hindrance, and therefore completely prevents the generation of the reactive and toxic semiquinone radicals by TCC/NaN_3. This represents the first report showing that an unexpected new protective mode of action for the copper "specific" chelating agent 2,9-Me_2OP by using its steric hindrance effect of the two CH_3 groups not only to chelate copper, but also to "chelate" a catechol through multiple H-bonding. These findings may have broad biological implications for future research of this widely used copper-chelating agent and the ubiquitous catecholic compounds.

Introduction

The use of selective transition-metal chelating agents with a preference for the binding of a specific metal ion to investigate its role in oxidative stress reactions is becoming increasingly common.[1–5] 2,9-Dimethyl-1,10-phenanthroline (2,9-Me_2OP; also called neocuproine) and its water-soluble analogue bathocuproine disulfonate (BCS) are two such chelating agents. The cuprous complexes with these ligands possess greater stability than other biologically relevant complexes with other transition metals due to the steric hindrance provided by the two CH_3 groups at the 2,9 positions of the 1,10-phenanthroline ring structure.[3,4] Reductions in toxicity and decreased molecular damage, which are caused by addition of these chelators, have been used to support the involvement of copper in deleterious reactions in vivo and in vitro.[5–13] In one of these studies, 2,9-Me_2OP protected isolated rat hearts against ischemia/reperfusion-induced injury.[13] In another study, BCS reversed copper-mediated growth inhibition of L1210 cells.[4]

We found recently that the combination of tetrachlorocatechol (TCC) and sodium azide (NaN_3) caused a pronounced synergistic cytotoxicity in a bacterial model.[14, 15] During our study on the role of transition-metal ions, especially Cu and Fe, we found, unexpectedly, that only 2,9-Me_2OP, but not any other well-known metal chelating agents, markedly inhibited the synergistic toxicity induced by TCC/NaN_3. Further studies indicate that the protection by 2,9-Me_2OP may not be due to its chelation of copper, but possibly due to the formation of an unknown supramolecular complex with TCC and/or NaN_3. However, the exact chemical structure and the composition of such a complex remained unclear because we were unable to detect and identify this complex by the typical techniques applied in the characterization of organic compounds in solutions.

In this study, we plan to address the following questions by the complementary application of single-crystal X-ray diffraction, IR, and solid state NMR spectroscopic methods: 1) What is the exact chemical structure and composition of this complex? 2) What is the nature of the binding forces for this complex? 3) Could 2,9-Me_2OP form similar complexes with other polyhalogenated catechols? 4) Why are the two CH_3 substituents at the 2,9 positions in the 2,9-Me_2OP critical? 5) What is the underlying molecular mechanism of the protection of 2,9-Me_2OP against TCC/NaN_3 induced synergistic toxicity?

[a] Dr. Y. Li, Dr. C.-H. Huang, Dr. Y.-X. Liu, Dr. L. Mao, Prof. Dr. B.-Z. Zhu
State Key Laboratory of Environmental Chemistry
and Ecotoxicology, Research Center for Eco-Environmental Sciences
The Chinese Academy of Sciences, Beijing 100085 (P.R. China)
E-mail: bzhu@rcees.ac.cn

[b] Prof. Dr. B.-Z. Zhu
Linus Pauling Institute, Oregon State University, Corvallis, OR 97331 (USA)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201402833.

Experimental Section

Instrumentation

All the complexes were determined by single-crystal X-ray diffraction. Data collection was performed on an Agilent Gemini A Ultra diffractometer, using graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) for 2,9-Me_2OP/TCC and 2,9-Me_2OP/4,5-DCC or $Cu_{K\alpha}$ radiation ($\lambda = 1.5418$ Å) for other complexes. The determination of crystal class and unit cell parameters was carried out by CrysAlis (Agilent 2011) program package, raw frame data was processed using CrysAlis (Agilent 2011), and the structures were solved by use of SHELX97 program or new versions and refined by full-matrix least-squares on F values. Crystallographic data are summarized in Tables S1 and S2 (in the Supporting Information).

