NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Kinetic Analysis of CO-releasing From a Diiron Hexacanbonyl Complex Promoted by Amino Acids

Limei Chen [1, 2], Xiujuan Jiang [2]*, Xiu Wang [2], Li Long [2], Jiayao Zhang [2], Xiaoming Liu [1, 2]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

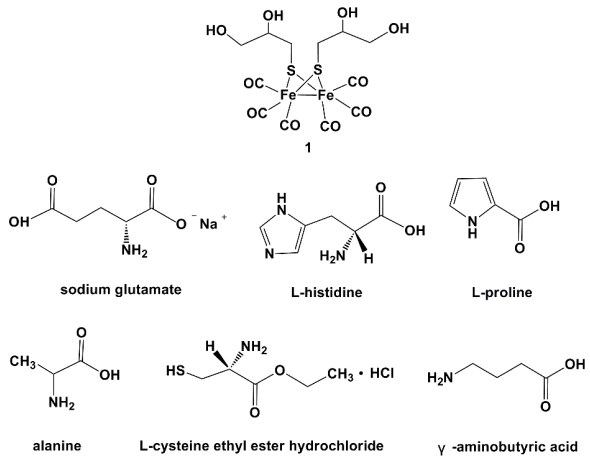
DOI: 10.1039/b000000x

A water-soluble diiron hexacarbonyl complex, $[Fe_2\{\mu\text{-}SCH_2CH(OH)CH_2(OH)\}(CO)_6]$ (**1**), was employed as carbon monoxide releasing molecule (CO-RM). The CO-releasing was initiated *via* substitution of the bound CO by amino acids. The kinetics of the decomposition of complex **1** was first-order process for both the complex and amino acids, respectively. Its CO-releasing rate varies with the amino acids. Six amino acids were examined, L-cysteine ethyl ester hydrochloride, alanine, γ-aminobutyric acid, L-histidine, L-proline, sodium glutamate, as a CO-releasing promoter. Among the examined promoters, sodium glutamate shows the highest efficiency in CO-releasing from complex **1**. The CO-releasing involves multiple mechanisms, an initially slow CO-releasing process followed by a much faster one. The period of the slow process is solvent-dependant. In D_2O and physiological saline (D_2O), this slow process lasted much longer compared to that in DMSO. Cytotoxic assessments of the systems containing glutamate and L-cysteine ethyl ester hydrochloride suggest that the examined amino acid and derivative showed stronger cytotoxicity than cysteamine.

Introduction

Like nitrogen analogue NO, carbon monoxide (CO) is believed an essential signalling molecule in human body [1, 2]. It is active in the cardiovascular system as a vasodilator [3]. In addition, CO possesses anti-inflammatory, anti-apoptotic and anti-proliferative properties and protects tissues from hypoxia and reperfusion injury. These properties render CO a great potential in medical applications [3, 4]. To deliver CO, one approach is inhaling directly the gas [1, 5]. In the early development of CO-RMs, CH_2Cl_2 was even employed to produce CO *via* metabolic pathway [6]. The shortcomings of these delivering manners are obvious because of potential poisoning by CO and unnecessary side-effects caused by the metabolising of the pro-drugs. Therefore, it is important to find alternative approaches to administrate CO. Using metal–carbonyl complexes is a solution to achieve safe and controllable CO-delivery in therapeutic applications due to the advantage of their high CO-capacity, possibly six CO per metal centre. In the past decade, investigating the potentials of metal–carbonyl complexes as CO-releasing molecules (CO-RMs) has attracted great attention [7-20].

A variety of transition metals, for example, rhenium, iron, manganese, molybdenum and tungsten, have been synthesized and tested as CO-RMs [21-28]. To achieve CO-releasing from metal carbonyl complexes, there are many approaches in consideration [29]. For example, ligand-substitution, enzymatic degradation, irradiation and redox reaction of CO-RMs can trigger CO-releasing. Of the various approaches is substitution reaction. Recently, we reported a water-soluble diiron hexacarbonyl complex, $[Fe_2\{\mu\text{-}SCH_2CH(OH)CH_2(OH)\}_2(CO)_6]$ (**1**), from which CO-releasing was achieved *via* substitution reaction by cysteamine (CysA) [30]. Cysteamine is a clinic medicine for the prevention and treatment of radiation sickness caused by X-ray or other radiation [31]. Therefore, it should be tolerable by human bodies.



Scheme 1 Structures of complex **1** and amino acids or their derivatives used in this work

To initiate CO-releasing *via* substitution reaction, it is essential to choose an appropriate promoter which initiates not only CO-releasing, but also show minimal toxicity to human bodies. Amino acids are a basic building block of proteins. In addition to the amino and carboxyl groups, there may be also other functional groups, for example, thiol, phenol, imidazole, which

are good nucleophiles to attack the complex to release CO. Herein, we report the CO-releasing of complex **1** triggered by six amino acids or their derivatives, alanine, γ-aminobutyric acid, L-proline, L-histidine, L-cysteine ethyl ester hydrochloride, sodium glutamate (Scheme 1), and its kinetic analysis. The CO-releasing investigation was performed in three media, DMSO / H_2O mixture (4:1), D_2O and deuterated physiological saline. The CO-releasing was monitored using infrared spectroscopy as used in our previous report [30].

