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Synthesis, Characterization and Immunoassay of The Artificial Antigen for Cadmium(II)

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Artificial antigens Cd-IEDTA-BSA (HSA) were synthesized using the bifunctional chelating agent IEDTA (isothiocyanobenzylethyl enediamine tetraacetic acid) coupling with cadmium and protein carrier. The artificial antigens were characterized by atomic absorption spectroscopy, ultraviolet spectrum, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) spectrometry and the comparison was taken among these methods. The animal immune was also done to verify immune effect of the artificial antigen. The consistent corroboration by these methods showed that the artificial antigen was synthesized successfully and the coupling rate of Cd-IEDTA-BSA, Cd-IEDTA-HSA were 11-13:1 and 14-16:1, respectively. The substitution extent of free amino groups for the artificial antigen was 23.12 % for Cd-IEDTA-BSA, 29.17 % for Cd-IEDTA-HSA. The experiment of animal immune indicated that the specific antiserum was generated after immunization of BALB/c mice with Cd-IEDTA-BSA and the titer of the antiserum reached the level of 12800:1. This work provides a helpful reference for further research of preparation on monoclonal antibodies against heavy metals.

Key Words: Cadmium, Artificial antigen, Conjugation, Antibody.

INTRODUCTION

Heavy metal ions pose a major threat to nature and mankind, they are classified as persistent environment toxins because they can not be completely degraded to relatively harmless products by chemical or biological remediation processes[1-3]. Cadmium is an environmental and occupational hazard that can cause serious health problems. It can accumulate in the body and has a biological half-life of more than 10 years in humans. Humans are exposed to cadmium mainly by ingestion of cadmium-contaminated food, dust and soil and by inhalation of cadmium-containing dusts. After ingestion or inhalation of cadmium, it accumulates in the kidney, liver, lungs and gastrointestinal tract where it can cause progressively toxic effects, including cancer and renal damage[4]. Hence, there is a need for a rapid, reliable and highly sensitive analytical method for trace cadmium detection. Traditional methods of trace metal ion detection include AAS, inductively coupled plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES), *etc*. Although these methods are excellent

for Cd(II) analysis, the instrumentations are expensive, analyses are time-consuming, labour-intensive and the data does not provide any information about metal oxidation state or speciation[5-7].

Immunoassays offer an alternative approach and have significant advantages over the traditional instrument-intensive methods of metal analysis. They are remarkably quick, inexpensive, easily performed, reasonably portable and also highly sensitive and selective. Nowadays most environmental immunoassays are direct towards pesticides, herbicides[8-12] and plant hormones[13,14], the immunoassay also provides a new strategy for the metal detection since the antibodies of metal chelates were successful prepared by Reardan[15]. The immunoassay for metal ions was based on the specific metal-chelator complex of monoclonal antibody and the successful synthesis of heavy metals antigen is the key for preparation of monoclonal antibodies. However metal ions are low molecular weight and tend to interact strongly and irreversibly with biological molecules (the same properties that are responsible for the toxicity of heavy metals). Sequestering a metal ion in the form of its coordination complex with a chelating agent reduces the activity of the resulting molecule and provides an outer shell of organic material that should be processed by the immune system in much the same way as any other organic hapten. The complete antigen can be obtained by coupling the metal-chelator with carrier protein such as HSA (human serum albumin), KLH (keyhole limpet hemocyanin), BSA (bovine serum albumin) and OVA (ovalbumin). Then, high titer and specificity of the antibodies for heavy metal can be generated with this antigen and a reliable immunological detection technology could be established. Therefore, the synthesis of artificial antigen is one of the hot areas nowadays. The preparation of metal ion artificial antigen has been brought forward in some reports[16,17], but the attempts that comparison of different methods about the coupling results of artificial antigen have been made limited. In this paper, artificial antigens Cd-IEDTA-BSA(HSA) were synthesized using the bifunctional chelating agent (IEDTA) coupling with cadmium and protein carrier and the products were characterized by atomic absorption spectroscopy (AAS), ultraviolet (UV), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), trinitrobenzenesulfonic acid (TNBS) and the immunological characteristics were tested by competitive indirect enzyme-linked immunosorbent assay (ELISA).

