Biol Trace Elem Res (2011) 144:854–864
DOI 10.1007/s12011-011-9031-z

Development of ELISA for Detection of Mercury Based on Specific Monoclonal Antibodies Against Mercury-Chelate

Yi Zhang · Xiaobing Li · Guowen Liu · Zhe Wang · Tao Kong · Jiajia Tang · Peng Zhag · Wei Yang · Dongna Li · Lei Liu · Guanghong Xie · Jianguo Wang

Received: 9 February 2011 / Accepted: 8 March 2011 /
Published online: 25 March 2011

Abstract Immunoassays for heavy metals offer an alternative approach to traditional techniques for detection of mercury. In this study, a mercury-chelate was prepared with 1-(4-aminobenzyl) ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (aminobenzyl-EDTA). The resulting complex was linked to keyhole limpet hemocyanin (KLH) or bovine serum albumin via the amino group and used as the immunizing antigen or detection antigen, respectively. BALB/c mice were immunized with KLH-aminobenzyl-EDTA-Hg and spleen cells from BALB/C mice were fused with Sp2/0 cells. One cell line (5F7) produced monoclonal antibodies with preferential selectivity and sensitivity for aminobenzyl-EDTA-Hg. This cell line had an affinity constant of 4.31×10^9 L/mol and its cross-reactivity (CR) with other metals was <2%. The antibody was used for competitive indirect ELISA (CI-ELISA) for Hg^{2+} measurements. The detection range was 0.087–790.4 μg/L and the lower limit of detection was 0.042 μg/L. The concentrations of mercury in environmental water samples obtained by CI-ELISA correlated well with graphite furnace atomic absorption spectrometry (GFAAS), and the mean recovery was 88.82% to 104.64%. These results indicate that this method could be used for monitoring mercury of water.

Xiaobing LI and Guowen LIU contributed equally to this study.

Y. Zhang · X. Li (✉) · G. Liu · Z. Wang (✉) · T. Kong · J. Tang · P. Zhag · W. Yang · D. Li · L. Liu · G. Xie · J. Wang
College of Animal Science and Veterinary Medicine, Jilin University, 5333 Xi'an Road, Changchun 130062, China
e-mail: sihuo12345@yahoo.com.cn
e-mail: Wangzhe500518@sohu.com

G. Liu
e-mail: liuguowen2008@163.com

Y. Zhang
College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110161, China

Keywords Mercury · Chelator · Monoclonal antibody · Immunoassay · Competitive indirect ELISA

Introduction

Environmental pollution by heavy metals is a growing problem worldwide, especially in developing countries. At least 20 metals are known to be toxic and half of these, including mercury, lead, copper, arsenic, nickel, cadmium, selenium, silver, and zinc, are heavy metals [1]. Mercury is a toxic metal that exists naturally in the environment, and its pollution can threaten the health of humans and wildlife [2–9]. Monitoring of mercury levels in water, soil, paint, and other media is important for health and safety. Current analytical methods for detection of mercury include atomic sbsorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectrometry, and atomic fluorescence spectrometry, which are sensitive and reliable [10, 11]. However, these methods are expensive and require highly specialized apparatus and trained operators, which makes them unsuitable for onsite detection. By contrast, immunoassays using specific monoclonal antibodies are rapid, inexpensive, simple, and highly sensitive [12]. Because metal ions are too small to elicit an immune response, they are usually chelated with isothiocyanate derivatives of bifunctional chelating agents, and conjugated to a carrier protein via the isothiocyanate [13–16]. This process is usually conducted by adjusting the pH to 9.0, but at this pH mercury would precipitation. This makes it difficult to produce a mercury antigen using isothiocyanate derivatives of chelators. Amino derivatives of chelators are much cheaper than the isothiocyanate derivatives, and can be linked to carrier proteins at pH 7.0 using glutaraldehyde. In the present study, a complete antigen and specific monoclonal antibody (mAb) for mercury were prepared using an amino derivative of EDTA (1-(4-aminobenzyl)ethylenediamine-*N*, *N*,*N*′,*N*′-tetraacetic acid (aminobenzyl-EDTA)). ELISA for the detection of mercury based on specific monoclonal antibodies was developed.