Experimental

Materials and instrumentations

Alanine, γ-aminobutyric acid, L-proline, L-histidine, L-cysteine ethyl ester hydrochloride and sodium glutamate were purchased from Aladdin and used as supplied. Complex **1** was synthesized using the procedure we reported recently [30]. FTIR spectra were recorded on Agilent 640 using a CaF_2-cell with a spacer of 0.1 mm. (wavelength range: 400 – 4000 cm^{-1}; repetitive scans: 8; resolution: 1 cm^{-1}). ^{13}C NMR spectra were measured on Bruker Avance (400 MHz) with tetramethylsilane as internal standard.

Monitoring the CO-releasing

A typical procedure for the monitoring is as follows: To a solution of complex **1** (17 mg, 0.0345 mmol) in DMSO (2.4 mL) was added an appropriate volume of an aqueous solution of L-cysteine ethyl ester hydrochloride (0.6 mL, 0.0115 mol L^{-1}). In the reaction mixture, the final concentrations of the two species are 0.0276 and 0.0023 mol L^{-1}, respectively. The ratio in volume of DMSO over water is 4 at the end. The reaction was maintained at 37 ℃ and regularly monitored using infrared spectroscopy. The CO-releasing initiated by alanine, γ-aminobutyric acid, L-proline, L-histidine and sodium glutamate, respectively, was analogously performed. The CO-releasing assessment carried out in either D_2O or physiological saline in D_2O (0.9%, 0.15 mol L^{-1}) was completed using the same procedure as described above. But in the two media, a minimum DMSO (50 μL) was added to dissolve complex **1**.

Cytotoxicity evaluations using MTT assay

QSG-7701 cell (100 μL, 5×10^{-3} cells mL^{-1}) were seeded into 96 well microtitre plates and left to adhere for 6 h. The media was removed from the wells after cultivation 6 h and replaced with filtered and sterilised complete media containing a mixture of complex **1** and L-cysteine ethyl ester hydrochloride or sodium glutamate in which the concentration of complex **1** was 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μmol L^{-1}, respectively, and the ratio of complex **1** over L-cysteine ethyl ester hydrochloride or sodium glutamate was kept at 1 : 6. The cells were then incubated for another 24 h before the incubation media were replaced with the complete medium and MTT (10 μL, 5 mg mL^{-1} in phosphate buffer solution, PBS) was added to each well of the plate. The cells were further incubated for 4 h before the media were replaced with DMSO (100 μL). Absorbance at 490 nm for each well of the plates was read with a microplate reader. In the MTT assay, DMSO (100 μL) in a well was used as blank and cells in well without the addition of CO-releasing agents (complex **1** and L-cysteine ethyl ester hydrochloride or sodium glutamate) were taken as a control (100 % in cell viability). Relative cell viability is expressed as $(A_{obs} - A_b) / (A_c - A_b)$, where A_{obs}, A_b, A_c, are absorbance observed for the cells treated with the CO-releasing system (complex **1**-L-cysteine ethyl ester hydrochloride or sodium glutamate), blank, and control, respectively. Inhibiting rate (%) was calculated as $\{100 \times [(A_{obs} - A_b) / (A_c - A_b) \times 100]\}$. Each concentration was assayed in 5 wells of the same plate, which was repeated 3 times to examine the reproducibility of the assessment. IC_{50} values were estimated by using MTT assay.

Results and discussion

CO-releasing in DMSO / H_2O

Amino acids are a building block of proteins, in which there are amino, carboxylic groups, and even other additional functional groups, such as thiol. They are all good nucleophiles. Furthermore, many amino acids have medical applications, for example, cysteine can be used for detoxification [32]. Inspired by our recent report in which a water soluble diiron hexacarbonyl complex, $[Fe_2\{\mu\text{-}SCH_2CH(OH)CH_2(OH)\}_2(CO)_6]$ (**1**), releases CO triggered by the initiation of cysteamine (CysA) [30], we explored further using amino acids or their derivatives as nucleophiles to promote CO-releasing from complex **1**. In this investigation, alanine, γ-aminobutyric acid, L-proline, L-histidine, L-cysteine ethyl ester hydrochloride and sodium glutamate were employed. For the convenience of monitoring the process *via* infrared spectroscopic technique, CO-releasing assessment was carried out in a mixture of DMSO / H_2O (v / v = 4 / 1). Considering strong interference in the range of 2000-1900 cm^{-1} from the absorption of water, the usage of water was kept minimal.

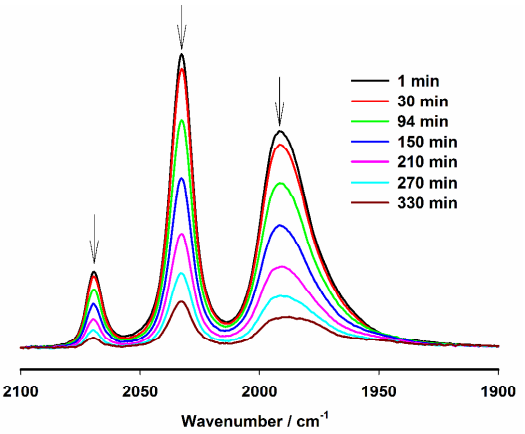


Fig. 1 Infrared spectral variation during the CO-releasing process ([**1**] = 0.0115 mol L^{-1} and [sodium glutamate] = 0.0345 mol L^{-1}) in DMSO/H_2O mixture at 37 ℃ under open atmosphere.