EXPERIMENTAL

Isothiocyanobenzyl-EDTA (IEDTA) was purchased from Dojindo Laboratories (J&K Chemical Ltd, Shanghai, China); bovine serum albumin, human serum albumin, 2,4,6-trinitro-benzenesulfonic acid (TNBS) and complete/incomplete Freund's adjuvant were obtained from Sigma Chemical Co. (J&K Chemical Ltd, Shanghai, China); SDS-PAGE gel preparation kit, silver nitrate, tetramethylethylenediamine (TEMED), β-mercaptoethanol, *o*-phenylenediamine (OPD) were purchased from Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). Low molecular weight calibration kit for SDS electrophoresis, goat anti-mouse IgG/HRP and cadmium

chloride were products of DingGuo Biotechnologies Co., Ltd (Beijing, China). 6-week-old female BALB/c mice were obtained from Zhejiang Academy of Medical Sciences (Hangzhou, China). The solutions of 0.1 mol L^{-1} potassium phosphate buffer of pH 9.5 was prepared. All regents were of analytical grade and fresh deionized distilled water (DDW) was used throughout. All plastic ware was soaked overnight in 3.0 mol L^{-1} HCl and rinsed gently with DDW.

All the atomic absorption measurement was carried out on an AA6650 atomic absorption spectrometry (Shimadzu, Japan). An UV2550 recording spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm cuvette was used for scanning the ultraviolet spectrum. Electronic analytical balance (1/100000, Sartorius, Germany) was used for weighing the sample. All pH measurements were done with automatic potentiometric titration (Shanghai Precision Scientific Instrument Co., Ltd, China). SDS-PAGE experiments were performed on the vertical electrophoresis device (Bio-Rad, U.S.A). Centrifuge (Beijng Jingli Centrifuge Co., Ltd, China) and centricon-30 filter (6 mL, sartorius, Germany) were used to purify the artificial antigen. ELISA plates were washed with a plate washer (Beijing Tuopu Analytical Instruments, China), well absorbencies were measured with a 660 plate reader (Bio-Rad, U.S.A.).

Synthesis of artificial antigen: Bovine serum albumin (BSA) was dissolved in 0.1 mol L^{-1} potassium phosphate buffer (pH 9.5), the concentration of BSA is 10 mg mL^{-1}, IEDTA (10 mg, 90 %) was dissolved in DMSO (1 mL). An aliquot of IEDTA was mixed (1:1) with cadmium in 0.1 mol L^{-1} potassium phosphate buffer, stirred for 0.5 h and then the cadmium-IEDTA complex was added to the BSA solution. The resultant solution contained 3.0 mmol L^{-1} cadmium-IEDTA and the pH was readjusted to 9.5 with trisodium phosphate and then the solution was incubated at 4 °C overnight. The unreacted IEDTA and cadmium-IEDTA complex were removed form the protein conjugates solution system by centricon-30, which had been treated with 10 mmol L^{-1} EDTA and liberally rinsed before use. The product was stored at -20 °C. The Cd-IEDTA-HSA and IEDTA-HSA have been synthesized by the same method.

Identification of artificial antigen

Detection by atomic absorption spectrophotometer: The atomic absorption spectroscopy was used to detect the coupling effect of synthetic artificial antigen. The standard solutions of cadmium (0.1, 0.3, 0.5, 0.7, 0.9 mg mL^{-1}) were prepared and the corresponding standard curve (concentration *vs.* absorbance) was constructed. Then, the conjugates were identified by AAS after diluted and the coupling ratio was calculated.

Detection by UV spectrophotometer: The concentration of protein in artificial antigen was detected by BCA kit *versus* a blank *Tris*-HCl (pH 7.4, 0.1 mol L^{-1}) buffer. Cd-IEDTA, BSA, HSA and artificial antigen were prepared at the certain concentration in *Tris*-HCl buffer and the characteristic spectrum was scanned with UV spectrophotometer, respectively. The degree of couple was judged according to the changes of UV absorption peak.

SDS-PAGE Procedure: SDS-PAGE was accomplished by a modification of a previously described method[18] by using *Tris*-HCl (glycine) buffer system, 5 % concentration gel, 10 % separation gel. The electrophoresis current was kept at 20 mA and 30 mA when samples in the zone of separation gel and concentration gel, respectively. Electrophoresis was stopped when BPB (bromophenol blue) was migrated to the 1 cm distance from the bottom of separation gel. Then, the gel was fixed for 40 min by fixative and sensitized for 0.5 h, washed four times, stained by $AgNO_3$, coloured and imaged.