Materials and Methods

Reagents

Aminobenzyl-EDTA was obtained from Dojindo Laboratories (Gaithersburg, MD). Mercury (II) nitrate monohydrate (99.99%), goat anti-mouse horseradish peroxidase conjugate (GAM-HRP) hypoxanthine-aminopterin-thymidine medium (HAT), and hypoxanthine-thymidine medium (HT), bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), Freund's complete and incomplete adjuvants, 3,3′,5,5′-tetramethylbenzidine (TMB), mouse monoconal antibody isotyping kit, and atomic absorption standard metals (1,000 μg/mL) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Fetal bovine serum (FBS) was obtained from Tianjin Haoyang Co. (Tianjin, China). ELISA 96-well plates and tissue culture plates were purchased from Corning Co. (Cambridge, MA). Water used for reagent preparation was purified by a Millipore (MUL-TYPE9000) water purification system. A BIO-TEK ELX800 plate reader was purchased from Bio-Tek Instruments Inc. (Winooski, VT). Sp2/0 cells were preserved by our laboratory. BALB/c mice were obtained from the Experimental Animal Center of Bethune Medical College (Changchun, China). All glassware and plasticware was treated with HNO_3 (H_2O/HNO_3, 1:1, *v*/*v*) overnight and rinsed with ultrapure water. Amicon

Centricon 30 concentrator tubes (Millipore Co., Bedford, MA) were treated with 100 mmol/L EDTA and rinsed with ultrapure water before use.

Antigen Preparation and Animal Immunization

Mercury ions were conjugated to the carrier protein (BSA or KLH) via the bifunctional chelating reagent [17]. The amino group of the bifunctional EDTA derivative was linked to the carrier protein using glutaraldehyde.

Mercury(II) nitrate monohydrate (5.14 mg, 15 μmol) was dissolved in 100 μL of ultrapure water, and mixed with a solution of aminobenzyl-EDTA (3.98 mg, 10 μmol) in dimethyl sulfoxide (50 μL). The pH of the mixed solution was adjusted to 5.5 with 1 mol/L KOH. Then 1 mL of glutaraldehyde (mass fraction, 1%) was added dropwise to the solution and the pH was adjusted to 7.0. After reaction at room temperature for 2 h, the solution was added dropwise to 1 mL of carrier protein solution (KLH or BSA, 10 mg/mL, pH 7.0, in 0.1 mol/L HEPES) with stirring. The pH was adjusted to 7.0, and the mixture was left to react for 18 h at 37.5°C. Then the conjugate (KLH-aminobenzyl-EDTA-Hg or BSA-aminobenzyl-EDTA-Hg) was purified by removing low-molecular-weight reagents via centrifugation (Centricon 30), and washed thoroughly six times with HEPES buffer (0.1 mol/L, pH 7.0). BSA-aminobenzyl-EDTA was prepared as described above without the addition of Hg^{2+}. All the conjugates were adjusted to a final concentration of 1 mg/mL using metal-free HEPES buffer (0.1 mol/L, pH 7.0).

Four 8-week-old female BALB/c mice were injected intraperitoneally with KLH-aminobenzyl-EDTA-Hg (100 μg/mouse) at 2-weekly intervals [18]. The immunogen was emulsified in complete Freund's adjuvant for the first injection and in incomplete Freund's adjuvant for the subsequent injections. One week after the sixth immunization, tail vein blood was collected for polyclonal antibody titer testing by indirect ELISA, with unimmunized mice as the control. Ninety-six-well polystyrene plates were coated with 1.25 μg/mL BSA-aminobenzyl-EDTA-Hg or BSA-aminobenzyl-EDTA (100 μL/well), washed three times with PBS containing 0.05% Tween 20, and blocked with 3% BSA in PBS. After a washing step, the immobilized antigens were incubated with 10,000 times diluted sera of the immunized mice. PBS-diluted serum from a nonimmunized mouse was used as the control. After incubation for 1 h at 37°C, the 96-well polystyrene plates were washed again and incubated sequentially with GAM-HRP and TMB microplate substrate as described previously [19]. The absorbance of each plate was measured by a microplate reader at 450 nm. The data are expressed as the mean±SD of triplicate measurements.

Hybridoma Production and Screening

The mouse that showed the highest antibody reactivity to BSA-aminobenzyl-EDTA-Hg and the lowest serum antibody reactivity to BSA-aminobenzyl-EDTA-Hg was injected with 100 μg of KLH-aminobenzyl-EDTA diluted in 100 μL of HEPES buffer (0.1 mol/L, pH 7.0).