The infrared spectra of the obtained complexes were recorded using KBr discs on Perkin–Elmer 1430 Infrared Spectrophotometer, in the range 4000–400 cm^{-1}. The solid-state NMR experiments were performed on a Bruker AVANCE III 400 MHz solid-state NMR spectrometer with Total Sideband Suppression (TOSS) pulse sequence at 5 KHz magic angle spinning (MAS) rate. The dissolved oxygen was measured by Thermo Scientific Eutech DO2700 dissolved oxygen meter. ESR spectra were obtained using Bruker ER 200 D-SRC spectrometers and calibrated with DPPH ($g = 2.0037$).

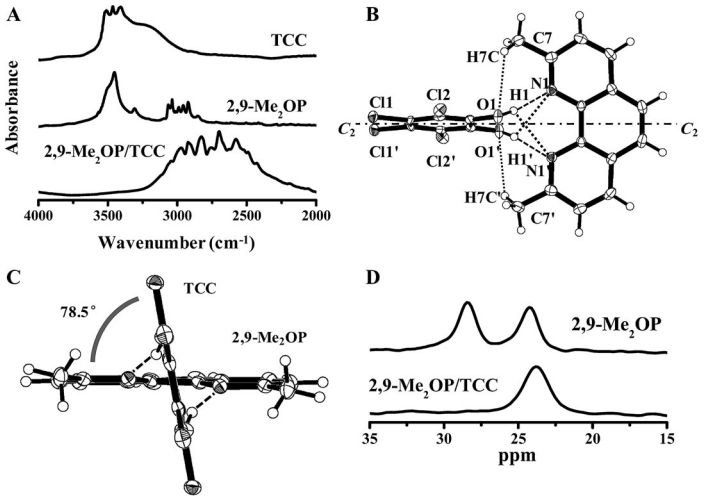


Figure 1. A multiply H-bonded complex with an unusual perpendicular conformation was formed between 2,9-Me_2OP and TCC. A) IR spectra; B) and C) Crystal structures of the 2,9-Me_2OP/TCC complex by ORTEP drawing with 30% thermal ellipsoids, N···H–O H-bond is indicated by the dashed line, the secondary attractive N···H–O interactions and C-H···O H-bond are indicated by the dotted line (all other structures were drawn in the same form); B) View face-on to the aromatic rings of 2,9-Me_2OP and C) View along the edge of both TCC and 2,9-Me_2OP; D) Solid-state ^{13}C NMR spectra.

Synthesis

2,9-Me_2OP (50 μL, 100 mM in methanol) were mixed in the phosphate buffer (pH 7.4). After 10 min of vigorous stirring, the resultant white precipitate was filtered, then washed with water and methanol and dried in vacuum. The clean precipitate was then redissolved in methanol/dichloromethane. After 3 days, single crystals suitable for X-ray analysis were grown at room temperature. All other complexes mentioned in this study were also synthesized and crystallized by the procedure as described above.

Crystallographic data

CCDC-980526 (2,9-Me_2OP/quercetin), -980527 (2,9-Me_2OP/TCC), -980528 (4,7-Me_2OP/TCC), -980529 (5,6-Me_2OP/TCC), -980530 (2,9-Me_2OP/TBrC), -980531 (2,9-Me_2OP/TFC), -980532 (2,9-Me_2OP/3,5-DCC), -980533 (2,9-Me_2OP/4,5-DCC), -980534 (2,9-Me_2OP/4-CC), and -980535 (2,9-Me_2OP/Cat) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Results and Discussion

A multiple hydrogen-bonded complex with an unusual perpendicular conformation was formed between 2,9-Me_2OP and TCC

Our preliminary studies demonstrated that a white precipitate was formed when a solution of 2,9-Me_2OP was mixed with TCC, but not with NaN_3. We also found that the typical analytical methods used for solutions were not suitable for structure determination of the unknown precipitate mainly because of its poor solubility in both water and organic solvents. Further IR studies suggest that the original weak hydrogen-bonds (H-bonds) in the individual TCC or 2,9-Me_2OP (the wide peak at 3451 cm^{-1} for 2,9-Me_2OP was attributed to the N···H and O–H H-bonds because of the presence of trace amount of water) were no longer detected when they combined together, possibly forming new N···H–O H-bonds between 2,9-Me_2OP and TCC (Figure 1A; for detailed descriptions, see the Supporting Information). To obtain the unequivocal evidence for the formation of such H-bonds and to further characterize the chemical structure of the complex, single crystals of the complex were grown (for details, see "Synthesis" in the Experimental Section). The solid-state structure was determined by using single-crystal X-ray diffraction. The solution of the diffracted data clearly showed the formation of a H-bonded complex between 2,9-Me_2OP/TCC with a 1:1 stoichiometry (Figure 1B and Table 1; for detailed crystal data, see Table S1 in the Supporting Information).