Steady progress of the reaction is shown in Fig. 1 during the reaction course with the presence of sodium glutamate. The characteristic infrared absorption peaks of complex **1** (2068, 2032, 1989 cm^{-1}) decreased continuously with the reaction time, which indicates that the reaction between complex **1** and sodium glutamate proceeds steadily to release CO. Increasing the concentration of sodium glutamate accelerates the decomposition of complex **1**. When the same equivalent γ-aminobutyric acid was added instead of sodium glutamate, the decomposition rate of

complex **1** slowed down considerably (Fig. 2). The IR spectral variations of the CO-releasing of complex **1** promoted by L-histidine, L-proline, alanine, L-cysteine ethyl ester hydrochloride are shown in Figs. S1-S4. Therefore, the amino acids can trigger CO-releasing *via* substitution reaction. Owing to poor solubility of the promoters in water and DMSO, only L-cysteine ethyl ester hydrochloride was examined how the decomposition is affected by varying the concentrations. The other amino acids were only examined at one concentration (three equivalents of the iron-carbonyl complex).

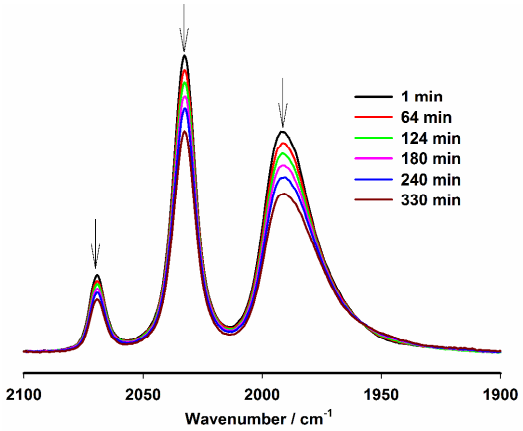


Fig. 2 Infrared spectral variation during the CO-releasing process ([**1**] = 0.0115 mol L^{-1} and [γ-aminobutyric acid] = 0.0345 mol L^{-1}) in DMSO / H_2O mixture at 37 ℃ under open atmosphere.

As widely known, the bound CO of diiron carbonyl compounds can be replaced by nucleophiles [33]. Undoubtedly, the functional groups of the amino acids serve as good nucleophiles to replace the bound CO of complex **1**, which induces its decomposition to release CO. In our previous study, it has been reported that complex **1** releases CO with the rate constant of 0.94 s^{-1} under the initiation of cysteamine *via* decomposition [30]. The decomposition undergoes a number of stages involving several intermediates such as diiron tetracarbonyl and monoiron dicarbonyl and Fe(0) species [30, 34]. Spectrally, the process becomes much simpler when the CO-releasing is carried out without the protection of inert atmosphere [30]. As we reported previously [30], the CO-releasing involves oxidative decomposition. When the decomposition process was exposed to air, the destruction of complex **1** accelerated due to the involvement of O_2. The influence of O_2 on the CO-releasing is confirmed by varying qualitatively the content of O_2 in the assessment, Table 1. The observation is in agreement with what we reported recently [30]. But contrary to the CO-releasing caused by CysA, the CO-releasing substitution by the compounds examined in this work does not produce any detectable intermediates at the spectroscopic time scale under either anaerobic or aerobic conditions. Our experience and report in the literature [35] indicate that the stability of diiron hexacarbonyl complexes could be considerably compromised due to the presence of carboxylic acid in the bridging linkages. Although, it may not be possible to exclude other either kinetic or thermodynamic causes, the carboxylic group in these compounds except cysteine ethyl ester must have played an important role in destabilising these intermediates generated during the CO-releasing.

Table 1 The kinetic data of the decomposition of complex **1** in DMSO / H_2O mixture at 37℃ ([**1**] = 0.0115 mol L^{-1}, [L-cysteine ethyl ester hydrochloride] = 0.0345 mol L^{-1}) under different conditions.

	N_2	N_2 + [a] O_2	open atmosphere
$k_{obs} \times 10^{-3}$	0.8	1.2	1.7
$t_{1/2}$ (min)	866	578	408

[a] 100 ml O_2 was injected into a reaction flask (10 ml) sealed with a suba-seal.

In addition to infrared spectroscopic technique, we also monitored the CO-releasing initiated by L-cysteine ethyl ester hydrochloride in deuterated DMSO using ^{13}C NMR spectroscopy, Table 2 and Fig. S5. As the data suggested, the NMR spectroscopic monitoring is not quite informative in terms of identifying any species involved in the process. But the monitoring results did show the steady progress of the reaction as indicated by the variation of chemical shifts at approximately 70, 60, 50 and 40 ppm and ultimate disappearance of the characteristic bands at 210.3 and 209.1 ppm of the bound CO in complex **1** after 24 hours' reaction, which implied the complete decomposition of complex **1**. And the second, paramagnetic species were involved in the process since once the reaction started, the field-locking of the system became difficult in one hour in collecting NMR data.

Table 2 ^{13}C NMR spectroscopic variation with reaction time of complex **1** under the substitution of L-cysteine ethyl ester hydrochloride (for comparison, the chemical shifts of free 1-thioglycerol, L-cysteine ethyl ester hydrochloride and complex **1** are also included).