Myeloma cells (Sp2/0) with high viability and rapid growth were prepared before cell fusion. Three days after the last injection the mouse was killed. Then the spleen cells were harvested aseptically, washed in RPMI 1640 medium, and fused with Sp2/0 cells at a ratio of 10:1 (spleen/myeloma) using 50% polyethylene glycol (PGE 2000). The cell pellets were suspended in HAT selective medium (RPMI 1640+20% FBS+HAT), and then added dropwise to 96-well cell culture plates (100 μL/well) that had been coated with mouse peritoneal cavity cells as feeder cells 1 day prior. The plates were incubated at 37°C in an

atmosphere with 5% CO_2 for 7 days. The HAT medium was changed every other day and switched to HT medium when most of the non-fused cells were eliminated [13].

When the hybridoma cells had grown to 20–30% confluence, the clone supernatant was screened for the ability to bind to immobilized conjugates (BSA-aminobenzyl-EDTA-Hg or BSA-aminobenzyl-EDTA) by the indirect ELISA method as described above.

The clone that showed high titer to BSA-aminobenzyl-EDTA-Hg and low titer to BSA-aminobenzyl-EDTA was selected to test whether the culture supernatant could bind with chelated mercury or aminobenzyl-EDTA by competitive ELISA. Aminobenzyl-EDTA at concentrations of 0, 1, 2, 4, 6, 8, 10, 12, 14, 16 mmol/L (50 μL/well) and culture supernatant (50 μL/well) were added to the 96-well plates previously coated with BSA-aminobenzyl-EDTA-Hg conjugate. The subsequent steps were the same as in the indirect ELISA method described above. The results were used to identify the antibodies that were not bound primarily to metal-free aminobenzyl-EDTA, and select an optimal concentration of aminobenzyl-EDTA for subsequent anti-mercury antibody screening.

For anti-mercury antibody screening, a mercury solution at concentrations of 0.001, 0.01, 0.1, 1, 10, 100, 1,000 μg/L was mixed with aminobenzyl-EDTA at the optimum concentration. These mixtures were used to inhibit antibody binding to BSA-aminobenzyl-EDTA-Hg immobilized in the 96-well plate with the protocol described above. This screen yielded positive hybridoma cell lines that did not bind to aminobenzyl-EDTA and Hg^{2+}, but specifically bound to aminobenzyl-EDTA-Hg^{2+}. These cell lines were subsequently subcloned by limiting dilution. The stable clones were expanded and preserved in liquid nitrogen.

Isotyping, Production and Purification of mAb

The immunoglobulin subclasses of the antibodies were determined using a mouse monoclonal antibody isotyping kit according to the manufacturer's recommendations. Only the cell line secreting IgG was expanded for ascites production.

Female 10-week-old BALB/c mice, pre-treated with incomplete Freund's adjuvant, were intraperitoneally injected with hybridoma cells. Ascitic fluid was collected and clarified by centrifugation at 3,000×g for 10 min, and then initially purified using saturated ammonium sulfate solution [20]. Samples were further purified through Hi Trap Protein G HP. The protein concentration of the purified antibody was determined by BCA protein assay.

Determination of Affinity Constants for BSA-aminobenzyl-EDTA-Hg

The affinity constant (K_{aff}) of the monoclonal antibody was determined by indirect ELISA according to Beatty et al. [21]. Ninety-six-well plates were coated with BSA-aminobenzyl-EDTA-Hg at concentrations of 2.0, 1.0, 0.5 μg/mL, and then incubated with serial concentrations of purified mAb. The indirect ELISA was performed as described above. Three sigmoid curves were constructed of OD_{450} against concentration of the antibody added to the wells.

Competitive Indirect ELISA Optimization

Optimum concentrations of mAb and the coating antigen were determined by indirect ELISA. The optimum incubation time of mAb with aminobenzyl-EDTA-Hg (30, 45, 60, and 90 min), concentration of GAM-HRP (2,500, 5,000, and 10,000 times dilution), and time of color development (5, 10, 15, and 20 min) were determined by indirect ELISA. To evaluate the effect of the pH on assay performance, HBS buffer solutions (137 mM NaCl,

3 mM KCl, and 10 mM phosphate) with pH values of 4, 5, 6, 7, 8, and 9 were prepared. Then the optimum conditions determined above were used for all other assay conditions. Standard curves (data not shown) were constructed using the mean of triplicate results to evaluate the effect of the pH on assay performance.