Table 1. Selected angles (∢, [°]) and distances (*d*, [Å]) of the complexes formed between Me_2OP and TCC.

Complex	Inter-planar angle[a]	N···H–O H bonds		Secondary electrostatic interactions		C–H···O H bonds	
		d(N O)	∢(NHO)	*d*(N···O)	∢(NHO)	*d*(C···O)	∢(CHO)
2,9-Me_2OP/TCC	78.55	2.709	163.72	3.092	117.40	3.384	128.18
		2.709	163.72	3.092	117.40	3.384	128.18
4,7-Me_2OP/TCC	59.83	2.640	156.54	3.452	127.17	–	–
		2.655	151.86	3.281	123.06	–	–
5,6-Me_2OP/TCC	52.66	2.656	149.57	3.450	132.78	–	–
		2.656	149.57	3.450	132.78	–	–

According to the H-bond distance and angle, the strength of the two primary H-bonds was moderate. Interestingly, 2,9-Me_2OP and TCC bound in the complex adopt an unusual perpendicular relative arrangement, which was C_2 symmetrical (further supported by data from solid-state ^{13}C NMR spectroscopy, Figure 1 D; for detailed descriptions, see the Supporting Information), probably to minimize the steric hindrance caused by the two CH_3 substituents of 2,9-Me_2OP (Figure 1 C). The geometry of the complex allowed the hydroxyl H atom to also form a H-bond with N atom on the other side and established two secondary attractive H-bonding interactions between the two adjacent primary H-bonds. Furthermore, the distance between the H atoms of the two CH_3 substituents and the two adjacent oxygen atoms in the TCC was found to be as short as 2.690 Å, and this may lead to the formation of two weak C–H···O H-bonds, which should further stabilize the 2,9-Me_2OP/TCC complex. The blueshift observed for the C–H stretching vibration of the two CH_3 groups in the IR spectrum of the complex provides further support to this hypothesis (from 2954 to 2965 cm^{-1}; Figure 1 A).

Therefore, the combination of two primary N···H–O H-bonds with two additional attractive secondary H-bonding interactions and two weak C–H···O H-bonds, should provide a high thermodynamic stability to the 2,9-Me_2OP/TCC complex.

Similar H-bonded complexes were formed between 2,9-Me_2OP and other polyhalogenated catechols

We found that similar H-bonded complexes were formed when TCC was substituted by other tetrahalogenated catechols such as tetrabromocatechol (TBrC) and tetrafluorocatechol (TFC; Supporting Information, Figure S2). The two 2,9-Me_2OP/TBrC and 2,9-Me_2OP/TFC complexes also adopted a perpendicular arrangement of the binding partners and featured similar intermolecular distances to those described for 2,9-Me_2OP/TCC complex (the Supporting Information, Figure S2 and Table S2). Interestingly, we found that 2,9-Me_2OP is also able to form analogous H-bonded complexes with catechols featuring a reduced number of chlorine substituents as 3,5-diclorocatechol (3,5-DCC), 4,5-diclorocatechol (4,5-DCC), and 4-chlorocatechol (4-CC). Even the un-substituted catechol (Cat) formed a H-bonded complex with 2,9-Me_2OP. However, the lengths measured for the primary H-bonding interactions in the solid-state structures of these complexes increase as the substitution level of the catechol is reduced. This finding suggests that the thermodynamic stability of the complexes is proportional to the acidity of the phenolic groups of the catechol (the Supporting Information, Figure S2 and Tables S2 and S3).

The two CH_3 substituents at the 2,9 positions in 2,9-Me_2OP are critical to stabilize the H-bonded complex with TCC due to steric hindrance

To investigate the effect of the two CH_3 substituents in 2,9-Me_2OP on the thermodynamic stabilization of the complex 2,9-Me_2OP/TCC, we evaluated the binding properties of two regioisomers of 2,9-Me_2OP, 4,7- and 5,6-dimethyl-1,10-phenanthroline (4,7-Me_2OP and 5,6-Me_2OP). The only difference between the three regioisomers is the substitution positions of the two CH_3 groups on the 1,10-phenanthroline (OP) structure (the Supporting Information, Figure S1). The unsubstituted OP was selected as a reference model system.

