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Synthesis and Characterization of Artificial Antigens for Nickel

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A novel artificial antigen (AA) for Ni^{2+} was prepared by designed route, which coupled diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic dianhydride (DTPAA) with bovine serum albumin (BSA) and human serum albumin (HSA), and then chelated the DTPAA–BSA/HSA with Ni^{2+}. The AA was characterized by bicinchoninic acid (BCA) protein assay, atomic absorption spectroscopy (AAS), ultraviolet (UV) spectrum, fluorescence spectrum, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and 2,4,6-trinitrobenzenesulfonic acid assay (TNBS). The consistent results showed that the synthetic route different from the traditional method was effective, and the AA was synthesized successfully.

Currently, heavy metal pollution is becoming serious in the environment. Traditional detection techniques for heavy metal are difficult to adapt to on-site rapid-tests. Enzyme-linked immunosorbent assay (ELISA) is a new rapid test technique. The key with ELISA is to obtain a high titer antibody, and successful synthesis of artificial antigen is a prerequisite. This work developed a novel synthesis method (Figure 1) for artificial antigens.

After a preliminary solid-phase reaction of protein and DTPAA, the mixture was kept in reaction in $NaHCO_3$ solution to produce protein–DTPAA, the Ni^{2+} salt was added to initiate chelation, and then the protein–DTPAA–Ni was obtained by means of purification. The hapten (DTPAA–Ni) was prepared by traditional method.

The concentration of BSA/HSA–DTPAA–Ni was determined by BCA protein assay. They were 1.42 ± 0.08 and $1.22 \pm 0.05\ mg\ mL^{-1}$ respectively.

The Ni^{2+} concentrations of two artificial antigens detected by AAS were 15.986 and $14.724\ mg\ L^{-1}$ respectively. The coupling ratios (ε) were calculated as 12–13:1 and 10–11:1 according to eq 1.

$$\varepsilon = [(c_a v)/m_a]/[(c_b v)/m_b] = (c_a/m_a) : (c_b/m_b) \quad (1)$$

Where c_a and c_b are the nickel and protein concentrations. v is the solution volume. m_a and m_b represent the average molecular weights of nickel and the carrier protein.

The maximum UV absorption peak of BSA/HSA–DTPAA–Ni is between BSA and hapten with the range of 260–280 nm (Figure 2). It has the characteristics of both hapten and carrier protein absorption.

BSA shows a strong fluorescence emission peak at 343 nm (Figure 3),[1,2] but the fluorescence intensity (F) of BSA–DTPAA–Ni at the same concentration is weaker. This explains that DTPAA was coupling with a fluorescence chromophore or their adjacent groups. The same change occurred between HSA and HSA–DTPAA–Ni.

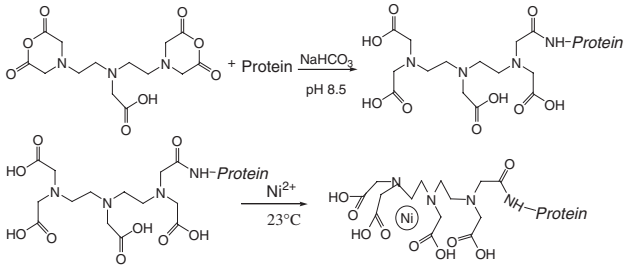

Figure 1. Synthesis of artificial antigens.

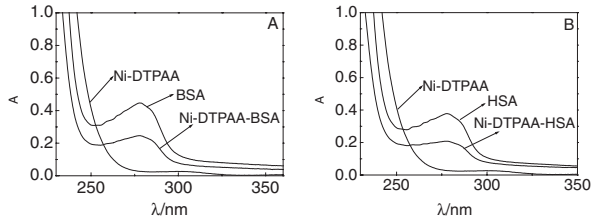

Figure 2. UV scanning spectra of BSA/HSA–DTPAA–Ni.