Serially diluted mercury standard solutions (0.001, 0.01, 0.1, 1, 10, 100, 1,000, and 10,000 μg/L) were mixed with equal volumes of HBS containing 2 mmol/L aminobenzyl-EDTA. The mixtures were incubated with equal volumes of diluted mAb. The subsequent steps were the same as those in the competitive indirect ELISA (CI-ELISA) described above, and with the determined optimal assay conditions. In the CI-ELISA, analytes that do not react with the mAb have absorbances close to 100% that of the zero analyte control, and analytes that reacted with the mAb will decrease the absorbance. The standard inhibition curve of the CI-ELISA was constructed by plotting the absorbance against the logarithm of the analyte concentration. These curves were fitted to a four-parameter logistic equation, $y=(A–D)/[1+(x/C)B+D]$, where x is the concentration of analyte, y is the absorbance at 450 nm (OD_{450}), A is the maximum absorbance with no analyte, B is the curve slope at the inflection point, C is the half maximal inhibitory concentration (IC_{50}), and D is the minimum absorbance with an infinite concentration of analyte [22].

CR with Other Metals

CR of the mAb with other metals (Cu^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Ag^{+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, and Fe^{3+}) was determined by CI-ELISA. The 96-well plates were coated with BSA-aminobenzyl-EDTA-Hg and blocked with 1% BSA. Each metal was diluted to the concentration range of 10^{-7} to 1 mmol/L in HBS containing the metal-free aminobenzyl-EDTA (2 mmol/L). Then the metal solutions (50 μL/well) and an equal volume of culture supernatant were added to the 96-well plates. The subsequent steps were same as for the indirect ELISA described above. The CR was obtained from the IC_{50}, which was defined as the concentration of inhibitor r to inhibit color development by 50% compared with control wells containing no competitor, of the mercury standard ($IC_{50\ mercury}$) and of the other metal ($IC_{50\ other\ metal}$) from the same plate as follows: $CR\%=(IC_{50\ mercury}/IC_{50\ other\ metal})\times 100$.

Measurement of Hg^{2+} in Environmental Water Samples

Water samples were collected from the Songhua River (Jilin, China), Yitong River (Changchun, China), and Xinlicheng Lake (Changchun,China), and the mercury ion concentrations were measured by CI-ELISA. The water samples were adjusted to pH 7.0, and then they were mixed with equal volumes of HBS containing 2 mmol/L aminobenzyl-EDTA. The mixtures were incubated with equal volumes of diluted mAbs. The subsequent steps were the same as those used in the CI-ELISA standard curve construction. The mercury contents of the water samples were also measured by GFAAS. All the samples were prepared and analyzed in triplicate.

Results

Antigen Preparation and Animal Response

In this study, aminobenzyl-EDTA was used to prepare mercury antigens. The complete immunogen (KLH-aminobenzyl-EDTA-Hg) was used to immunize BALB/c mice. One

week after the sixth immunization, blood from each mouse was collected from the tail vein, and the sera were tested for antibody responses by indirect ELISA. As shown in Table 1, all the mice showed much higher antibody reactivity to immobilized BSA-aminobenzyl-EDTA-Hg than immobilized BSA-aminobenzyl-EDTA. Among the mice, mouse 1 showed the highest antibody reactivity to immobilized BSA-aminobenzyl-EDTA-Hg and the lowest reactivity to immobilized BSA-aminobenzyl-EDTA.

Hybridoma Production and Screening

The mouse 1 was injected with KLH-aminobenzyl-EDTA (100 μg in 100 μL of HEPES buffer). Three days later, the mouse was killed and the spleen cells were harvested for fusion with Sp2/0 cells. After fusion, indirect ELISA was used to screen the culture supernatants of 517 hybridoma clones for their ability to bind to immobilized BSA-aminobenzyl-EDTA-Hg and BSA-aminobenzyl-EDTA. The supernatant from Sp2/0 was used as a negative control. Two strains of hybridoma cell lines (3D9 and 5F7) showed high binding to BSA-aminobenzyl-EDTA-Hg and poor binding to BSA-aminobenzyl-EDTA.

The supernatants from the 3D9 and 5F7 cell lines were tested for binding with chelated mercury and aminobenzyl-EDTA by CI-ELISA. Aminobenzyl-EDTA concentrations >6 mmol/L did not inhibit reaction of the antibodies with the immobilized BSA-aminobenzyl-EDTA-Hg (Fig. 1). Consequently, 2 mmol/L aminobenzyl-EDTA was selected for subsequent experiments.

