Journal of Thermal Analysis and Calorimetry, Vol. 83 (2006) 1, 181–186

# A MICROCALORIMETRIC STUDY OF THE ACTION OF MERCURIC CHLORIDE ON THE METABOLISM OF MITOCHONDRIA ISOLATED FROM *CYPRINUS CARPIO* LIVER TISSUE

*J. C. Zhu*[1], *C.-H. Li*[2], *Y. Liu*[1,3*], *Z.-H. Zhang*[1], *A.-X. Hou*[1] *and S.-S. Qu*[1]

[1]Department of Chemical Biology, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China
[2]School of Stomatology, Wuhan University, Wuhan 430072, P. R. China
[3]State Key Laboratory of Virology, Wuhan University, Wuhan 430072, P. R. China

After the occurrence of 'Minamata disease' in 1950, mercury aroused much more attention, and lots of studies concerned have been made. The purpose of the present paper is to study the effect of mercuric chloride on the mitochondria suspension isolated from the liver tissue of *Cyprinus carpio* from the direct viewpoint of energy by using the microcalorimetric method. The metabolic thermogenic curves of the mitochondria suspension at 25°C were obtained, and the mitochondria metabolic thermokinetic equations were established, from which we obtained the thermodynamic and thermokinetic parameters: thermogenic rate constant ($k$), heat output ($Q$), average heat power ($P_{av}$), etc. Experimental results indicated that low concentration of mercuric chloride (5 nmol $Hg^{2+}$/(mg protein)) stimulates the thermogenesis of mitochondria, suggesting a strong effect of uncoupling action, while high concentration of mercuric chloride (20 nmol $Hg^{2+}$/(mg protein)) inhibits the metabolism of mitochondria completely, suggesting a fatal effect on the phosphorylation system. The effect of $Hg^{2+}$ on mitochondria is concentration-depended, from which the probable reaction mechanism of $Hg^{2+}$ to the mitochondria was proposed. So the microcalorimetric method can be used in the toxicology research.

***Keywords:*** *Cyprinus carpio, mercuric chloride, microcalorimetry, mitochondria*

## Introduction

As an obvious toxicant, elemental, inorganic, and organic forms of mercury exhibit toxicological characteristics including neurotoxicity [1, 2], nephrotoxicity [3–5], immunotoxicity [6–9] and hepatic toxicity [10–15], etc. After the occurrence of 'Minamata disease' in 1950, mercury aroused much more attention, and lots of studies concerned have been made. So far, many biochemical effects of Hg have been found, including interfering with the synthesis of proteins and nucleic acids, inhibiting several membrane-bound or cytosolic enzymes, diminishing albumin secretion and altering porphyrin metabolism so as to cause cellular damage and lipid peroxidation in vitro and increase the levels of reactive intermediates in tissue cells by the depletion of cellular antioxidants [11]. Most of these studies are focused on the organic Hg, and there has been relatively little systematic study of the effect of inorganic $Hg^{2+}$ as opposed to the organic mercurials. The role of $Hg^{2+}$ in cell injury has been under intensive investigation in recent years. Mitochondria were found to be one of the most primary targets of Hg and may be studied as a marker of cellular injury. And perturbation of mitochondrial function has been shown to be a key event in the apoptotic cascade [7]. So, most studies in this area focused on the mitochondria level [3, 4, 6, 12–14, 16].

As one of the most important cell organs in the eukaryotic cell, mitochondria can provide most of the energy for cell's various activities (including biological synthesis, respiration, endocrine secretion, etc.) by oxidative phosphorylation. In addition to ATP synthesis, mitochondria are also critical to the modulation of cell redox status, osmotic regulation, pH control, and cytosolic calcium homeostasis, and they play an important role in metabolic cell signaling pathways and in the regulation of cell morphology, mobility, multiplication and apoptosis [17, 18]. Accordingly, it is of great interest and significance to evaluate the hepatotoxic effects of $HgCl_2$ on the basis of mitochondrial bioenergetic perturbations.