TNBS Assay procedure: The solution of protein in different concentration (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mg mL^{-1}) were prepared, respectively. To a series of cells, 1 mL solution of protein for each concentration was titrated into these cells, then 1 mL phosphate buffer (pH 9.3, 0.1 mol L^{-1}) and 1 mL (0.1 %) TNBS solution were added in order. The reaction was lasted for 0.5 h at room temperature (25 °C), then 0.5 mL HCl (1.0 mol L^{-1}) was added to prevent its precipitation. The absorbance at 420 nm was read against a blank of water and the standard curve was constructed according to absorption *vs.* protein concentration. The standard curve of artificial antigen was obtained by the same way. The regression equation could be made by means of data-handling, the slope of regression equation could be considered as absorbance of unit concentration and the substituting degree of free amino groups can be calculated as follows:

$$r = (A_a - A_b)/A_a \quad (1)$$

while r is the extent of substitution of free amino groups, A_a is the absorbance of BSA, A_b is the absorbance of cadmium artificial antigen.

Immunization of mice and preparation of anti-serum: The artificial antigen was mixed with adjuvant (complete and incomplete, 1/1 volume ratio) by syringe and the mixture should be as homogeneous as possible. Eight 6-week-old female BALB/c mice were injected (abdominal cavity, skin and neck) with 0.2 mL of the artificial antigen emulsified with Freund's adjuvant. The injections were repeated after 3 weeks using the same dose in the same way, but different adjuvant (incomplete adjuvant). From then on, the mixture of artificial antigen with incomplete adjuvant was injected to the mice every 2 weeks. The antibody responses were measured by detection the titer after the fourth injection and the best one was taken to strengthen immune. The artificial antigen without any adjuvant was injected to the mice at the last immune.

The mice blood was taken out and the antiserum was collected to sterile centrifuge tube 7 d after its last immune. The tube was kept at room temperature for 2 h, then moved in refrigerator at 4 °C until the serum fully precipitated (overnight). Finally, the serum was centrifugated by 3000 rpm for 10 min, the supernatant was collected and preserved at -20 °C.

Analysis the titer of antibody by indirect ELISA: The 96-well microwell plates were coated overnight at 4 °C with 2 μg mL^{-1} coating antigen in carbonate coating buffer (pH 9.6, 5.0×10^{-2} mol L^{-1}, 100 μL/well). The liquid in the wells was

dumped next day and the plate was washed three times with PBS-T (pH 7.4, 0.15 mol L^{-1}, 0.5 % Tween) at an interval of three minutes, then the plate was taped clean and blocked with 0.5 % BSA in carbonate coating buffer (200 µL/well), incubated 3 h at 37 °C and washed as before. A series of multiple diluted antiserums was added to the coated, blocked microplate and negative comparison and blank comparison were set up in parallel. After 1h incubation at 37 °C, the plate was washed, IgG-HRP (1:5000) was added to this microplate (100 µL/well), which was incubated 1 h at 37 °C, then washed again. The *o*-phenylenediamine was added to the plate containing antiserum and negative comparison (100 µL/well), respectively. After 15 min of chromogenic reaction at 37 °C, 2.0 mol L^{-1} H_2SO_4 was added in the volume of 50 µL/well and kept 5 min to end this chromogenic reaction. The absorption (optical density) was measured at 492 nm and when the optical density value was greater than twice the control, it is defined as positive.

RESULTS AND DISCUSSION

Synthesis reaction of artificial antigen: It is the key point to choose an appropriate bifunctional chelating agent in this work because of its vital role in the synthesis of artificial antigen. On the one hand, the heavy metal ions can be coordinated with appropriate bifunctional chelating agent to form a chelate complex, which can avoid a direct and strong non-reversible reaction between heavy metal ion and biological molecules. The chelate complex can also provide an organic shell, which can be recognized by the immune system. On the other hand, the chelate complex can be coupled to protein molecule through the function reaction of bifunctional chelating agent. If the conjugates own the immunogenicity, then the expected artificial antigen should have been produced[19]. Commonly used of metal ion chelating agent are these which derivative with nitrobenzene, benzenesulfonic acid on the basis of ethylenediamine tetraacetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA).

IEDTA is the derivate of EDTA, while artificial antigen containing IEDTA is synthesized, the stable six-member metal chelate can be formed between the EDTA substructure on the IEDTA molecule and Cd^{2+}, at the same time, the chain connection that reactive isothiocyanate coupling to protein can also be occurred, then the metal-IEDTA-protein complex was obtained. The principle for its reaction is shown in Fig. 1.