Compounds	δ (ppm)				
1-thioglycerol		72.9, 64.3			28.1
L-cysteine ethyl ester hydrochloride	168.1		62.4, 54.3		24.6, 14.4
Complex **1**		72.7, 72.6, 67.5, 65.1, 65.0, 64.9		43.3, 41.9	29.3, 25.6
[a]Complex **1** (1 min)	168.6, 168.3	72.7, 72.6, 67.5, 65.1, 64.9 **68.8**	62.5, 62.6, 54.4 **51.9**	43.4, 42.0	29.4, 25.6, 24.8, 14.5
[a]Complex **1** (1 h)		72.7, 72.6, 67.6, 65.1 **70.5**,	62.8, 51.8,	**43.8, 43.4**	29.4, 25.7, 14.7
[a]Complex **1** (2 h)		72.8, 72.9, 72.6, 67.6, 65.1, 64.9 **70.6**,	**63.2**		29.5, 25.8, 15.0
[a]Complex **1** (22 h)		70.4, 64.8			

[a]Containing the ligand, L-cysteine ethyl ester hydrochloride.

Variations of the absorbance at 1900 cm^{-1} with the reaction course are shown in Fig. 3. Unlike the analogous plot we reported earlier [30], the exponential decline in absorbance is not as obvious as what was observed in our earlier report probably due to the much slower reaction. Kinetic analysis was performed when amino acids were presented in excess. The logarithmic plots of

different amino acids *versus* reaction time are shown in Fig. 4. The linear relationship observed for these reactions suggests that the CO-releasing progress is first-order reaction for complex **1**. All the kinetic data were summarized in Table 3. The results indicate that the CO-releasing rate for complex **1** differs significantly from the amino acids. Among the examined compounds, sodium glutamate promotes CO-releasing most efficiently whereas γ-aminobutyric acid shows the lowest CO-releasing efficiency. Compared with glutamate, the half-life time of its reaction with complex **1** is about 7-fold longer (Table 3). The drastic variation in decomposing rates of complex **1** should be certainly associated with the number of functional groups and the structural nature of the compounds. It is interesting to note that both glutamate and histidine possess an extra functional group (carboxylic and imidazolyl group, respectively). And the two amino acids promote most efficiently CO-releasing from complex **1**. For both proline and alanine, no additional functional group is available and they have comparable k_{obs} values which are smaller compared with those observed for both glutamate and histidine. The half-life time of the CO-releasing reactions promoted by both cysteine ethyl ester and γ-aminobutyric acid are approximately 3 and 7 times longer than that by glutamate, respectively. It is particularly noteworthy that cysteine ethyl ester is significantly less efficient in the promotion of CO-releasing than cysteamine [30] although they possess the same functional groups of the same moiety ($HSCH_2CH_2NH_2$) compared with CysA. It seems that the additional hydrophilic and electron-withdrawing ethyl ester skeleton has profound influence on its reaction with complex **1**. It would be imaginable that cysteine itself could be efficient on inducing CO-releasing. Unfortunately, assessing its CO-releasing capability is hindered by its extremely poor solubility in the media employed in this work. At first glance, γ-aminobutyric acid should have comparable efficiency of CO-releasing to that of either proline or alanine. But, in fact, its half-life time is about 4-fold longer, Table 3. Further looking into the other five bidentate ligands, we could notice that all the ligands except γ-aminobutyric acid can form either six-member ring or five-member ring with the diiron center or one of the two iron atoms. This chelating effect is certainly beneficial to the substitution reaction which leads to the relatively fast CO-releasing. Apparently, γ-aminobutyric acid does not have this advantage. Probably, being lack of this effect may be the major cause of its low efficiency on promoting CO-releasing.

As aforementioned, only L-cysteine ethyl ester hydrochloride was examined at various concentrations in DMSO / H_2O medium due to poor solubility of the ligands. The results of the kinetic analysis are shown in Table 4, Fig. 5 and Fig. S6, respectively. As shown in Table 4, the values of k_{obs} increase steadily with the increasing of the concentration of the ligand (L-cysteine ethyl ester hydrochloride). Plotting the observed rate constant k_{obs} against the concentration of the ligand gave a linear relationship between k_{obs} and the concentration of the ligand (Fig. S6). From the linear plot, the absolute rate constant k of the reaction was derived as 0.0099 s^{-1}. The kinetic behaviours are in agreement with those we reported recently [30]. Overall, for both the diiron complex and L-cysteine ethyl ester hydrochloride, the first-order kinetics applies. As being pointed out above, despite their possessing the same functional groups, cysteamine promotes CO-releasing about 100 times faster than cysteine ethyl ester hydrochloride (0.94 *versus* 0.0099), which is further indicative of the influence of a carboxylic acid on destructing the metal carbonyl complex as mentioned earlier.

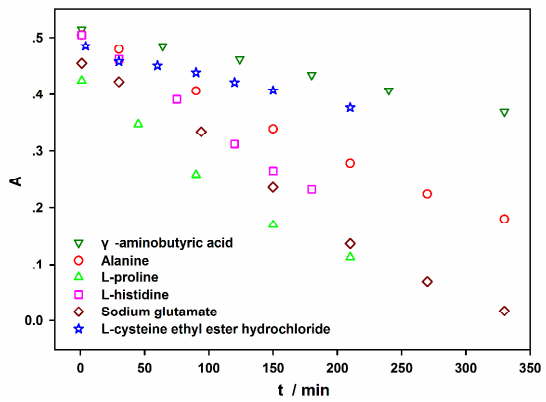


Fig. 3 Plots of concentrations of complex **1** against reaction time in the presence of different amino acids ([**1**] = 0.0115 mol L^{-1} and [compound] = 0.0345 mol L^{-1}) in DMSO / H_2O mixture (4 : 1) at 37 ℃ under open atmosphere (the absorbance used for the kinetic analysis was used at 1990 cm^{-1}).