Similar to our previous observations with 2,9-Me_2OP, we found that both 4,7-Me_2OP and 5,6-Me_2OP, but not the unsubstituted OP, could also form H-bonded complexes with TCC, and the two primary N···H–O H-bonds of the three Me_2OP/TCC complexes were similar to each other (Table 1 and Figure 2). However, for geometrical reasons the TCC complexes formed with Me_2OP without the CH_3 substituents at the 2,9 positions cannot establish C–H···O interactions between the CH_3 groups and the O atoms of the TCC.

Furthermore, the solid-state binding geometries of the complexes of 4,7-Me_2OP/TCC and 5,6-Me_2OP/TCC showed that the binding partners are arranged in a less perpendicular orientation. The observed change in the binding geometry of these complexes is a consequence of a more parallel orientation adopted by the two N···H–O intermolecular H-bonds. The H-bonding interactions are energetically more favored when the atoms involved are linearly oriented. The absence of substituents at the 2,9 positions on the OP made the complex structurally flexible, which most likely made the complex less stable and more prone to dissociation with competing agents like NaN_3 or solvent molecules (see below). We also observed that the secondary H-bonding interactions were significantly reduced in these complexes because the less perpendicular orientation made the hydroxyl group far away from the N atom on the other side.

Taken together, the results described above give a significant thermodynamic and kinetic advantage to the 2,9-Me_2OP/TCC H-bonded complex.

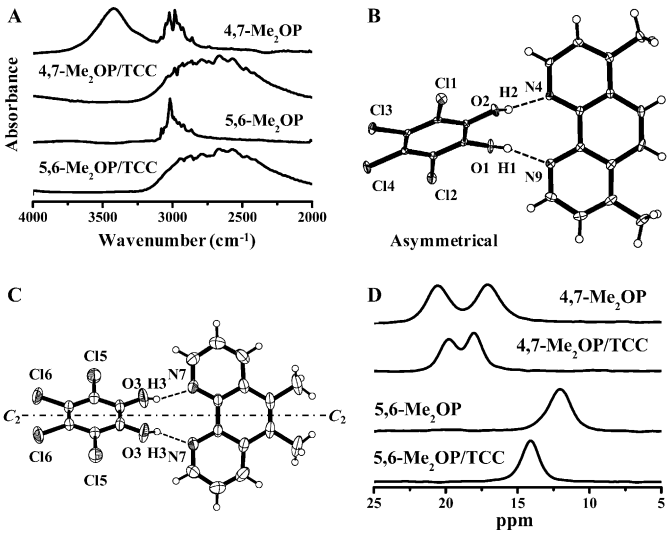


Figure 2. Less stable H-bonded complexes with TCC could be formed by 4,7-Me_2OP and 5,6-Me_2OP, the two regioisomers of 2,9-Me_2OP. A) IR spectra; B) and C) Crystal structure of the 4,7-Me_2OP/TCC and 5,6-Me_2OP/TCC complex; D) Solid-state ^{13}C NMR spectra.

Mechanism of protection by 2,9-Me_2OP against the synergistic toxicity induced by TCC/NaN_3: Inhibition of the reactive and toxic semiquinone radical generation by forming a stable and redox-inactive H-bonded complex

It has been shown that both the chemical structure and reactivity of different functional groups within a molecule could be modulated by forming H-bonded complexes.[16,17] Since 2,9-Me_2OP was found to form a rather thermodynamically stable H-bonded complex with TCC, we wondered whether it could be effective in reducing the known redox-activity of TCC. We expected that the trapping of TCC in the H-bonded complex may inhibit its reaction with NaN_3. Our previous work showed that the reaction between TCC and NaN_3 was accompanied with enhanced oxygen consumption (from 100 to 60%, Figure 3A: Control) and the formation of *O*-tetrachlorosemiquinone anion radical (*O*-$TCSQ^{\bullet-}$) and other semiquinone radicals ($SQ^{\bullet-}$; Figure 3C), which were considered to be the reactive and toxic species responsible for cell death.[15] We found that both the oxygen consumption and semiquinone radicals generation (as monitored by direct ESR) was inhibited by 2,9-Me_2OP in a dose-dependent manner. When the molecular ratio of [2,9-Me_2OP]/[TCC] = 1:1, no oxygen consumption was observed and the semiquinone radicals relative value was reduced to the base line, indicating that the reaction between TCC and NaN_3 was completely inhibited by 2,9-Me_2OP (Figure 3A and D). Even when 2,9-Me_2OP was added 3 min after the reaction was initiated, a sharp decrease of semiquinone radical signal close to the base line values was observed (Figure 3E).

