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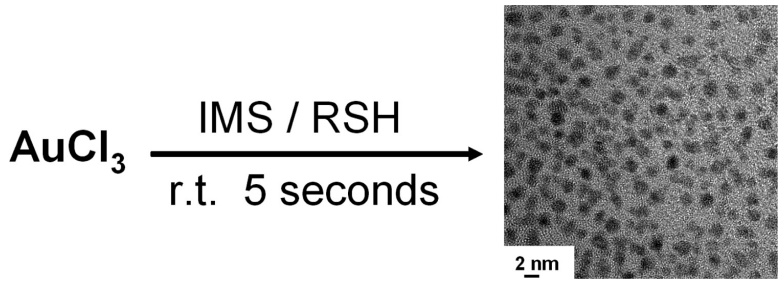

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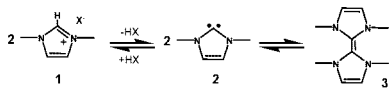
Imidazolium Salts: A Mild Reducing and Antioxidative Reagent

Lan Zhao, Chunyan Zhang, Lang Zhuo, Yugen Zhang,* and Jackie Y. Ying*

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669

Received May 29, 2008; E-mail: jyying@ibn.a-star.edu.sg; ygzhang@ibn.a-star.edu.sg

Imidazolium salts (IMSs) are well-known as room-temperature ionic liquids (RTILs) that can be used as electrolytes or green solvents because of their low vapor pressure and wide chemical stability.[1] IMSs are also known as precursors for stable carbenes or bisimidazolidine with many applications in organic synthesis.[2,3] Most of the *N*-heterocyclic carbenes (NHCs) are sensitive to moisture.[4] However, a natural analogue of this group, thiamine or vitamin B, plays a very important biological role in the aqueous environment.[5] Although the physical behavior of IMSs in aqueous media has been widely studied, their chemical properties and applications have seldom been mentioned.[6] Amyes and co-workers have reported the carbon acid pK_a of a series of imidazolium cations in aqueous solutions.[7] The equilibrium in eq 1 is shown to always exist. Therefore, bisimidazolidine (**3**) is known as a superelectron-donor (S.E.D.) reagent and has been used as a reducing agent in some reactions.[8] These studies prompted us to investigate the redox properties of IMSs.



(1)

First, IMS (1,3-dibenzylimidazolium bromide (**4**)) was employed in the synthesis of gold particles. It was found that ultrafine gold nanoparticles could be very efficiently derived in IMS/thiol solution in seconds at room temperature. The small gold nanoparticles were well dispersed in the solvent to form a clear solution that was stable for at least 6 months at 4 °C. Transmission electron microscopy images showed that uniform gold nanoparticles (1−2 nm in size) were produced. Similar products were obtained by using different IMSs, solvents, and thiol/Au ratios.[9] Compared to the commonly used borane or borohydride reduction processes,[10] no strong reducing reagent was added in this case. The ultrafine gold nanoparticles were obtained under a very mild reaction condition with remarkable efficiency. This new method could be easily scaled up for industrial applications.[11,12]

Further studies showed that there were large amounts of 1-benzylimidazole, benzyl chloride (bromide), and disulfide present in the reaction system. Combined with other observations (see Supporting Information (SI)), it was proposed that the carbene derivative[7] of **4** coordinated with and reduced the gold cations, followed by decomposition to benzyl chloride (bromide) through a radical pathway (SI, Scheme S1). The thiol further reduced gold complex to Au(0) and released benzylimidazole, which played a very important role as a ligand generated in situ in coordination with and protection of Au(0) to form stable, ultrafine nanoparticles. This radical intermediate pathway was also commonly accepted in the carbene or bisimidazolidine reduction processes.[13,8]

On the basis of the understanding of IMS-promoted gold reduction, it was proposed that stable IMS could also be a potential candidate as a radical scavenger antioxidant in a cellular system.[14] Thiamine deficiency has been known to be related to oxidative

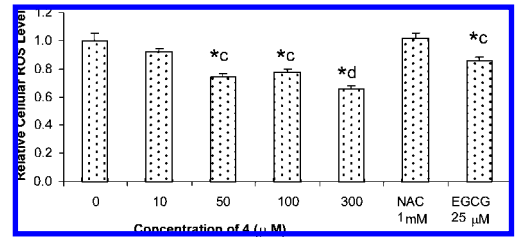


Figure 1. IMS attenuated cellular ROS level. HSC-T6 cells were incubated with **4** of different concentrations, NAC (1 mM) and EGCG (25 μM). Data were presented as relative value after normalization against vehicle control, and as mean and standard error of mean (SEM), $N = 6$. *^{c} $P < 0.005$; *^{d} $P < 0.0005$.