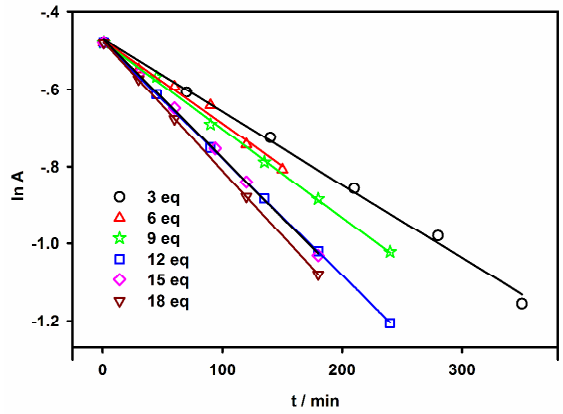


Fig. 4 The logarithmic plots of concentrations of complex **1** against reaction time in the presence of various concentration of L-cysteine ethyl ester hydrochloride in DMSO / H_2O mixture (4:1) at 37 ℃ under open atmosphere ([**1**] = 0.0115 mol L^{-1}, the absorbance used for the kinetic analysis was used at 2032 cm^{-1}).

CO-releasing in D_2O and physiological saline

Clinic application of any CO-RMs requires that they are operational in water. Furthermore, examination of CO-releasing kinetics under physiological condition is also desirable. For the convenience of applying infrared spectroscopic technique to monitor the CO-releasing, deuterated water was used. Again, due to the insolubility of other compounds in the media, only L-cysteine ethyl ester hydrochloride in D_2O and physiological saline (in D_2O) were examined. The CO-releasing behaviours of complex **1** upon the substitution reaction by the ester are shown in Figs 6 and 7, respectively. Compared with the kinetic behaviours in DMSO, the decomposing kinetic of complex **1** is strikingly different. At all the concentrations examined, an initial stage was observed along the reaction coordinate in both media, during which decomposition is very slow. The only difference is

that the stage lasted about 100 min in D_2O whereas in physiological saline, it took a longer time (*ca.* 150 min). After this stage, the decomposition became significantly faster. The two reaction stages may be attributed to the different reaction mechanisms of the CO-releasing. When further looking into the data-set of other amino acids, for example, praline and histidine (Fig. 8), it seems that such mixed mechanism exist also. Since the ligands examined so far are bidentate, we wondered whether such stage-wise decomposition is associated with this nature. In other words, initially only one of the functional groups plays a role and the two functional groups act concertedly when the reaction proceeds to certain stage. To verify this, a monodentate ligand, pyridine, was employed to decompose complex **1**. Indeed, no such two-stage decomposition was observed (Fig. S7).

Table 3 The half-life time of the decomposition of complex **1** in DMSO / H_2O (v / v = 4 / 1) at 37 ℃ ([**1**] = 0.0115 mol L^{-1}, [Compound] = 0.0345 mol L^{-1}).

Compound	$k_{obs} \times 10^{-3}$	$t_{1/2}$ (min)
Sodium glutamate	5.7	127
L-histidine	4.9	139
L-proline	2.9	190
Alanine	3.0	217
L-cysteine ethyl ester hydrochloride	1.7	408
γ-aminobutyric acid	0.9	818

Table 4 The kinetic data of the decomposition of complex **1** in DMSO / H_2O (v / v = 4 / 1) at 37℃ ([**1**] = 0.0115 mol L^{-1}, [L-cysteine ethyl ester hydrochloride] / [**1**] = 3, 6, 9, 12, 15 and 18, respectively).

$t_{1/2}$ (min)	408	315	301	231	224	204
$k_{obs} \times 10^{-3}$	1.7	2.2	2.3	3.0	3.1	3.4

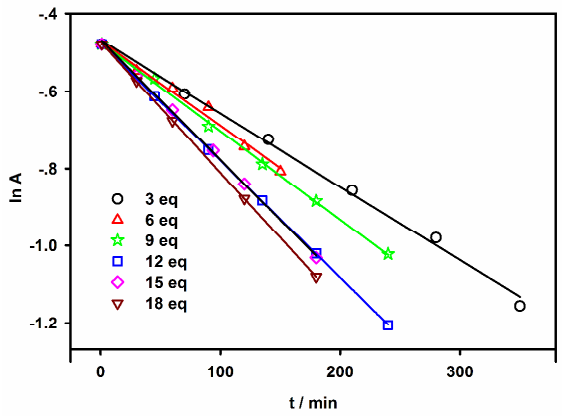


Fig. 5 The logarithmic plots of concentrations of complex **1** against reaction time in the presence of various concentration of L-cysteine ethyl ester hydrochloride in DMSO / H_2O mixture (4:1) at 37 ℃ under open atmosphere ([**1**] = 0.0115 mol L^{-1}, the absorbance used for the kinetic analysis was used at 2032 cm^{-1}).

Kinetic data can be estimated for each stage. In the initial stage, the reaction rate is much slow and little variation takes place for both media (k_{obs} (D_2O) = (1.3 ± 0.2) × 10^{-3}, k_{obs} (physiological saline) = (0.3 ± 0.2) × 10^{-3}). For the second stage, the reaction rate became faster and the decomposition of complex **1** accelerated with the increasing concentration of L-cysteine ethyl ester hydrochloride (Table S1). Furthermore, linear plots are observed *via* plotting k_{obs} against the concentration of L-cysteine ethyl ester hydrochloride in both media in the second stage, (R = 0.98, Figs. S8 and S9), which indicates that they are also first-order reaction for L-cysteine ethyl ester hydrochloride in these systems. Furthermore, the values of the absolute rate constant k can be estimated from these plots are 0.12 s^{-1} and 0.05 s^{-1} in D_2O and physiological saline, respectively. The CO-releasing rate initiated by L-cysteine ethyl ester hydrochloride in both media is faster than in DMSO / H_2O. This improvement in CO-releasing can be attributed to the hydrolysing capability of water *via* nucleophilic reaction. But the improvement is less significant in the deuterated physiological saline. This suggests that chloride may affect the decomposing of complex **1** due to probably its increasing the ionic strength.