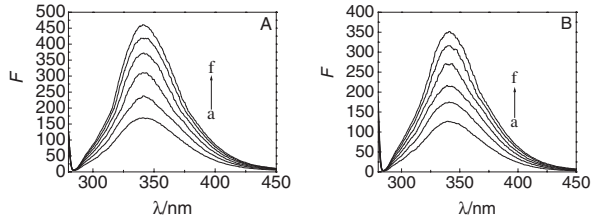

Figure 3. Fluorescence emission spectra of BSA (A) and BSA–DTPAA–Ni (B). c ($\times 10^{-6}\ mol\ L^{-1}$), a–f: 1.55, 2.34, 2.88, 3.48, 4.04, and 4.56.

Table 1. Comparison of fluorescence emission intensity between BSA and BSA–DTPAA–Ni (c: $\times 10^{-6}\ mol\ L^{-1}$)[a]

	The concentrations of BSA–DTPAA–Ni (c)					
	1.55	2.34	2.88	3.48	4.04	4.56
F	168.2	233.4	309.1	370.3	418.1	457.4
F'	126.8	173.1	214.4	269.4	316.1	349.0
ΔF	41.40	60.30	94.70	100.90	102.0	108.4
f	0.2462	0.2584	0.3064	0.2725	0.2440	0.2370

a: $\Delta F = F - F'$, $f = \Delta F/F$.

The fluorescence intensity data and quantitative results from the characteristic spectrum of BSA and BSA–DTPAA–Ni are shown as Table 1.

The averages of f of BSA–DTPAA–Ni and HSA–DTPAA–Ni are 26.8% and 16.4%, respectively. The data provide evidence for the synthesis of artificial antigens.

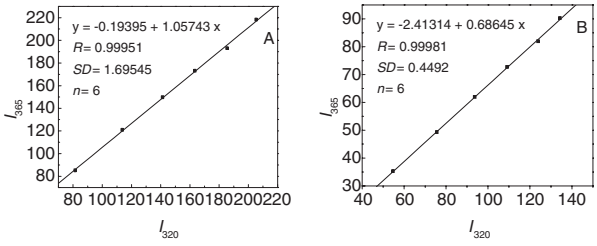

Figure 4. Fluorescence phase diagram of BSA–DTPAA–Ni (A) and HSA–DTPAA–Ni (B).

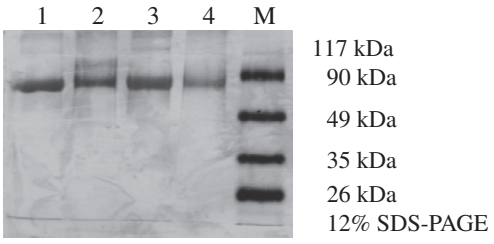

Figure 5. The SDS-PAGE electrophoretogram. **1**: BSA, **2**: BSA–DTPAA–Ni, **3**: HSA, **4**: HSA–DTPAA–Ni, and **M**: Maker.

Table 2. P and r of polarization spectra (c: $\times 10^{-6}$ mol L^{-1})

Sample	c	G	P	r
BSA	4.56	1.18	0.0697	0.0476
BSA–DTPAA–Ni	4.56	1.10	0.1161	0.0805
HSA	4.01	1.14	0.0827	0.0567
HSA–DTPAA–Ni	4.01	1.15	0.1207	0.0839

A fluorescence phase diagram was used to characterize the microconformational change of the folding conformation of the carrier protein in the artificial antigens synthesis (Figure 4). The I_{320}–I_{365} phase diagram shows superior linearity (with interpretation of the correlation coefficient R and standard deviation (SD)), which explains the changes in the protein environment leading to the "all-or-none" transition between two different conformations.