In contrast, 4,7-Me_2OP, 5,6-Me_2OP, and OP only partially inhibited the oxygen consumption and semiquinone radical generation under the same experimental conditions used with 2,9-Me_2OP (Figure 3B and F). These results are in good agreement with our previous finding in the bacterial system.[15]

Based on previous studies,[15] we proposed that for TCC to be oxidized to generate the reactive and toxic semiquinone radicals, it is necessary that the hydroxyl group of TCC be first deprotonated to form its anionic form TCC^-(Scheme 1).

As mentioned above, the steric hindrance effect caused by the two CH_3 substituents at the 2,9 positions in 2,9-Me_2OP plays a critical role in stabilizing the H-bonded 2,9-Me_2OP/TCC complex. The combination of two primary N···H–O H-bonds with two additional attractive secondary H-bonding interactions and two weak C–H···O H-bonds, should provide a high thermodynamic stability to the 2,9-Me_2OP/TCC complex. These special features may completely inhibit the deprotonating process of TCC, and therefore inhibiting further oxidation of TCC (with or without NaN_3) to produce toxic semiquinone radical species.

In contrast, due to the absence of the two CH_3 groups at the 2,9 positions, although 4,7-Me_2OP and 5,6-Me_2OP can form similar two primary N···H–O H-bonds with TCC, they cannot form the two weak C–H···O H-bonds with TCC; and the secondary attractive interactions in the two 4,7-Me_2OP/TCC and 5,6-Me_2OP/TCC complexes are also markedly reduced because the less perpendicular orientation makes the hydroxyl group far away from the N atom on the other side (Figures 1 and 2, and Table 1). Thus the two 4,7-Me_2OP/TCC and 5,6-Me_2OP/TCC complexes are less stable as compared with 2,9-Me_2OP/TCC, and therefore, TCC may be partly deprotonated and more prone to be attacked by $N_3^-$to generate the reactive and toxic semiquinone radicals.

In summary, we have discovered that the steric hindrance provided by the two CH_3 substituents in 2,9-Me_2OP assists in

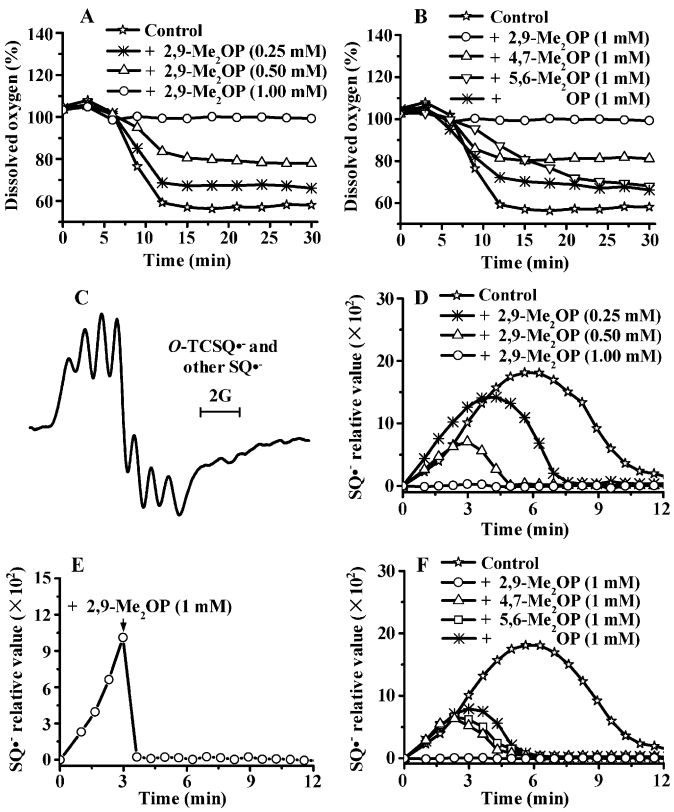
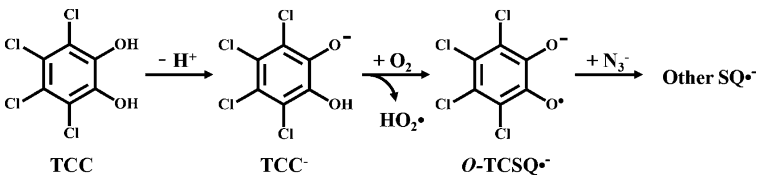
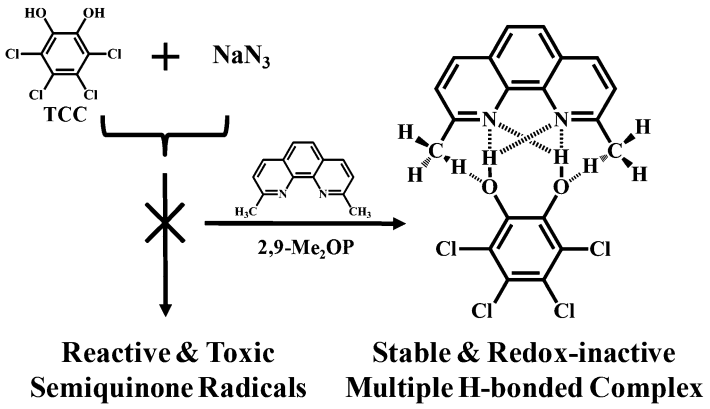