When Hg^{2+} was serially diluted with 2 mmol/L aminobenzyl-EDTA and incubated with the immobilized BSA-aminobenzyl-EDTA-Hg in the presence of hybridoma culture supernatant from the 3D9 and 5F7 cell lines, the ability of mercury to inhibit the antibody binding to BSA-aminobenzyl-EDTA-Hg depended on the mercury concentration (Fig. 1).

The two positive hybridoma cell lines (3D9 and 5F7) were subcloned by limiting dilution and expanded to produce ascites.

Subclass Determination and Production of Monoclonal Antibody

The immunoglobulin subclasses of the antibodies were determined using a mouse monoclonal antibody isotyping kit. The antibody isotyping secretions by 3D9 and 5F7 were IgM and IgG1, respectively. Only the cell line (5F7) secreting IgG was expanded for ascitic fluid production. The mAb produced from the ascitic fluid in mice (2.80 mg/mL) was purified and stored at −20°C for further characterization studies.

Table 1 Serum reactivity of BALB/c mice injected with KLH-aminobenzyl-EDTA-Hg [a]

Mouse number	Abs_{450} against BSA-minobenzyl-EDTA-Hg	Abs_{450} against BSA-minobenzyl-EDTA
1	1.854±0.091	0.297±0.046
2	1.653±0.103	0.365±0.024
3	1.799±0.012	0.421±0.019
4	1.633±0.092	0.294±0.025
Unimmunized	0.235±0.014	0.2295±0.009

[a] Sera from blood samples collected from the tail vein of each mouse were diluted 4,000 times with PBS (pH 7.4) and used for indirect ELISA. Sera from blood samples collected from unimmunized mice were used as the control. The data represent the mean±SD of triplicate measurements

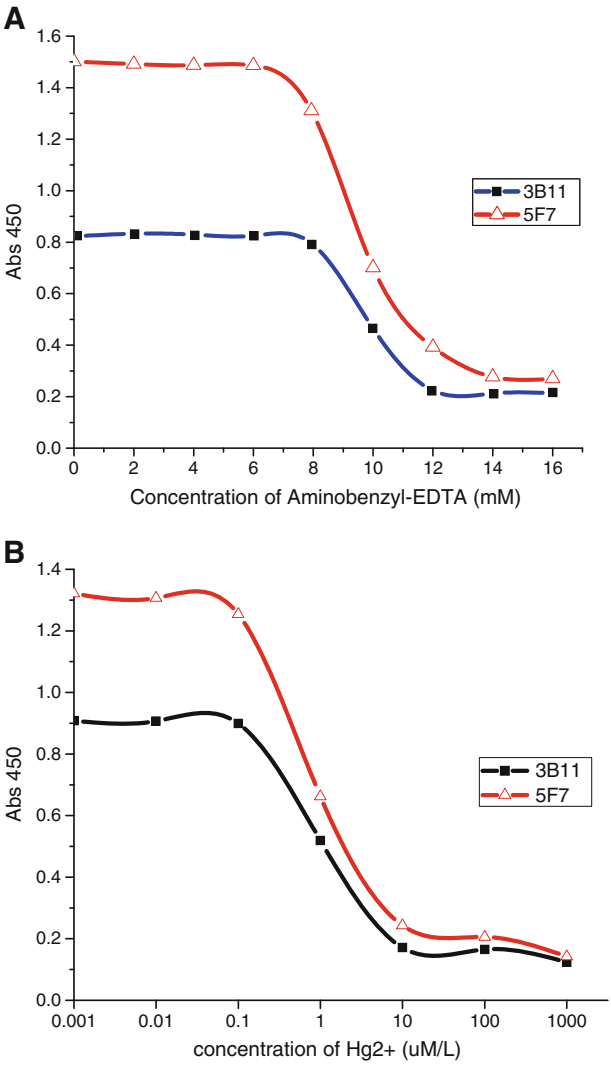

Fig. 1 CI-ELISA with 3D9 and 5F7. **a** Inhibition by aminobenzyl-EDTA, and **b** inhibition when Hg^{2+} was serially diluted into 2 mmol/L EDTA. Each point represents the mean of triplicate determinations

Determination of K_{aff} for BSA-aminobenzyl-EDTA-Hg

The K_{aff} was determined by indirect ELISA using serial dilutions of the purified antibody and three concentrations of immobilized BSA-aminobenzyl-EDTA-Hg (Fig. 2). K_{aff} was calculated using $K_{aff}=(n-1)/2(n[Ab']-[Ab])$, where [Ab] and [Ab′] are the antibody concentrations at OD_{50} (half maximal optical density) and OD_{50}' of the 96-well plate coated with [Ag] and [Ag′], with $[Ag']=[Ag]/n$. The K_{aff} of mAb 5F7 was 4.31×10^9 L/mol.