Fluorescence polarization (P) and fluorescence anisotropy (r) can be measured by the fluorescence polarization spectrum, which can be used to estimate the degree of the coupling reaction. The P is given as:[3]

$$P = (I_{VV} - I_{VH} \times G)/(I_{VV} + I_{VH} \times G) \quad (2)$$

Where I_{VV} and I_{VH} are fluorescence intensities when the excitation polarizer is perpendicular, and the emission polarizer is parallel and perpendicular, respectively. G ($G = I_{HV}/I_{HH}$) is the correction factor. The I_{HV} and I_{HH} are fluorescence intensities when the excitation polarizer is parallel, and the emission polarizer is perpendicular and parallel, respectively. The r, is measured as follows:

$$r = (I_{VV} - I_{VH} \times G)/(I_{VV} + 2I_{VH} \times G) \quad (3)$$

The data of determined P and r are shown as Table 2.

From Table 2, P and r of artificial antigens both increased. This illustrates that micro-area conformation changes of fluorescence chromophore bring about the conformation changes of carrier protein after synthesis,[4] and different carrier proteins lead to the difference of P and r. The structure difference of proteins may generate the discrepancy of the fluorescent complex.

SDS-PAGE can also characterize the synthesis of artificial antigens. Although the molecular weight increase of the artificial antigens was limited for carrier proteins, the rate of migration showed slight differences (Figure 5). The artificial antigens contained metal ion and the subunits of carrier proteins participated in chelation, which resulted in the emergence of hysteresis of the artificial antigens stripe.

2,4,6-Trinitrobenzenesulfonic acid can react with the free amine of protein to form an intermediate complex with an absorption peak at 420 nm. In the synthesis, the content of free amine reacting with TNBS decreased, thus the absorption of the intermediate complex was reduced. The content of free amine can be detected by the absorption.[5] The extent of amine group substitution (ε) of BSA–DTPAA–Ni and HSA–DTPAA–Ni calculated by eq 4 were 30.77% and 37.53%, respectively.

$$\varepsilon = (A_a - A_b)/A_a \quad (4)$$

Where A_a and A_b are the absorbance of carrier protein and artificial antigen.

Heavy metal ions (M^{n+}) have no immunogenicity and tend to react strongly and irreversibly with biological molecules. They do not couple with carrier protein directly, and those heavy metal ions can only be recognized by the antibody through the coupling of a chelator and carrier proteins.[6] Nowadays, new synthesis methods for artificial antigens are few, and most use haptens to react with a carrier protein, such as isothiocyanobenzyl–EDTA–M^{n+}, or isothiocyanobenzyl–DTPA–M^{n+}.[7–9] In this study, DTPAA was selected to couple with protein first, which is different from the traditional method. The coupling ratio of artificial antigens prepared by us is suitable for the range reported. This work can be an important reference for the synthesis of other artificial antigens and the preparation on monoclonal antibodies against heavy metals.

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References

1 S. Pihlasalo, J. Kirjavainen, P. Hänninen, H. Härmä, *Anal. Chem.* **2009**, *81*, 4995.
2 P. L. Gentili, F. Ortica, G. Favaro, *J. Phys. Chem. B* **2008**, *112*, 16793.
3 Y. Yan, J. G. Xu, *Sci. China, Ser. B (Chin. Ed.)* **1997**, *27*, 16.
4 Y. V. Il'ichev, J. L. Perry, J. D. Simon, *J. Phys. Chem. B* **2002**, *106*, 452.
5 V. L. Hegde, Y. P. Venkatesh, *Immunobiology* **2007**, *212*, 119.
6 T. Kong, G. W. Liu, X. B. Li, Z. Wang, Z. G. Zhang, G. H. Xie, Y. Zhang, J. Sun, C. Xu, *Food Chem.* **2010**, *123*, 1204.
7 I. A. Darwish, D. A. Blake, *Anal. Chem.* **2002**, *74*, 52.
8 R. C. Blake, A. R. Pavlov, M. Khosraviani, H. E. Ensley, G. E. Kiefer, H. N. Yu, X. Li, D. A. Blake, *Bioconjugate Chem.* **2004**, *15*, 1125.
9 X. X. Zhu, B. S. Hu, Y. Lou, L. N. Xu, F. L. Yang, H. N. Yu, D. A. Blake, F. Q. Liu, *J. Agric. Food Chem.* **2007**, *55*, 4993.