Figure 3. Inhibition of the reaction between TCC and NaN_3 by 2,9-Me_2OP and its isomers. The 40% oxygen consumption caused by 1 mM TCC and 2 mM NaN_3 (as a control) was completely inhibited by 1 mM 2,9-Me_2OP (A), but only partly inhibited by its two regioisomers 4,7-Me_2OP, 5,6-Me_2OP and OP under the same condition (B); The ESR signal of the *O*-$TCSQ^{\bullet-}$ and other $SQ^{\bullet-}$ radicals (C); The semiquinone radical generation was completely inhibited by 1 mM 2,9-Me_2OP (D), but only partly inhibited by its two isomers 4,7-Me_2OP, 5,6-Me_2OP and OP under the same condition (F); The semiquinone radical generation could be inhibited by 2,9-Me_2OP even after the reaction was initiated for 3 min (E). D–F were drawn according to the relative value of the highest peak of C with the time interval of 1.66 min. All the reaction mixtures contain 1 mM TCC and 2 mM NaN_3 (also as control), and the reaction was conducted in phosphate buffer (100 mM, pH 7.4) at room temperature.



Scheme 1. Proposed possible reaction pathway for TCC/NaN_3 to produce a toxic semiquinone radical species.



Scheme 2. Proposed mechanism of protection by the copper-chelating agent 2,9-Me_2OP against the synergistic toxicity induced by TCC/NaN_3: Inhibition of the reactive and toxic semiquinone radicals generation by forming a stable and redox-inactive multiple H-bonded complex with TCC.

the formation of a multiple H-bonded complex with TCC. The 2,9-Me_2OP/TCC complex features an unusual perpendicular arrangement of binding units. The complex is thermodynamically highly stable and is capable of inhibiting the reaction between TCC and NaN_3, and the subsequent generation of the reactive and toxic semiquinone radicals. In short, 2,9-Me_2OP completely eliminates the synergistic toxic effect induced by TCC/NaN_3 through multiple H-bonding with TCC (Scheme 2).

H-bonding is a contemporary research interest because of its fundamental importance in many branches of science.[18–21] Usually, the formation of H-bond would enhance chemical and enzymatic reactions by stabilizing the transition states or reaction intermediates.[22–24] However, in this study, we found an unique example where the H-bonding between 2,9-Me_2OP and TCC is so tight that it will make the reactive catechol group too stable to go further redox reactions.

Comparison between 2,9-Me_2OP/TCC and (2,9-Me_2OP)$_2$$Cu^I$ complexes

Compared with OP, 2,9-Me_2OP has two more CH_3 groups at positions 2 and 9. The steric hindrance of these two CH_3 groups leads it to bind favorably with cuprous ion to form the preferred tetrahedral structure, which is typical of many Cu^I complexes. Due to its high redox potential (+510.59 mV), as well as its high stability constant [$\log\beta_2 = 19.1$],[25] once (2,9-Me_2OP)$_2$$Cu^I$ is formed, copper will be stabilized at the Cu^I state, thus will be unable to allow redox-cycling between Cu^I and Cu^{II}.