CI-ELISA Optimization

The optimal concentrations of mAb and coating antigen were determined to be 70 ng/mL and 1 μg/mL, respectively, by ELISA. The indirect ELISA showed that the optimal

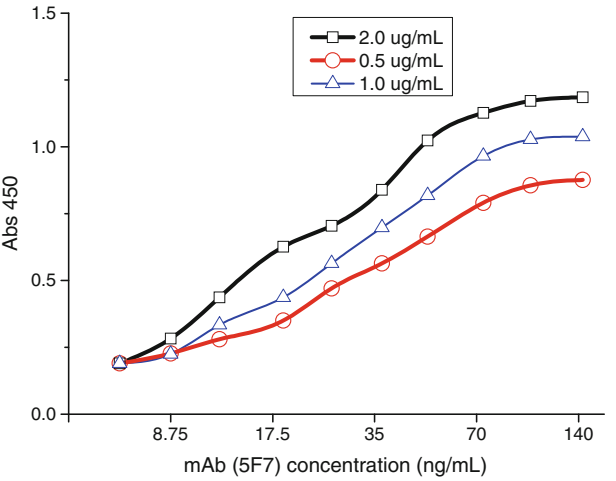

Fig. 2 Affinity constant curve of mAb 5F7 against aminobenzyl-EDTA-Hg. Each point represents the mean of triplicate determinations

incubation time of mAb with the mixture of aminobenzyl-EDTA was 60 min, the optimal dilution of GAM-HRP was 5,000 times, and the optimal time of color development was 10 min. The pH range suitable for the CI-ELISA was 5.5–7.0 and the optimal pH was 7.0.

Standard curves of the CI-ELISA were constructed at the optimal assay conditions. As shown in Fig. 3, the IC_{50} of the optimized assay was 2.955±0.0041 μg/L, and the detection range ($IC_{20–80}$) was 0.087–790.4 μg/L. The lower limit of detection, which was defined as two standard deviations (SDs) above the lowest detectable level, was estimated as 0.042 μg/L.

CR with Other Metals

The mAb 5F7 was highly selective for Hg^{2+}. The CRs with Cd^{2+}, Pb^{2+} were 1.87% and 0.381%, respectively, and with other metals were less than 0.2% (Table 2). These results indicate that the mAb 5F7 has high specificity for aminobenzyl-EDTA-Hg.

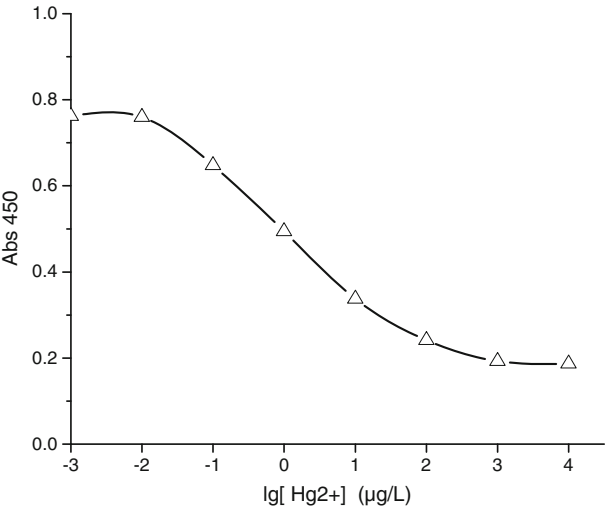

Fig. 3 Standard inhibition curve of the CI-ELISA

Table 2 Cross-reactivity (CR) of other metals with mAb (5F7)

Metal	IC_{50} (μM/L)	CR (%)[a]
Hg^{2+}	1.93±0.041	100
Cu^{2+}	>1,000	<0.193
Zn^{2+}	>1,000	<0.193
Cd^{2+}	103±0.939	1.87
Cr^{3+}	>1,000	<0.193
Pb^{2+}	506±3.18	0.381
Co^{2+}	>1,000	<0.193
Ni^{2+}	>1,000	<0.193
Ag^{+}	>1,000	<0.193
Ca^{2+}	>1,000	<0.193
Mn^{2+}	>1,000	<0.193
Mg^{2+}	>1,000	<0.193

[a] $(IC_{50\ mercury}/IC_{50\ other\ metals})\times 100$

Measurement of the Hg^{2+} in Environmental Water Samples

The mercury ion concentrations in water samples from the Songhua River (Jilin, China), Yitong River (Changchun, China), and Xinlicheng Lake (China) were measured by CI-ELISA and GFAAS. The concentrations obtained by CI-ELISA correlated well with those obtained by GFAAS, and the mean recovery was 88.82% to 104.64% (Table 3).