Atomic absorption spectroscopy analysis of the artificial antigen: AAS can used to infer the coupling extent of artificial antigen by detection of its cadmium, because the uncoupling small molecule was removed by a centricon-30 filter in the process of conjugate purification, the protein and conjugate are still reserved.

The artificial antigen Cd-IEDTA-BSA and Cd-IEDTA-HSA were detached by AAS and the concentration of cadmium ion were 20 and 26 mg L^{-1}, respectively. These results demonstrated that the synthesis of artificial antigen was successful. The formula of coupling rate can be described as:

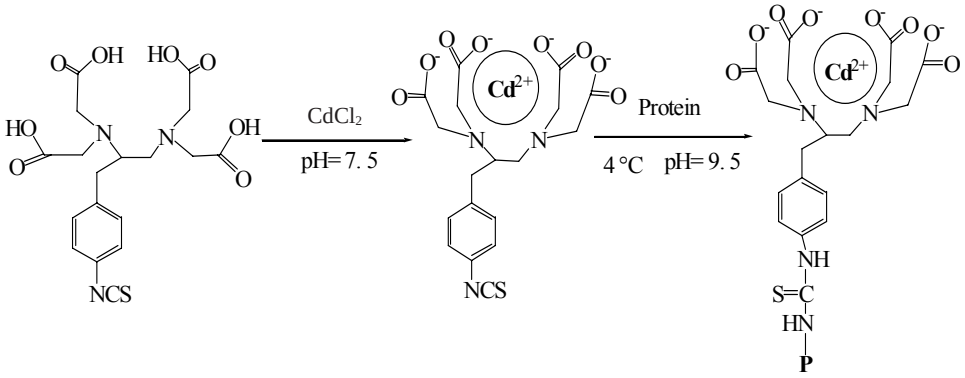

Fig. 1. Synthetic route to the antigen conjugates

$$\varepsilon = \frac{c_a \nu}{m_a} : \frac{c_b \nu}{m_b} : \frac{c_a}{m_a} : \frac{c_b}{m_b} \quad (2)$$

here ε is coupling rate, c_a is the concentration of cadmium, c_b is the unreacted protein concentration, ν is the final volume, m_a, m_b are the cadmium and protein molecular weight, respectively.

The coupling rate of Cd-IEDTA-BSA and Cd-IEDTA-HSA are 11-12:1 and 14-15:1, respectively.

Analysis of UV spectroscopy: UV spectrophotometry is the common method used to detect antigen, hapten (Cd-IEDTA) and carrier protein (BSA, HSA), which show different ultraviolet absorption peak. The conjugate will own some characteristics of both hapten and protein. We can judge whether the coupling is successful from the change of the absorption peak of coupling product.

The UV spectroscopy of artificial antigen, hapten and carrier protein are shown in Fig. 2. From Fig. 2, we can find that the characteristic absorption peak of the BSA appear at near 278 nm, the maximum absorption peak of the hapten (Cd-IEDTA) at near 260 nm. Compare to the scope of absorption curve from 260 to 280 nm, the wave shape of coupling product (Cd-IEDTA-BSA) tends to be gentle and it also has the absorption characteristic of hapten and carrier protein and HSA and its coupling product are the same shape. These analysis results indicate that the anticipant conjugate is a successfully obtained preliminarily.

The concentration of hapten is denoted as c_h (0.01 mg mL^{-1}), protein and artifical antigen are denoted as c_p (0.3 mg mL^{-1}) and c_a (0.3 mg mL^{-1}), respectively. The coupling rate (ε) of artifical antigen can be written as:

$$\varepsilon = (A_{a\text{-}266} \times K_{p\text{-}278} - A_{a\text{-}278} \times K_{p\text{-}266}) / (A_{a\text{-}278} \times K_{h\text{-}266} - Aa_{\text{-}266} \times K_{h\text{-}278}) \quad (3)$$

where, $A_{a\text{-}266}$ is the absorbance of artifical antigen at 266 nm, $A_{a\text{-}278}$ is the absorbance of artifical antigen at 278 nm. $K_{p\text{-}278}$, $K_{p\text{-}266}$, $K_{h\text{-}266}$, $K_{h\text{-}278}$ are molar extinction coefficient of protein and hapten at 278 nm, 266 nm, respectively.