Interestingly, these two CH_3 groups also lead 2,9-Me_2OP to bind favorably with TCC to form the preferred perpendicular structure. Due to its high stability through multiple H-bonding, once 2,9-Me_2OP/TCC was formed, TCC would be stabilized at the catechol state, thus will be unable to allow redox-cycling between catechol, its corresponding *O*-semiquinone radical and *O*-quinone (see above). In other words, 2,9-Me_2OP could not only chelate the inorganic Cu^I by forming a tetrahedral coordination structure, but could also "chelate" the organic catecholic compounds by forming perpendicular conformation through multiple H-bonding. Due to its unique steric hindrance effect of the two CH_3 groups at 2,9 positions, both of the two complexes were restricted, and the catechol group and/or Cu^I were also protected against the attack from other competing agents.

To our knowledge, this represents the first report showing that an unexpected new protective mode of action for the copper "specific" chelating agent 2,9-Me_2OP by using its steric hindrance effect of the two CH_3 groups not only to chelate copper, but also to "chelate" a catechol through multiple H-bonding (Scheme 1).

Potential biological implications

We found that the formation of the unusual H-bonded 2,9-Me_2OP complex is not only limited to TCC, but it is also a general mechanism for all polyhalogenated catecholic compounds. Therefore, our findings may have interesting biological and environmental implications because these polyhalogenated catecholic compounds are the reactive and toxic metabolites, or degradation products for many widely used polyhalogenated aromatic compounds (such as pentachlorophenol, Agent Orange, and hexachlorobenzene), which are considered probable human carcinogens and have also been detected in discharges from pulp and paper mills.[26–28]

Polyphenolic compounds, which are found in large amounts in fruits and vegetables, have been reported to exhibit beneficial antioxidant and anticancer activities.[29,30] However, they could also exert deleterious effects by generating reactive phenoxyl or semiquinone radicals.[31] Interestingly, 2,9-Me_2OP was also found to efficiently inhibit cytotoxic effects induced by some of these polyphenolic compounds.[32, 33] Since many of them contain the characteristic catecholic structure, we speculate that they probably underwent similar reaction with 2,9-Me_2OP to form H-bonded complexes. Indeed, we found that 2,9-Me_2OP could combine with quercetin, a typical polyphenolic compound, to form 2:1 2,9-Me_2OP/quercetin complex (the Supporting Information, Figure S3), which was stable enough to inhibit radical generation from the oxidation of quercetin (the Supporting Information, Figure S4 and Table S4). Therefore the formation of H-bonded complexes with catecholic compounds may serve as a general, but previously unrecognized copper-independent new detoxication mechanism for the widely used 2,9-Me_2OP. We suggest that special care should be paid when 2,9-Me_2OP was used to study the role of copper in the toxicity induced by polyphenolic compounds, especially when they possess a catecholic structure.

Therefore, our findings may have broad chemical, biological, and environmental significance for future research on both polyhalogenated aromatic pollutants and natural polyphenolic compounds, which are two important classes of catecolic compounds of major environmental and biomedical concern that

have been attracting the attention of both academic researchers and the broader general public.

Acknowledgements

The work in this paper was supported by the Strategic Priority Research Program of CAS Grant No. XDB01020300; NSF China Grants (21237005, 21321004, 20925724); and NIH Grants (ES11497, RR01008, and ES00210) (B.Z.).