stress.[15] There have also been attempts to investigate the biological effects of some NHC−metal complexes in a cell-free system.[16] To examine if the metal-free IMSs possessed beneficial biological properties, herein we investigated the antioxidative capacity of IMSs by accessing the cellular oxidative stress level in cultured hepatic stellate cells (HSC-T6) treated with **4**. Cultured HSCs are known to be routinely activated when cultured on the plastic surface of the culture flask and are therefore used as an *in vitro* model of liver fibrosis. Oxidative stress resulting from the metabolic generation of reactive oxygen species (ROS) is believed to be implicated in HSC activation and liver fibrogenesis and many other diseases, such as cancer, autoimmune disorders, neurodegeneration, and aging.[17] Cells treated with **4** (10, 50, 100, and 300 μM) for 48 h in full serum medium were assayed for ROS. Cells treated with *N*-acetyl-L-cystein (NAC) (1 mM) and (−)-epigallocatechin gallate (EGCG) (25 μM) separately were included as references.[18] ROS level was significantly attenuated under the treatment of **4**. As shown in Figure 1, ROS was dose-dependently suppressed (25% for 50 μM ($P < 0.005$) and 34% for 300 μM ($P < 0.0005$)). In comparison, EGCG (25 μM) was able to attenuate 14% ROS ($P < 0.005$), while NAC (1 mM) did not show any apparent inhibition of ROS level under the same scheme of treatment. In addition, **4** exhibited a much lower cytotoxicity ($IC_{50} = 1.7$ mM) as compared to the natural antioxidant EGCG ($IC_{50} = 30$ μM) assayed on the same HSC-T6 cells (Figure 2). A full characterization of **4** and additional IMSs in cultured HSCs will be presented in detail elsewhere.[19b]

This study demonstrated IMSs as a promising antioxidant that could effectively attenuate ROS in a dose-dependent manner. Other assays (glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD)) were also conducted, and similar effects were achieved with IMSs.[19] The antioxidative power of IMSs was proposed to be through the direct neutralization of hydrogen peroxide or other radical species by carbene or bisimidazolidine (see eq 1). However, the interactions between carbene and radicals were not well-known.[13] To support this hypothesis, two model reactions between stable carbenes and radical oxidants were conducted (see Scheme 1). Stable carbene mesitylimidazolinylidene

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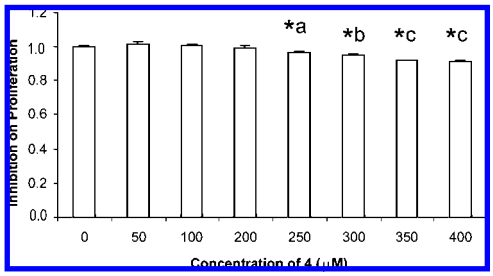
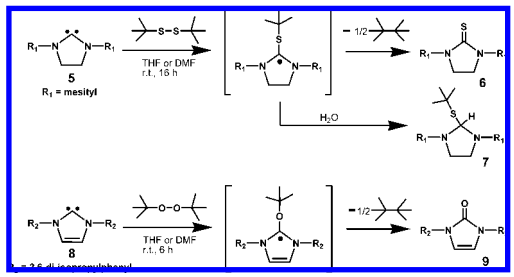


Figure 2. Cytotoxicity of **4**. HSC-T6 cells were seeded at a density of 5000 cells/well in 96-well plate, and adapted for 18−24 h in Dulbecco modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), before the addition of compounds of various concentrations (0−400 μM) in DMEM containing 10% FBS for 48 h. They were then assayed for proliferation. Signal was normalized against vehicle treatment (0 μM compound). Data were obtained from four independent experiments, and presented as mean and SEM. $*^a$ $P < 0.01$; $*^b$ $P < 0.005$; $*^c$ $P < 0.0005$.

Scheme 1



5 derived from 1,3-bis-(2,4,6-trimethylphenyl)imidazolinium (SIMes) reacted with *tert*-butyldisulfide to form a radical intermediate, which further converted to a thiourea product **6**. When this reaction was quenched by water, hydrogenated intermediate product **7** was also isolated. Stable carbene 2,6-bisisopropylphenylimidazolylidene **8** derived from 1,3-bis-(2,6-bisisopropylphenyl)imidazolium (IPr) was less active in this reaction, and heating was necessary to promote the reaction. When more active peroxide was mixed with carbene species, the reaction occurred much faster than the disulfide system. Carbene **8** reacted with *tert*-butylperoxide to form a urea compound **9**, and underwent further decomposition. Here, a radical intermediate was also proposed, however, the analogue compound of **7** could not be isolated after quenching the reaction, probably due to the short lifetime of this intermediate. The reaction between electron-rich carbene **5** and peroxide was faster and directly resulted in the structure decomposition of the five-membered ring.[9] These results clearly illustrated that NHC could trap and react with radicals or peroxides. They further explained the antioxidative power of IMSs in biological systems.

In conclusion, on the basis of the equilibrium between IMSs, carbene, and bisimidazolidine, the concept of directly using IMSs as mild reducing and antioxidative reagents was proposed and investigated. A simple and robust protocol for the synthesis of stable, ultrafine gold nanoparticles has been established using IMSs under mild conditions. The mechanism of this new protocol was also examined. IMS **4** showed remarkably lower toxicity but greater antioxidative power than EGCG or NAC on HSC-T6 cells. These findings illustrated that the simple and inexpensive IMSs represent a new type of antioxidant with potential biomedical applications. The antioxidative property of IMSs was proposed to be through the neutralization of peroxide or radicals, which was further confirmed by the model reaction between stable carbene with disulfide or peroxide. Additional mechanistic studies on both the chemical and biological aspects of IMSs' actions in vitro and in vivo are under way.

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Supporting Information Available: Synthesis of gold nanoparticles, antioxidative properties of IMSs, toxicity of **4** on HSC-T6 cells, and reaction between stable carbenes with disulfide and peroxide. This material is available free of charge via the Internet at http://pubs.acs.org.

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