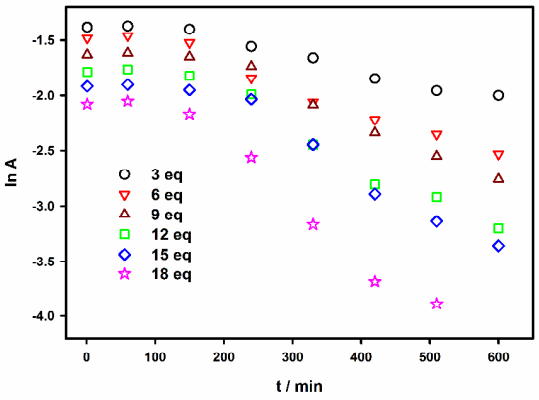


Fig. 6 The logarithmic plot of various concentration of L-cysteine ethyl ester hydrochloride in D_2O conditions at 37 ℃ under open atmosphere, ([**1**] = 0.0115 mol L^{-1}). For clarity, except the plot of 3 eq. of cysteine ethyl ester hydrochloride, the rest of the plots are shifted negatively by adding −0.1, −0.2, −0.3, −0.4, −0.5 to the kinetic data, respectively.

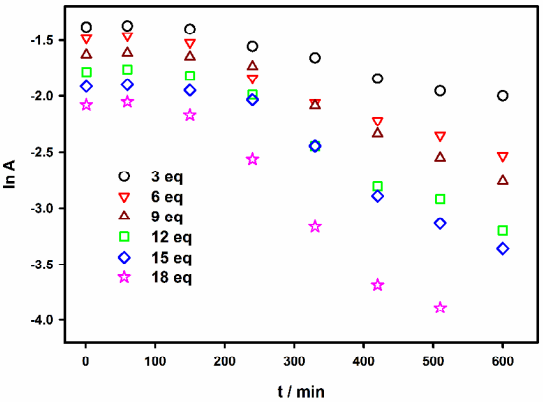


Fig. 7 The logarithmic plot of various concentration of L-cysteine ethyl ester hydrochloride in physiological saline (in D_2O) conditions at 37 ℃ under open atmosphere. ([**1**] = 0.0115 mol L^{-1}). For clarity, except the plot of 3 eq of cysteine ethyl ester hydrochloride, the rest of the plots are shifted negatively by adding −0.2, −0.4, −0.6, −0.8, −1.0 to the kinetic data, respectively.

Cytotoxicity of the CO-releasing system

To examine the biocompatibility of the CO-releasing systems,

normal human liver cell line (QSG-7701) was employed to assess the cytotoxicity using MTT assay. In the assessment, L-cysteine ethyl ester hydrochloride and sodium glutamate were selected, and the ratio of complex **1** over compounds was kept at 1 : 6. The variation of cell viability with the change of the concentration of complex **1** is shown in Fig. 9. IC_{50} values for the CO-releasing systems of L-cysteine ethyl ester hydrochloride and sodium glutamate were estimated at 52 and 53 μmol L^{-1}, respectively. Compared with the cytotoxicity (QSG-7701) of the CO-releasing system activated by cysteamine we reported recently [30], the systems examined in this work show stronger cytotoxicity towards the same cell line we examined. The results show that both sodium glutamate and L-cysteine ethyl ester hydrochloride are more cytotoxic than cysteamine, which is somewhat beyond our expectation.

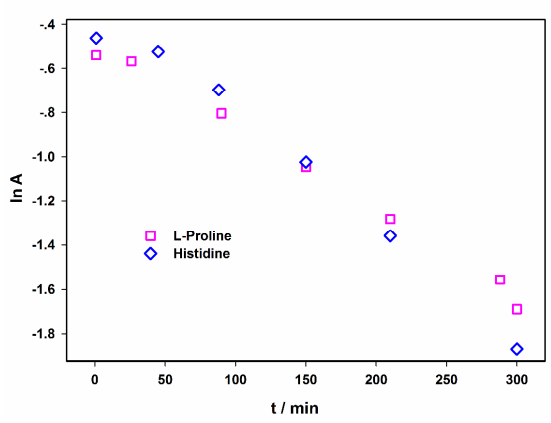


Fig. 8 The logarithmic plot of concentrations of L-Proline and Histidine *versus* reaction time, respectively.

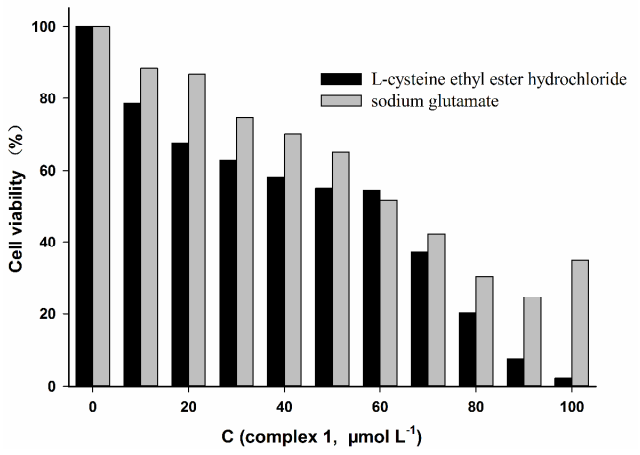


Fig. 9 Cell viability at various concentrations of complex **1** in the presence of L-cysteine ethyl ester hydrochloride and sodium glutamate, respectively. Please note that the ratio of [**1**] : [**Compound**] was kept at 1 : 6 throughout the assessment.