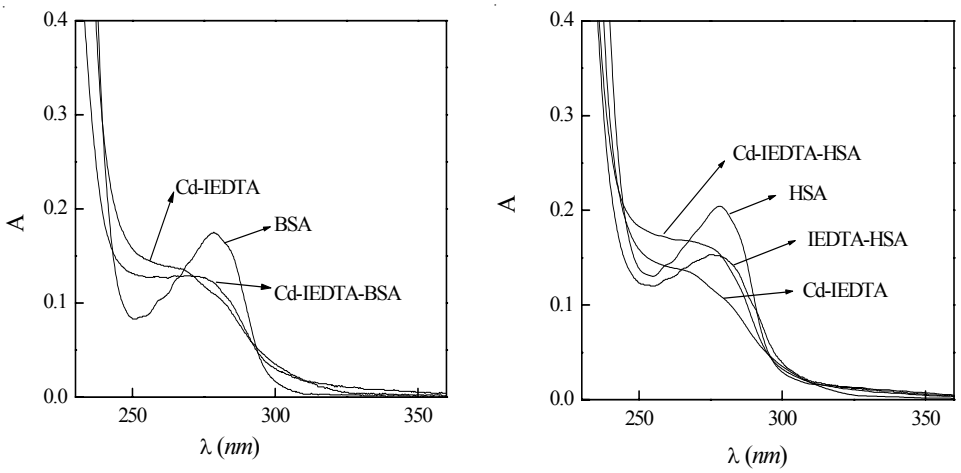

Fig. 2. UV scanning spectra of BSA, HSA and artificial antigens

The absorbance data (A) was obtained from Fig. 2 and the coupling rate of Cd-IEDTA-BSA, Cd-IEDTA-HSA can be calculated according to eqn. 3 and was shown in Table-1.

TABLE-1
UV SPECTRAL ANALYSIS OF HAPTEN, CARRIER-PROTEIN AND CONJUGATES

Sample	A (278 nm)	A (266 nm)	Coupling rate
BSA	0.1750	0.1280	–
HAS	0.2041	0.1645	–
Cd-IEDTA	0.1090	0.1360	–
Cd-IEDTA-BSA	0.1210	0.1280	11
Cd-IEDTA-HAS	0.1524	0.1680	16

The molar extinction coefficient of BSA and HSA are 3.850×10^4 L mol^{-1} cm^{-1} and 4.694×10^4 L mol^{-1} cm^{-1} at 278 nm, 2.816×10^4 L mol^{-1} cm^{-1} and 3.783×10^4 L mol^{-1} cm^{-1} at 266 nm, respectively. The molar extinction coefficient of hapten (Cd-IEDTA) are 6.018×10^3 L mol^{-1} cm^{-1} at 278 nm and 7.509×10^3 L mol^{-1} cm^{-1} at 266 nm, respectively. From Table-1, the coupling rate of Cd-IEDTA-BSA and Cd-IEDTA-HSA are 11:1 and 16:1, respectively.

Results of SDS-PAGE analysis: For the system of SDS-PAGE, the migration speed of migrator depends only on its molecular weight[19]. In the examination of synthetic artificial antigen, if the hapten coupling with protein in a specific rate, its molecular weight will change, we can judge weather the coupling is successful by comparison the migration speed between protein and artificial antigen.

After the coupling reaction occurred and coupling products (Cd-IEDTA-BSA, Cd-IEDTA-HSA, IEDTA-HSA) were synthesized in this experiment the SDS-PAGE was used to verify whether hapten had been attached to the carrier. Fig. 3 shows the

separation spot of SDS-PAGE. From Fig. 3, we can find that the speed of the coupling product (Cd-IEDTA-BSA, Cd-IEDTA-HSA, IEDTA-HSA) is slower than both BSA and HSA, which indicates that the molecular weight increases after coupling.