Up to now people have found that at low level $Hg^{2+}$ induces marked stimulation of state 4 respiration and mild inhibition of state 3 respiration while at high level it can inhibit the respiration completely [3, 12, 13]. $Hg^{2+}$ can result in the releasing of cytochrome c from mitochondria isolated from mice liver, following by mitochondria permeability transition (PT) which was inhibited by cyclosporin A [6]. And treatment of T-cells with $HgCl_2$ resulted in reduced $pH_i$ from 7.0 to 6.7, a decrease in the $\Delta\Psi_m$, increased reactive oxygen species (ROS) generation [7] and ultrastructural alterations of the mitochondria [13].

But almost all of these studies are based on the mitochondrial respiration state 3 and 4, in which many in-

* Author for correspondence: prof.liuyi@263.net, liuyi@chem.whu.edu.cn

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

fluence factors included, so that the mechanism of these actions remains hypothetical. And how about the action of $Hg^{2+}$ to the mitochondrial respiration state 1 is? The common viewpoint is that when the mitochondria are in the state 1, the respiration is so slow that can hardly be detected. For the common study method such as electrochemical methods or spectroscopy methods it is do so, but for the microcalorimetric method it is not. Through microcalorimetric method, we have found that the suspension mitochondria can take a remarkable endogenesis respiration resulting in an integrated thermogenic curve. And when the xenobiotics added, the thermogenic curves changed accordingly. Through the thermokinetic equation established, we can quantitate the reaction of the xenobiotics such as $Hg^{2+}$.

The major objective of our study was to research the effect of $Hg^{2+}$ on mitochondria in the state 1, so as to enrich the study $Hg^{2+}$ on the mitochondria bioenergetics. In the present paper, the microcalorimetric method was selected. By using a microcalorimetry LKB2277, ample method, the effect of mercuric chloride on mitochondria suspensions, which were in the state 1, from *Cyprinus carpio* liver tissue was studied. The mitochondria metabolic thermokinetic equations were established and the thermodynamic and thermokinetic parameters were obtained, from which the affection of $Hg^{2+}$ to the mitochondria was discussed. And the probable reaction mechanism of $Hg^{2+}$ to the mitochondria was proposed.

## Experimental

### *Materials*

*Cyprinus carpio*, about 0.5 kg/one, supplied by College of Life Sciences, Wuhan University, P. R. China. Homogenization (suspension) medium contained 0.25 M sucrose (HPLC, SIGMA), 1.0 mM EDTA (A. R., Chemical Reagent Factory of Shantou), 10.0 mM Tris-HCl (A. R., Xinhua Chemical Reagent of Shanghai), adjusted pH 7.4. The medium was sterilized at 120°C in an autoclave for 30 min. Mercuric chloride (A. R., No. 3 Chemical Reagent Factory of Tianjin). Biuret reagent contained per 1000 mL $CuSO_4 \cdot 5H_2O$ (A. R., No. 3 Chemical Reagent Factory of Shanghai) 1.5 g, $NaKC_4H_4O_6 \cdot 4H_2O$ (A. R., Medicine Corporation of China) 6.0 g, and NaOH (A. R., No. 3 Chemical Reagent Factory of Tianjin) 10.0 g.

### *Instrumentation*

LKB-2277 Bioactivity Monitor, a type of heat-conduction microcalorimeter, was used to obtain the thermogenic curves of mitochondria metabolism. It is designed to monitor continuously a wide variety of processes and complex systems over the temperature range of 20–80°C. To minimize the systematic errors and disturbance effects, a differential or twin detector system is used. The detection limit is 0.15 μW, and the baseline stability (over a period of 24 h) is 0.2 μW. There are three operating modes: ampoule mode, flow-through mode and flow-mixed mode. The ampoule mode was used in this experiment. The microcalorimeter was thermo-stated at 25°C. The signal was recorded by means of an LKB 2210 recorder (1000 mV range). For more details of the performance and construction of the instrument [19].