Discussion

The molecular masses of metal ions are too low and their structures are too simple to induce an immune response. Consequently, preparation of a complete immunogen is critical for antibody-based immunoassays of metals. In this study, an amino derivative of EDTA (aminobenzyl-EDTA) was used to prepare mercury antigens. Mercury ions were chelated with aminobenzyl-EDTA and the resulting chelator-metal complexes were conjugated to a carrier protein (KLH or BSA) to produce complete antigens for mercury (KLH-aminobenzyl-EDTA-Hg and BSA-aminobenzyl-EDTA-Hg). After the sixth immunization, all the mice showed much higher antibody reactivity to immobilized BSA-aminobenzyl-EDTA-Hg than immobilized BSA-aminobenzyl-EDTA (Table 1), immunogens prepared from aminobenzyl-EDTA could stimulate production of specific antibodies against chelated mercury.

Metal antigens have been prepared from isothiocyanate derivatives of chelators in other studies, but required a pH adjustment to 9.0 [13–16]. If applying the reported

Table 3 Hg^{2+} concentrations in three environmental water samples obtained by CI-ELISA and GFAAS

Water sample	GFAAS (μg/L)	CI-ELISA (μg/L)	Mean recovery (%)
Songhua River	0.237±0.0323	0.248±0.0167	104.64
Yitong River	0.314±0.0297	0.279±0.0241	88.85
Xinlicheng Reservoir	0.278±0.0265	0.283±0.02317	101.80

[a] The data represent the mean±SD of triplicate measurements

isothiocyanate derivatives of chelators to prepare antigen for mercury, the mole of mercury can't surpass that of chelator because the mercury that not chelated by chelator would precipitate when the pH was adjusted to 9.0. In the case, there would be some metal-free aminobenzyl-EDTA sites of the antigen, which will induce production of nonspecific polyclonal antibody against metal-free chelator but not against the chelated mercury. In this study, aminobenzyl-EDTA, which is much cheaper alternative to the isothiocyanate derivatives, was linked to carrier protein at pH 7.0. This avoided the potential precipitation of mercury with pH adjustment to 9.0. The concentration of mercury(II) (5.14 mg, 10 μmol) was 1.5 times that of aminobenzyl-EDTA (3.98 mg, 10 μmol), which guarantees that all the aminobenzyl-EDTA sites of the antigen will be occupied by mercury. This ensures that the immunogen (KLH-aminobenzyl-EDTA-Hg) will induce production of specific polyclonal antibody against chelated mercury but not against the metal-free chelator. Immunoassays offer an alternative approach, and have significant advantages over, traditional instrument-intensive methods of metal analysis. They are remarkably quick, simple, transportable, require minimum sample pretreatment, and have high throughput. Furthermore, studies have shown that the use of immunoassays can reduce the analysis cost by 50% or more [23, 24]. Our results revealed that the mAb 5F7 prepared from aminobenzyl-EDTA displayed high affinity and specificity for the aminobenzyl-EDTA-Hg complex. Subsequently, the mAb was used to establish a CI-ELISA for mercury ions. The lower limit of detection of the assay was 0.042 μg/L, which is much lower than the World Health Organization threshold for mercury in drinking water (0.5 μg/L). The assay was successfully applied to the measurement of mercury ion concentrations in three environmental water samples. The concentrations of mercury in the water samples determined by CI-ELISA correlated well with those obtained by GFAAS, and the mean recovery was 88.82% to 104.64% (Table 3). This indicates the method could be used for monitoring of mercury in water. Furthermore, the time for preparing sample and aminobenzyl-EDTA complex of the immunoassay were shorter than the traditional methods. The immunoassay is a promising analytical tool for rapid and sensitive determination of mercury ion concentrations in agricultural products and the environment.

Acknowledgment This work was supported by the National "863" Project (2007AA10Z440) of China and the National Natural Science Foundation of China (Nos. 30972224, 31072178, and 30871897).