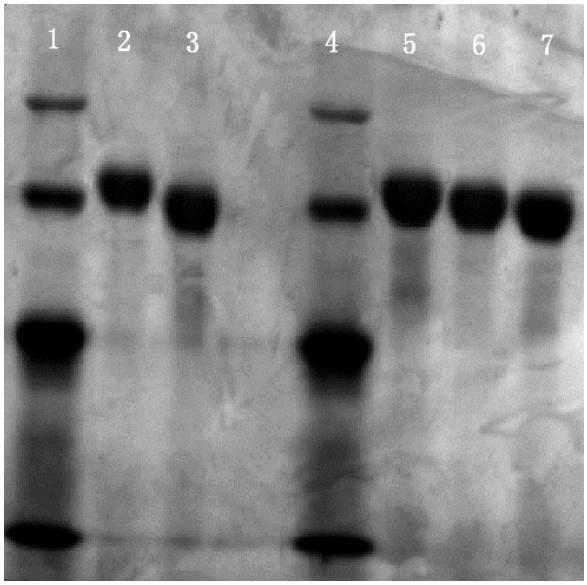

Fig. 3. Analysis of BSA, HSA and artificial antigen by SDS-PAGE, 1, 4 = Marker; 2 = Cd-IEDTA-BSA; 3 = BSA; 5 = Cd-IEDTA-HSA; 6 = IEDTA-HSA; 7 = HSA

Some literatures reported that if the molecular weight of the hapten is smaller, with the low rate of coupling, it is difficult to find the difference between antigen and carriers, but the macromolecule could be departed[20]. In this work, the molecular weight of the hapten is about 551.8, the data of atomic absorption spectroscopy shows the coupling scale of artificial antigen is in the scope of 11-15, the increase of the molecular weight is about 6000, so this result also confirmed the result of atomic absorption spectrophotometer.

Analytical results of TNBS method: The reagent 2,4,6-trinitrobenzenesulfonic acid (TNBS) has been used as a chromophore to determine primary amino groups in proteins, artificial antigen, *etc*. The reaction process is shown in Fig. 4.

2,4,6-TNBS reacts with free amino groups of protein molecules and the optical density is generally evaluated at 340 nm or 420 nm. The former value corresponds to the maximal absorption of the final product, N-trinitrophenylamines. The latter value is relative to the mesenheimer *p*-complex, the well-known intermediate of the overall reaction. Both wavelengths are suitable for quantification of amines, but 420 nm seems to be the best[21]. According to the value of absorbance, we can detect the content of free amino groups in protein molecules[22]. For the synthetic artificial

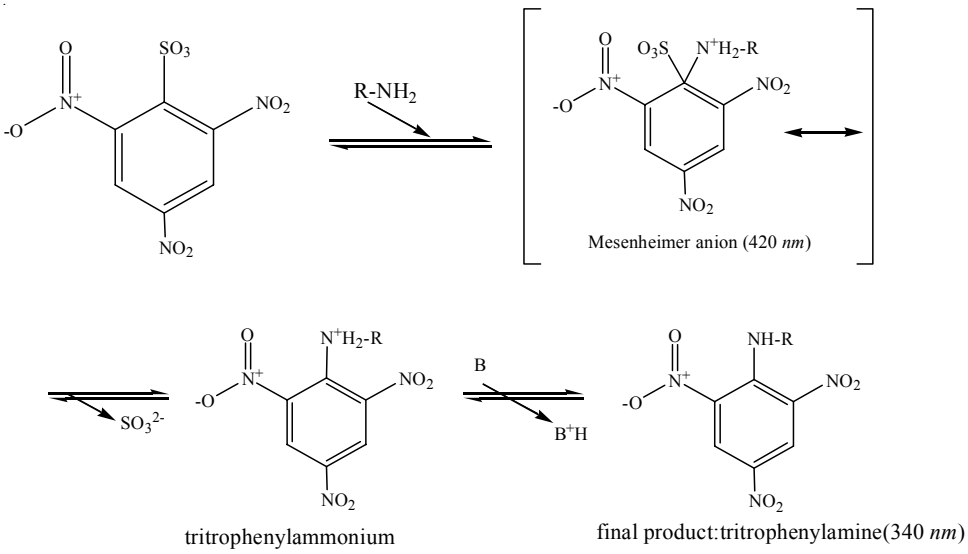

Fig. 4. Reaction mechanism of 2,4,6-TNBS and protein

antigen, bifunctional chelating agent makes the content of amino group which react with TNBS reduce through the reaction between isothiocyanic acid phenyl and the free amino group of carrier protein. In this case, the absorbance of the derivative in 420 nm was reduced. The replacement level of amino group also reflects coupling degree between hapten and protein.

The amino group substitution degree of artificial antigen was calculated by eqn. 1 and the results are shown in Table-2. The amino group consumption rate of Cd-IEDTA-BSA and Cd-IEDTA-HSA were 23.12 and 29.17 %, the coupling rate was 13:1 and 16:1, respectively. The results also reflect the artificial antigens were synthesized successfully.