721-UV spectrophotometer (Third Analytic Apparatus Factory of Shanghai, China) was used to measure protein concentration of mitochondria suspension.

### *Isolation of mitochondria*

The mitochondria were isolated according to the method of Sehneider and Hogeboom [20]. Firstly removed liver tissue from *Cyprinus carpio* and weighed, the liver was then washed with sterilized isolating medium, homogenized, and centrifuged at 3500 rpm (900 g) for 10 min. Abandoned the sediment, the clean supernatant was centrifuged twice at 3500 rpm for 20 min each time. The sediment was discarded, and the clean liquid was centrifuged twice at high speed (10000 rpm) 10 min each time to deposit the mitochondria as sediment. This was suspended again in the isolating medium for calorimetric measurements. All the above operations were performed aseptically at 273–277 K. Finally quantified the suspension and there are mitochondria from 0.4 g liver tissue per mL. Using UV spectrophotometer and biuret reagent [21], we maintained the equal concentration of mitochondria protein at 5 mg protein $mL^{-1}$ by adding the suspension medium.

### *Monitoring the bioactivity of the mitochondria*

The thermogenic curves of mitochondria metabolism were recorded using sealed glass ampoules, one containing a reference solution (isolating medium), and another containing the sample (suspension of mitochondria). Each ampoule contained a 1 mL sample or reference and 2 mL of air. Setting the measuring amplifier at 300 μW, the mercuric chloride was added into the sample at the beginning of the experiment. The final concentration of $Hg^{2+}$ is 0, 5, 10, 15, 20 nmol $Hg^{2+}$/(mg protein), respectively.

## Results

### *Thermogenic curves of mitochondrial metabolism*

From the microcalorimetric experiments, we obtained the metabolic thermogenic curves of mitochondria sus-

pension under the action of different concentration of mercuric chloride, which are shown in Figs 1 and 2.

From Fig. 1, which represents the control, we can see that the thermogenic curve includes four phases: lag phase, activity recovery phase, stationary phase and decline phase. It showed that after a long accommodation procedure (about 1500 min) in this condition, the mitochondria suspension have a normal but slow metabolism, and the metabolism result from the use of the inherent nutrients. This is different from the common opinion that when mitochondria are in state 1, there is almost no respiration. Contrasting with the control curve, the change of the thermogenic curves can indicate the interaction between the mercuric chloride and the mitochondria. Adding different concentration of mercuric chloride to the mitochondria suspension, different thermogenic curves were obtained. When the concentration changed from 5 nmol $Hg^{2+}$/(mg protein) to 15 nmol $Hg^{2+}$/(mg protein), the heights of the curves decreased from 252.4 to 171.6 μW and the time of the peak value delayed from 2265 to 4785 min. The result showed in Fig. 2, from which we can see that the regularity with the gradually increased concentration of $HgCl_2$ existed.

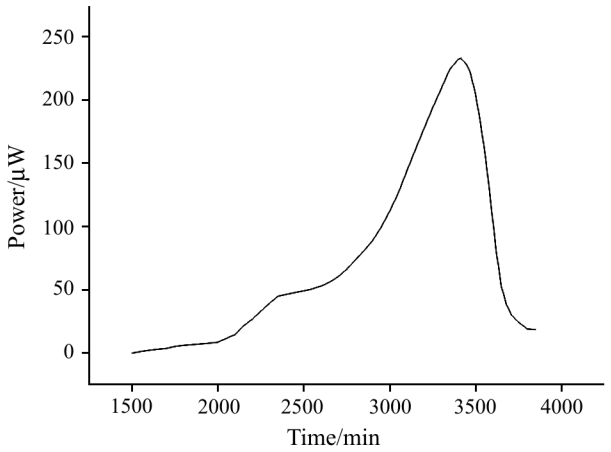


**Fig. 1** Thermogenic curve (control) for mitochondria recording by LKB2277 Bioactivity monitor, ampoule method, at 25°C, repeating for three times