Conclusions

In summary, complex **1**, $[Fe_2\{\mu\text{-}SCH_2CH(OH)CH_2(OH)\}_2(CO)_6]$ can decompose to release CO under the initiation of the substitution reaction by various amino acids or their derivatives. The CO-releasing rate of these reactions varies with the amino acids. The results showed that sodium glutamate is the most efficient CO-releasing promoter. Kinetic analysis suggested that for both diiron complex and the substitution reagent, the CO-releasing reactions were first-order. By comparison, it is believed that the stage-wise mechanisms can be attributed to the bidentate nature of the promoters. In the CO-releasing, both solvent and chelating effect exert significant influence on the reaction. As reported before [30], the presence of oxygen can speed up the CO-releasing progress. Finally, the results described in this work suggest also that both sodium glutamate and L-cysteine ethyl ester hydrochloride are more cytotoxic than cysteamine

Acknowledgements

We thank the Natural Science Foundation of China (Grant Nos. 21102056, 21171073), the Government of Zhejiang Province (Qianjiang Professorship for XL) and the Graduate Innovation Fund of Jiangxi University of Science and Technology (Grant No. YC2013-X06) for supporting this work. We also thank Dr Wei Zhong and Mr Zhiyin Xiao for collecting the ^{13}C NMR data.

Notes

[a]*School of Metallurgy and Chemical Engineering*
Jiangxi University of Science and Technology
Ganzhou 341000, China
[b]*College of Biological, Chemical Sciences and Engineering*
Jiaxing University, Jiaxing 314001, China
Tel. / Fax: +86 (0)573 83643937; E-mail: xiaoming.liu@mail.zjxu.edu.cn; jiangxj@mail.zjxu.edu.cn

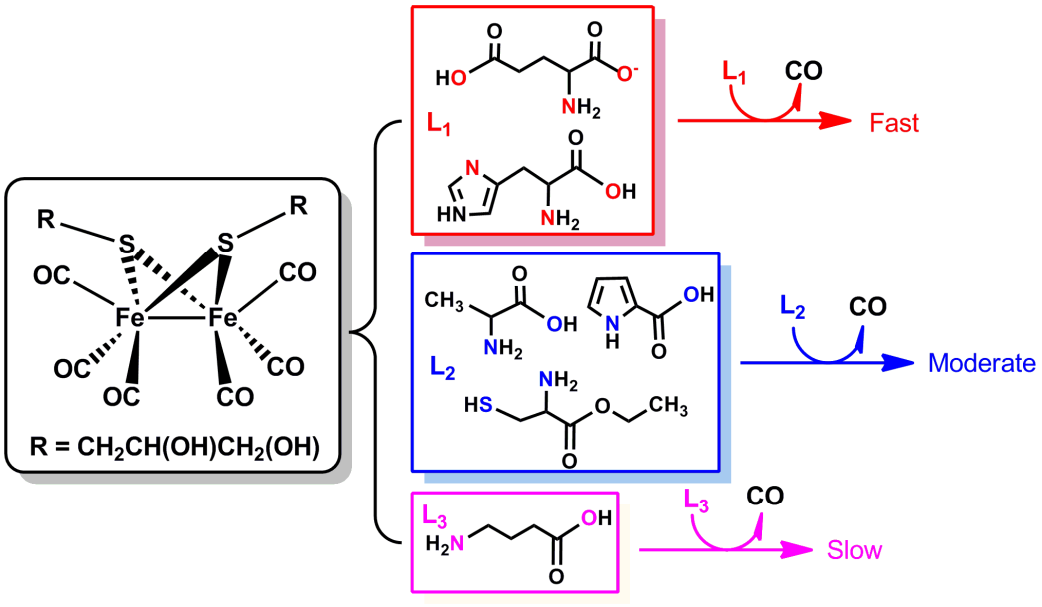
† Electronic Supplementary Information (ESI) available: [IR variation of other amino acids and the kinetic analysis the reaction in different solvents]. See DOI: 10.1039/b000000x/