TABLE-2
RESULTS OF ARTIFICIAL ANTIGENS BY
2,4,6-TRINITROBENZENE SULPHONIC ACID

Sample	Absorbance of unit concentration	Substitution degree of free amino groups (%)	Coupling rate
Cd-IEDTA-BSA	0.6330	23.12	13:1
Cd-IEDTA-HSA	0.5760	29.17	16:1

Results of indirect ELISA: Anti-serum was detected by encrusting substances, Cd-IEDTA-HSA and IEDTA-HSA and the normal BALB/c serum was used as negative control. The results of indirect ELISA shown in Table-3 indicated that the titer was more than 12800:1 and the OD value of Cd-HSA-IEDTA was significantly higher than HSA-IEDTA, the results demonstrated the synthesis of artificial antigen was successful, it also indicated that the mice produced antibodies against Cd^{2+}.

TABLE-3
RESULTS OF TITER BY INDIRECT ELISA

Coating antigen	Dilution multiplier							Blank control	Negative control
	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800		
Cd-IEDTA-HSA	1.858	1.232	0.854	0.562	0.302	0.251	0.139	0.043	0.052
IEDTA-HSA	1.476	1.023	0.622	0.402	0.288	0.204	0.115		

Conclusion

The core of immunoassay for heavy metal is to get the antibody with high titer and high specificity. High purity, moderate coupling ratio of the synthetic artificial antigen is as well as the key point to prepare antibodies. The artificial antigen can be synthesized through chemical methods, which couple the non-immunogenicity heavy metal ion and protein macromolecules by chemical reaction, the synthetic artificial antigen possess a full immunogenicity and will generate the anticipated antibody. In this work, the hapten molecule acquired in the first step through chelating reaction between IEDTA and Cd^{2+} and then the artificial antigen was synthesized successfully by coupling hapten molecule with carrier protein. The methods of AAS, UV, SDS-PAGE and TNBS were used to characterize synthetic antigen and to measure the coupling ratio. The consistent corroboration by those methods showed that the artificial antigen was synthesized successfully and the coupling rate of Cd-IEDTA-BSA, Cd-IEDTA-HSA was 11-13:1 and 14-16:1. The result from TNBS is a little higher than those from other methods. This may be due to the self-coupling of the protein at the same time, apart from ε-NH_3^+ of lysine, the hapten may interact with other groups of protein.

In the synthesis process of artificial antigen, it is not better for the protein molecule to hold more or less hapten, an appropriate ratio of hapten connected to the protein molecule is the basis of superior immunogenicity. As usual, for the immune-response procedure, T-cell recognizes carrier section of antigen and B-cell recognizes hapten section of antigen. But in the course of humoral immune response, T-cell must first identify carrier section of antigen and then B-cell could recognize hapten section of antigen, *i.e.*, B-cell does its work *via* T-cell on its effect. The distinguishable recognition function of T-cell will promote the antigen recognition reactions of B-cell. The excess number of the hapten connected to carrier protein will affect T-cell to identify the carrier section of antigen, *vice versa*, the response of B-cell will decrease. In general, for the artificial antigen which takes BSA as a carrier, its coupling ratio of 5-20:1 is suitable and there are also some reports showed that 10-30 hapten molecules linked to 100 kDa /carrier protein are more conductive to produce antibodies[23]. However, the idiographic coupling product must be tested through immunity experiment to see whether it can induce the organism to produce high titer antibodies. In this study, the immune experiment of mice has been carried out and the anti-serum was obtained, the titer of antiserum reached the level of 12800:1 by using competitive indirect ELISA. This result indicates

that the designed artificial antigen has been successfully synthesized and it owns a highly immunogenicity.

The development of heavy metal immunoassay, especially the successful use of enzyme linked immunoassary, provides a new idea for the detection of heavy metal contamination. The advantage of its rapidness, sensitivity, low cost, easy to operate online, dealing with a number of samples at the same time make itself as a better candidate to match the features of heavy tasks, high-demand in heavy metal analysis. Immunoassay has become a developmental technique for the rapid detection of heavy metal. The successful preparation of cadmium ion artificial antigen provides some references to analogy synthetic antigen for other heavy metals. It is also beneficial to antibody production, establishment of the immune analysis system and fast test the heavy metal in the field of foods and environments.

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