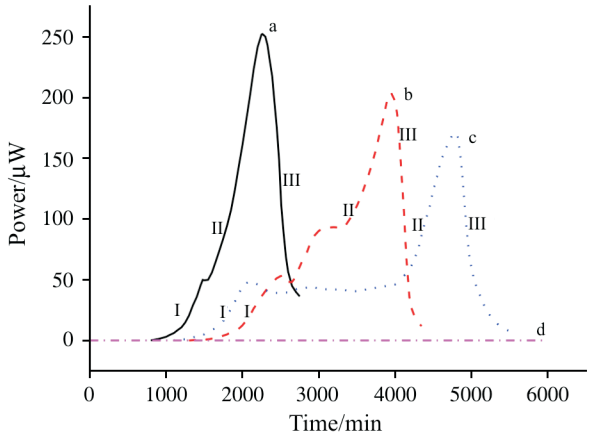


**Fig. 2** Thermogenic curves for mitochondria under different concentrations of mercuric chloride; a – 5 nmol $Hg^{2+}$/(mg protein), b – 10 nmol $Hg^{2+}$/(mg protein), c – 15 nmol $Hg^{2+}$/(mg protein), d – 20 nmol $Hg^{2+}$/(mg protein)), and the other conditions are the same to the control. In the curves I, II and III showed the activity recovery phase, the stationary phase and the decline phase, respectively, from which the corresponding rate constant $k$ were obtained

### *Calculation of the thermogenic rate constant of mitochondria*

Analyzing the shape of the thermogenic curves, we can see that the thermogenic curves are S-shape. And the feature of S-shape curves can be described by a logistic equation [22, 23]:

$$\frac{dP}{dt} = k_m P(1 - SP) \quad (1)$$

$$\ln\left(\frac{P}{1-SP}\right) = k_m t + \ln\left(\frac{P_0}{1-SP_0}\right) \quad (t = 0, P = P_0) \quad (2)$$

where $S$, $P$ and $P_0$ are restrictive factor, heat production power at time $t$ and heat production power at time $t$=0, respectively.

From Eq. (2), we can get that: 1–$SP$>0. So that 0<$S$<1/$P_{max}$. When $S$=0, Eq. (2) changed to be the logarithmic equation [22].

The $S$ value was optimized through loop computing function by computer according to the Eq. (2). By Eq. (2), we can get rate constant $k_1$ for activity recovery phase, $k_2$ for stationary phase and $k_3$ for decline phase. The result was showed in Table 1. We can see that, excepting the concentration of 20 nmol $Hg^{2+}$/(mg protein), with the increasing of mercuric chloride concentration, the value of $k_1$ decreased from $6.233 \cdot 10^{-3}$ to $5.084 \cdot 10^{-3}$ $min^{-1}$, and $k_3$ become higher from $-1.196 \cdot 10^{-2}$ to $-4.763 \cdot 10^{-2}$ $min^{-1}$.

### *The heat output Q, the maximum heat production power ($P_{max}$) and the corresponding time ($t_{max}$) for mitochondrial metabolism*

The heat output ($Q$), which has the same meaning as $\Delta H$ in this condition, released by *Cyprinus carpio* liver mitochondria during the metabolic process, was obtained by integrating the thermogenic curves. The heat output ($Q$) was calculated, because it is an important parameter to indicate the metabolism activity of the mitochondria, as well as the maximum heat production power ($P_{max}$) and the corresponding time ($t_{max}$). The values of $Q$, $P_{max}$ and $t_{max}$ are showed in Table 2.