Keywords: chelates · hydrogen bonds · radical reactions · reaction mechanisms · steric hindrance

[1] A. Barbouti, P. T. Doulias, B. Z. Zhu, B. Frei, D. Galaris, *Free Radical Biol. Med.* **2001**, *31*, 490 – 498.
[2] B. Z. Zhu, B. Kalyanaraman, G. B. Jiang, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 17575 – 17578.
[3] G. F. Smith, W. H. McCurdy, *Anal. Chem.* **1952**, *24*, 371 – 373.
[4] A. Mohindru, J. M. Fisher, M. Rabinovitz, *Nature* **1983**, *303*, 64 – 65.
[5] L. M. Sayre, *Science* **1996**, *274*, 1933 – 1934.
[6] H. H. Al-Sa′doni, I. L. Megson, S. Bisland, A. R. Butler, F. W. Flitney, *Br. J. Pharmacol.* **1997**, *121*, 1047 – 1050.
[7] B. Z. Zhu, M. Chevion, *Arch. Biochem. Biophys.* **2000**, *380*, 267 – 273.
[8] N. Ögülener, Y. Ergun, *Eur. J. Pharmacol.* **2004**, *485*, 269 – 274.
[9] C. Gocmen, B. Giesselman, W. C. de Groat, *J. Pharmacol. Exp. Ther.* **2004**, *312*, 1138 – 1143.
[10] T. O'Reilly-Pol, S. L. Johnson, *Zebrafish* **2008**, *5*, 257 – 264.
[11] N. Arnal, M. J. deAlaniz, C. A. Marra, *Chem.-Biol. Interact.* **2011**, *192*, 257 – 263.
[12] O. V. Patel, W. B. Wilson, Z. Qin, *BioMetals* **2013**, *26*, 415 – 425.
[13] Y. J. Appelbaum, J. Kuvin, J. B. Borman, G. Uretzky, M. Chevion, *Free Radical Biol. Med.* **1990**, *8*, 133 – 143.
[14] B. Z. Zhu, S. Levy, M. Chevion, *Free Radical Biol. Med.* **1999**, *27*, S127.
[15] S. Levy, M. Chevion, *Environ. Toxicol. Chem.* **2009**, *28*, 1380 – 1389.
[16] G. R. Desiraju, T. Steiner, *The Weak Hydrogen Bond: Structural Chemistry and Biology*, Oxford University Press, Oxford, **2001**.
[17] P. R. Schreiner, *Chem. Soc. Rev.* **2003**, *32*, 289 – 296.
[18] F. Weinhold, R. A. Klein, *Mol. Phys.* **2012**, *110*, 565 – 579.
[19] M. Meot-Ner, *Chem. Rev.* **2005**, *105*, 213 – 284.
[20] A. Shokri, J. Schmidt, X. B. Wang, S. R. Kass, *J. Am. Chem. Soc.* **2012**, *134*, 2094 – 2099.
[21] C. L. Perrin, *Acc. Chem. Res.* **2010**, *43*, 1550 – 1557.
[22] A. N. Thadani, A. R. Stankovic, V. H. Rawal, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5846 – 5850.
[23] W. K. Lim, J. Rösgen, S. W. Englander, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2595 – 2600.
[24] A. Warshel, P. K. Sharma, M. Kato, Y. Xiang, H. Liu, M. H. Olsson, *Chem. Rev.* **2006**, *106*, 3210 – 3235.
[25] B. R. James, R. J. P. Williams, *J. Chem. Soc.* **1961**, 2007 – 2019.
[26] N. Schweigert, A. J. Zehnder, R. I. Eggen, *Environ. Microbiol.* **2001**, *3*, 81 – 91.
[27] B. Z. Zhu, J. G. Zhu, L. Mao, B. Kalyanaraman, G. Q. Shan, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 20686 – 20690.
[28] B. Z. Zhu, L. Mao, C. H. Huang, H. Qin, R. M. Fan, B. Kalyanaraman, J. G. Zhu, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16046 – 16051.
[29] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouysegu, *Angew. Chem.* **2011**, *123*, 610 – 646; *Angew. Chem. Int. Ed.* **2011**, *50*, 586 – 621.
[30] J. M. Landete, *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 706 – 721.
[31] T. Miura, S. Muraoka, Y. Fujimoto, *Food Chem. Toxicol.* **2003**, *41*, 759 – 765.
[32] H. Y. Khan, H. Zubair, M. F. Ullah, A. Ahmad, S. M. Hadi, *Curr. Drug Targets* **2012**, *13*, 1738 – 1749.
[33] H. Y. Khan, H. Zubair, M. Faisal, M. F. Ullah, M. Farhan, F. H. Sarkar, A. Ahmad, S. M. Hadi, *Mol. Nutr. Food Res.* **2014**, *58*, 437 – 446.

Received: March 29, 2014
Revised: June 12, 2014
Published online on August 14, 2014