References

1. Rubio C, Gonzalez-Iglesias T, Revert C et al (2005) Lead dietary intake in a Spanish population (Canary Islands). J Agric Food Chem 53:6543–6549
2. Abernathy AR, Cumbie PM (1977) Mercury accumulation by largemouth bass (*Micropterus salmoides*) in recently impounded reservoirs. Bull Environ Contam Toxicol 17:595–602
3. Cox JA, Carnahan J, DiNunzio J et al (1979) Source of mercury in fish in new impoundments. Bull Environ Contam Toxicol 23:779–783
4. Verdon R, Brouard D, Demers C et al (1991) Mercury evolution (1978–1988) in fishes of the La Grande hydroelectric complex, Quebec, Canada. Water Air Soil Pollut 56:405–417
5. Tremblay A, Lucotte M, Rheault I (1996) Methylmercury in a benthic food web of two hydroelectric reservoirs and a natural lake of northern Quebec (Canada). Water Air Soil Pollut 91:255–269
6. Myers GJ, Davidson PW, Cox C et al (2003) Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361:1686–1692
7. Bloom NS, Gill GA, Cappellino S et al (1999) Speciation and cycling of mercury in Lavaca Bay, Texas, sediments. Environ Sci Technol 33:7–13

8. Chasar LC, Scudder BC, Stewart AR et al (2009) Mercury cycling in stream ecosystems. 3. Trophic dynamics and methylmercury bioaccumulation. Environ Sci Technol 43:2733–2739
9. Wang L, Jia G (2005) Progress in developmental toxicity of methylmercury. J Hyg Res 34:633
10. Jackson KW, Chen G (1996) Atomic absorption, atomic emission, and flame emission spectrometry. Anal Chem 68:231–256
11. Saouter E, Blattmann B (1994) Analyses of organic and inorganic mercury by atomic fluorescence spectrometry using a semiautomatic analytical system. Anal Chem 66:2031–2037
12. Szurdoki F, Jaeger L, Harris A et al (1996) Rapid assays for environmental and biological monitoring. J Environ Sci Health B 31:451–458
13. Khosraviani M, Blake RC II, Pavlov AR et al (2000) Binding properties of a monoclonal antibody directed toward lead-chelate complexes. Bioconjug Chem 11:267–277
14. Blake RC II, Pavlov AR, Khosraviani M et al (2004) Novel monoclonal antibodies with specificity for chelated uranium (VI): isolation and binding properties. Bioconjug Chem 15:1125–1136
15. Chakrabarti P, Hatcher FM, Blake RC et al (1994) Enzyme immunoassay to determine heavy metals using antibodies to specific metal-EDTA complexes: optimization and validation of an immunoassay for soluble indium. Anal Biochem 217:70–75
16. Xiang J, Zhai Y, Tang Y et al (2010) A competitive indirect enzyme-linked immunoassay for lead ion measurement using mAbs against the lead-DTPA complex. Environ Pollut 158:1376–1380
17. Mariani M, Bartolazzi A, Camagna M et al (1991) Monoclonal antibodies to a soluble metallic radioisotope chelator: development and characterization. Hybridoma 10:695–705
18. Kishiro Y, Kagawa M, Naito I et al (1995) A novel method of preparing rat-monoclonal antibody-producing hybridomas by using rat medial iliac lymph node cells. Cell Struct Funct 20:151–156
19. Blake DA, Chakrabarti P, Khosraviani M et al (1996) Metal binding properties of a monoclonal antibody directed toward metal-chelate complexes. J Biol Chem 271:27677
20. Reik LM, Maines SL, Ryan DE et al (1987) A simple, non-chromatographic purification procedure for monoclonal antibodies Isolation of monoclonal antibodies against cytochrome P450 isozymes. J Immunol Methods 100:123–130
21. Beatty JD, Beatty BG, Vlahos WG (1987) Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay. J Immunol Methods 100:173–179
22. Jones G, Wortberg M, Kreissig SB et al (1994) Extension of the four-parameter logistic model for ELISA to multianalyte analysis. J Immunol Methods 177:1–7
23. Darwish IA, Blake DA (2001) One-step competitive immunoassay for cadmium ions: development and validation for environmental water samples. Anal Chem 73:1889–1895
24. Wengatz I, Harris AS, Gilman SD et al (1996) Recent developments in immunoassays and related methods for the detection of xenobiotics. In: Environmental Immunochemical Methods, vol 646. ACS Symposium Series. ACS Publications, Washington. pp 110–126