**Table 1** The metabolism rate constant $k$ of the mitochondria and the related coefficients

| $C$/(nmol $Hg^{2+}$/mg protein) | $k_1$/$min^{-1}$ | $R$ | $k_2$/$min^{-1}$ | $R$ | $k_3$/$min^{-1}$ | $R$ |
|---|---|---|---|---|---|---|
| 0 | $3.886 \cdot 10^{-3}$ | 0.997 | $2.207 \cdot 10^{-3}$ | 0.997 | $-1.328 \cdot 10^{-2}$ | –0.988 |
| 5 | $6.233 \cdot 10^{-3}$ | 0.993 | $3.189 \cdot 10^{-3}$ | 0.999 | $-1.196 \cdot 10^{-2}$ | –0.996 |
| 10 | $5.543 \cdot 10^{-3}$ | 0.993 | $1.331 \cdot 10^{-3}$ | 0.997 | $-1.634 \cdot 10^{-2}$ | –0.991 |
| 15 | $5.084 \cdot 10^{-3}$ | 0.991 | $2.797 \cdot 10^{-3}$ | 0.994 | $-4.763 \cdot 10^{-3}$ | –0.996 |
| 20 | 0 | – | 0 | – | 0 | – |

**Table 2** The total heat output ($Q_{total}$) and the maximum heat production power ($P_{max}$); the corresponding time ($t_{max}$) and the average heat power ($P_{av}$) of the metabolism of mitochondria under different concentration of $Hg^{2+}$

| $C$/(nmol $Hg^{2+}$/mg protein) | $Q_{total}$/J | $P_{max}$/μW | $t_{max}$/min | $P_{av}$/μW |
|---|---|---|---|---|
| 0 | 10.302 | 233.4 | 3415 | 44.0 |
| 5 | 10.588 | 252.6 | 2265 | 64.2 |
| 10 | 12.364 | 204.2 | 3950 | 47.4 |
| 15 | 12.864 | 171.4 | 4785 | 39.0 |
| 20 | 0 | 0 | – | 0 |

*The average heat power for mitochondrial metabolism*

From the heat output ($Q$) and the time spending, we can get the average heat powers of mitochondrial metabolism according the following equation:

$$P_{av} = Q/t \quad (3)$$

where $t$ represents the total time of metabolism and $P_{av}$ represents the average heat power. Because the action of $HgCl_2$ can bring on the variation of $Q$ and $t$ at the same time, it is significant to study this action through the parameter $P_{av}$. Contrasting with the control, $P_{av}$ can represent the relative speed of heat production for mitochondria during the whole procedure of metabolism. The results are showed in Table 2.

## Discussion

In the present work, the thermogenic curves of the metabolism of mitochondria isolated from the liver tissue of *Cyprinus carpio* and the effect of $Hg^{2+}$ on it were studied by using an LKB-2277 Bioactivity Monitor, ampoule method, at 25°C. The mitochondria metabolic thermokinetic equations were established, from which we obtained the thermodynamic and thermokinetic parameters: thermogenic rate constant ($k$), heat output ($Q$) and average heat power ($P_{av}$), etc.

The thermogenic curve of the control indicated that, by using the inherent nutrition, the isolated mitochondria could metabolize normally and slowly after a long time of lag phase. In this experiment, the suspension medium just provided a constant pH and osmotic pressure for mitochondria, therefore the mitochondria should be in state 1. Without additional reductive substrate and ADP, the system can make the mitochondria an idea model to simplify the system and obtain more assured conclusion.

The experimental results showed that: (1) With the increasing of the concentration of $Hg^{2+}$ (from 5 nmol $Hg^{2+}$/(mg protein) to 15 nmol $Hg^{2+}$/(mg protein)), the lag phase and the stationary phase became longer, the metabolic rate constant of recovery phase increase evidently, and the maximum heat production power $P_{max}$ decreased while the $t_{max}$ increased evidently. All of these suggested an inhibition effect on the mitochondria with the increasing of $Hg^{2+}$ concentration. (2) When the concentration of $Hg^{2+}$ becomes 20 nmol $Hg^{2+}$/(mg protein), the metabolism of the mitochondria was inhibited completely, suggesting a completely inhibition of the respiratory chain. This is coincident well with the former report [3]. (4) When 5 nmol $Hg^{2+}$/(mg protein) was added, the value of $P_{max}$ became higher and the value of $t_{max}$ became shorter than that of the control, indicating an uncoupling effect on the mitochondria. (5) During the range of 5 to 15 nmol $Hg^{2+}$/(mg protein), the value of $Q$ increased from 10.588 to 12.864 J, but all of them are higher than the control, while the $P_{av}$ decreased from 64.2 to 39.0 μW, suggesting the concomitance of the uncoupling effect and the effect of inhibition to the respiration chain on the mitochondria and the uncoupling effect become less gradually.