References

1. R. Motterlini, J. E. Clark, R. Foresti, P. Sarathchandra, B. E. Mann and C. J. Green, *Circ. Res.*, 2002, **90**, E17-E24.
2. T. R. Johnson, B. E. Mann, J. E. Clark, R. Foresti, C. J. Green and R. Motterlini, *Angew. Chem. Int. Ed.*, 2003, **42**, 3722-3729.
3. B. E. Mann and R. Motterlini, *Chem. Commun.*, 2007, 4197-4208.
4. R. Motterlini and L. E. Otterbein, *Nat. Rev. Drug Discov.*, 2010, **9**, 728-743.
5. L. E. Otterbein, *Antioxid. Redox Signaling*, 2002, **4**, 309-319.
6. C. Chauveau, D. Bouchet, J. C. Roussel, P. Mathieu, C. Braudeau, K. Renaudin, L. Tesson, J. P. Soulillou, S. Iyer, R. Buelow and I. Anegon, *Am. J. Transplant.*, 2002, **2**, 581-592.
7. B. E. Mann, *Topics in Organometallic Chemistry* 2010, **32**, 247-285.
8. C. C. Romao, W. A. Blattler, J. D. Seixas and G. J. L. Bernardes, *Chem. Soc. Rev.*, 2012, **41**, 3571-3583.
9. A. J. Atkin, J. M. Lynam, B. E. Moulton, P. Sawle, R. Motterlini, N. M. Boyle, M. T. Pryce and I. J. S. Fairlamb, *Dalton Trans.*, 2011, **40**, 5755-5761.
10. J. Niesel, A. Pinto, H. W. Peindy N'Dongo, K. Merz, I. Ott, R. Gust and U. Schatzschneider, *Chem. Commun.*, 2008, 1798-1800.
11. R. D. Rimmer, H. Richter and P. C. Ford, *Inorg. Chem.*, 2010, **49**, 1180-1185.
12. U. Hasegawa, A. J. van der Vlies, E. Simeoni, C. Wandrey and J. A. Hubbell, *J. Am. Chem. Soc.*, 2010, **132**, 18273-18280.
13. J. B. Matson, M. J. Webber, V. K. Tamboli, B. Weber and S. I. Stupp, *Soft Matter*, 2012, **8**, 6689-6692.
14. M. A. Gonzalez, M. A. Yim, S. Cheng, A. Moyes, A. J. Hobbs and P. K. Mascharak, *Inorg. Chem.*, 2012, **51**, 601-608.
15. H.-M. Berends and P. Kurz, *Inorg. Chim. Acta*, 2012, **380**, 141-147.
16. P. C. Kunz, H. Meyer, J. Barthel, S. Sollazzo, A. M. Schmidt and C. Janiak, *Chem. Commun.*, 2013, **49**, 4896-4898.

17. S. Pai, K. Radacki and U. Schatzschneider, *Eur. J. Inorg. Chem.*, 2014, 2886-2895.
18. S. Pai, M. Hafftlang, G. Atongo, C. Nagel, J. Niesel, S. Botov, H. G. Schmalz, B. Yard and U. Schatzschneider, *Dalton Trans.*, 2014, **43**, 8664-8678.
19. R. Motterlini, P. Sawle, J. Hammad, S. Bains, R. Alberto, R. Foresti and C. J. Green, *FASEB J.*, 2005, **19**, 284-286.
20. M. A. Gonzales and P. K. Mascharak, *J. Inorg. Biochem.*, 2014, **133**, 127-135.
21. T. R. Johnson, B. E. Mann, I. P. Teasdale, H. Adams, R. Foresti, C. J. Green and R. Motterlini, *Dalton Trans.*, 2007, 1500-1508.
22. C. Bischof, T. Joshi, A. Dimri, L. Spiccia and U. Schatzschneider, *Inorg. Chem.*, 2013, **52**, 9297-9308.
23. S. Romanski, B. Kraus, U. Schatzschneider, J.-M. Neudörfl, S. Amslinger and H.-G. Schmalz, *Angew. Chem. Int. Ed.*, 2011, **50**, 1-6.
24. H. T. Poh, B. T. Sim, T. S. Chwee, W. K. Leong and W. Y. Fan, *Organometallics*, 2014, **33**, 959-963.
25. J.-D. Compain, M. Bourrez, M. Haukka, A. Deronzier and S. Chardon-Noblat, *Chem. Commun.*, 2014, **50**, 2539-2542.
26. G. Dördelmann, H. Pfeiffer, A. Birkner and U. Schatzschneider, *Inorg. Chem.*, 2011, **50**, 4362-4367.
27. W.-Q. Zhang, A. J. Atkin, I. J. S. Fairlamb, A. C. Whitwood and J. M. Lynam, *Organometallics*, 2011, **30**, 4643-4654.
28. C. Nagel, S. McLean, R. K. Poole, H. Braunschweig, T. Kramer and U. Schatzschneider, *Dalton Trans.*, 2014, **43**, 9986-9997.
29. U. Schatzschneider, *British Journal of Pharmacology*, 2014, doi:10.1111/bph.12688.
30. L. Long, X. Jiang, X. Wang, Z. Xiao and X. Liu, *Dalton Trans.*, 2013, **42**, 15663-15669.
31. G. A. Lukashin BP, *Radiats Biol Radioecol*, 2001, **41**, 310-312.
32. C.-M. G. Baker DH, *J. Nutr.*, 1987, **117**, 1003-1010.
33. Y. Wang, Z. Li, X. Zeng, X. Wang, C. Zhan, Y. Liu, X. Zeng, Q. Luo and X. Liu, *New J. Chem.*, 2009, **33**, 1780-1789.
34. X. J. Jiang, L. Long, H. L. Wang, L. M. Chen and X. M. Liu, *Dalton Trans.*, 2014, **43**, 9968-9975.
35. C. M. Thomas, O. Rüdiger, T. Liu, C. E. Carson, M. B. Hall and M. Y. Darensbourg, *Organometallics*, 2007, **26**, 3976-3984.

Graphic abstract



L_1 = Bidentate with chelating effect and additional functional group

L_2 = Bidentate with chelating effect

L_3 = Bidentate without chelating effect and additional functional group

CO-releasing from $[Fe_2\{\mu\text{-}SCH_2CH(OH)CH_2(OH)\}(CO)_6]$ initiated by amino acids depends highly on the nature of the acids. Among the examined amino acids, glutamate, the ligand with chelating effect and additional functional group, exhibits the best efficiency in promoting the CO-releasing.