Considering the changing regularity above, we can draw a conclusion that the adding of $HgCl_2$ result in a strong uncoupling action, following which is the directly inhibition to the respiratory chain. Because when the concentration changed from 5 nmol $Hg^{2+}$/(mg protein) to 15 nmol $Hg^{2+}$/(mg protein), the metabolism heat increased gently, but the increasing extent decreases gradually (according to the $P_{av}$). So, there must be two kinds of action for the mercuric chloride to the mito-

chondria, including the uncoupling action and the directly action to the respiratory chain. The uncoupling effect dissipate $\Delta\tilde{u}_H^+$ productively in the form of heat emission, wasting the metabolic energy of substrates, while the inhibition to the respiration chain can depress the metabolism of mitochondria so as to inhibit the production of the metabolic heat. And the results, especially the value of $P_{av}$, also indicated that during the range of 5 nmol $Hg^{2+}$/(mg protein) to 10 nmol $Hg^{2+}$/(mg protein), the uncoupling action is the dominant factor, but when the concentration of $HgCl_2$ was above 10 nmol $Hg^{2+}$/(mg protein) (such as 15 nmol $Hg^{2+}$/(mg protein)), the action to the respiratory chain become a dominant factor, so as to completely inhibited the metabolism of mitochondria when the concentration increased to 20 nmol $Hg^{2+}$/(mg protein).

The molecular basis for the toxicity of mercury remains unclear. The cytotoxicity can presumably result from binding to sulfhydryl groups of important sites involved in enzyme catalysis or ion transport. Palmeira *et al*. thought that the uncoupling effect is presumably caused either by the incorporation of $HgCl_2$ causing the permeabilization of mitochondrial membrane to $H^+$, or by inducing slippage of the proton pumps or by unspecific pore opening [12]. By swelling experiment, Bragadin reported that $CH_3HgCl$ induces opening of a permeability transition pore (PMT) [14]. Araragi *et al*. coworkers found the same effect for $HgCl_2$ on mitochondria isolated from mice liver, and the property of the PMT was the same to that result from $CH_3HgCl$, for all of them were inhibited by cyclosporin A [6]. Konisberg *et al*. suggest that one of the primer effects of $Hg^{2+}$ on mitochondria could be to react with sulfhydryl groups of membrane proteins, modifying their transport and altering the mitochondrial permeability. The collapse of the proton motive force and the loss of membrane permeability would account for acceleration of the oxygen consumption, increasing the rate of formation of reactive oxygen species and thus accounting for the lipid peroxidation [13].

The result of the present work is in well agreement with these reports. But the fact that in the condition of 20 nmol $Hg^{2+}$/(mg protein), the metabolism of the mitochondria suspension can be completely inhibited, suggesting that following the formation of the PMT, $Hg^{2+}$ can go into the mitochondria and directly reaction with it, otherwise the metabolism heat should exist if causing reactive oxygen species is the only reason for the effect of mercury toxicity.

The microcalorimetric technology has been widely performed in the biological area in these years [22–29]. The present work showed us that the microcalorimetric method might be a useful tool to study the toxicology of the xenobiotics in the cell level or the molecular level.

## Acknowledgements

We gratefully acknowledge the financial support of the National Natural Science Foundation of China, the New Century Outstanding Young Scientist Foundation of Hubei Province and the Teaching and Research Award Program for Outstanding Young Professors High Education Institute, Chinese Education Ministry.

## References

1 P. L. Goering, D. L. Morgan and S. F. Ali, J. Appl. Toxicol., 22 (2002) 167.
2 D. A. Kuznetsov, Neurochem. Res., 12 (1987) 751.
3 J. M. Weinberg, P. G. Harding and H. D. Humes, J. Biol. Chem., 257 (1982) 60.
4 J. M. Weinberg, P. G. Harding and H. D. Humes, J. Biol. Chem., 257 (1982) 68.
5 A. C. Santos, S. A. Uyemura, N. A. G. Santos, F. E. Mingatto and C. Curti, Mol. Cell. Biochem., 177 (1997) 53.
6 S. Araragi, M. Kondoh, M. Kawase, S. Saito, M. Higashimoto and M. Sato, Toxicology, 184 (2003) 1.
7 T. L. Guo, M. A. Miller, I. M. Shapiro and B. J. Shenker, Toxicol. Appl. Pharmacol., 153 (1998) 250.
8 B. J. Shenker, T. L. Guo and I. M. Shapiro, Environ. Res., Section A, 77 (1998) 149.
9 B. J. Shenker, T. L. Guo and I. M. Shapiro, Environ. Res., Section A, 84 (2000) 89.
10 A. L. Nieminen, G. J. Gores, T. L. Dawson, B. Herman and J. J. Lemasters, J. Biol. Chem., 265 (1990) 2399.
11 L. Bucio, C. Garcia, V. Souza, E. Hernandez, C. Gonzalez, M. Betancourt and M. C. Gutierrez-Ruiz, Mutation Res., 432 (1999) 65.
12 C. M. Palmeira and V. M. C. Madeira, Environ. Toxicol. Pharmacol., 3 (1997) 229.
13 M. Konigsberg, N. E. Lopez-Diazguerrero and M. L. Bucio, J. Appl. Toxicol., 21 (2001) 323.
14 M. Bragadin, D. Marrton, S. Manente, M. Grasso and A. Toninello, J. Inorg. Biochem., 89 (2002) 159.
15 L. Bucio, V. Souza, A. Albores, A. S. E. Charez, A. Carabez and M. C. Gutierrez-Ruiz, Toxicology, 102 (1995) 285.
16 S. J. Stohs and D. Bagchi, Bio. Med., 18 (1995) 321.
17 K. B. Wallace and A. A. Starkov, Annu. Rev. Pharmacol., 40 (2000) 353.
18 K. B. Wallace, J. T. Eells, V. M. C. Madeira, G. Cortopassi and D. P. Jones, Fundam. Appl. Toxicol., 38 (1997) 23.
19 J. Suurkuusk and I. Wadsö, Chemica Scripta, 20 (1982) 155.
20 Striebich, J. Biol. Chem., 196 (1952) 111.
21 A. G. Gornall, C. J. Bardawill and M. M. David, J. Biol. Chem., 177 (1949) 751.
22 Y. Liu, X. Li, S. S. Qu and P. Sheng, J. Biochem. Bioph. Methods, 45 (2000) 231.
23 Y. Liu, C. L. Xie, S. S. Qu, F. J. Deng and Y. Guo, Acta Phys.-Chim. Sinica, 11 (1995) 929.
24 S. C. Hand and E. Gnaiger, Science, 239 (1988) 1425.
25 P. Johansson and I. Wadsö, J. Therm. Anal. Cal., 57 (1999) 275.
26 Y. Yang, Y. Liu, J. C. Zhu and P. Shen, J. Therm. Anal. Cal., 75 (2004) 293.

27 Y. Yang, Y. Liu, J. Zhu, M. J. Li and P. Shen, J. Therm. Anal. Cal., 79 (2005) 645
28 S. M. McGuinmess and D. Roess, Thermochim. Acta, 172 (1990) 131.
29 M. L. C. Montanari, A. E. Beezer and C. A. Montanaric, Thermochim. Acta, 328 (1999) 91.

Received: March 25, 2005
In revised form: July 9, 2005

DOI: 10.1007/s10973-005-7020-1