Chinese Chemical Letters Vol. 13, No. 9, pp 845 – 848, 2002
http://www.imm.ac.cn/journal/ccl/html

A Novel Scheme for Production of Polyclonal Antibody against Estrogenic Bisphenols

Mei Ping ZHAO[1], Yuan Zong LI[1], Zhen Quan GUO[2], Xin Xiang ZHANG[1]
Wen Bao CHANG[1]*

[1]College of Chemistry and Molecular Engineering, Peking University, Beijing 100871
[2]College of Life Sciences, Peking University, Beijing 100871

Abstract: A polyclonal antibody against the currently concerned estrogenic bisphenol compounds was produced according to a new scheme. 4,4-Bis (4-hydroxyphenyl) valeric acid was used to synthesize the complete antigen in which the characteristic bisphenol structure was exposed to the largest extent. The produced polyclonal antibody showed high specificity and affinity for bisphenol A.

Keywords: Bisphenol A, antigen, polyclonal antibody.

Krishnan[1] reported in 1993 that bisphenol A (BPA) released from flasks made of polycarbonates had weak estrogenic property. Later on, Perez *et al*[2]. identified that besides bisphenol A, some other related hydroxylated diphenylalkanes are also estrogenic. Since these compounds are very widely used in industry, particularly in plastics, potential exposure of human beings to them is becoming a significant issue.

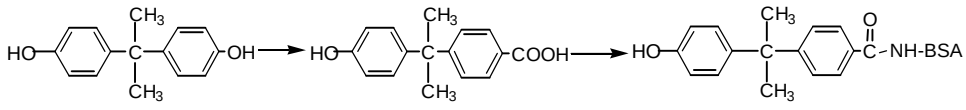
For the time being, chromatographic methods combined with solid phase extraction (SPE) and other chemical analytical techniques are the most commonly used methods to measure the bisphenols in environmental and biological samples[3-5]. As it is known, such methods usually suffer from laborious sample-pretreatment steps and the limit of detection was hindered by the interference of other phenolic compounds with similar structures but at a much higher concentration in the matrix. Regarding of the biological activity of bisphenols, immunoassay is supposed to be a specific and sensitive tool to detect them. There have been some reports concerning the production of antibodies against BPA[6,7]. However, in these research, one of the two hydroxy groups of BPA was carboxylated so as to conjugate bovine serum albumin (BSA) **(Scheme 1,** conjugate **1)**. Thus part of the structural characteristics of BPA was lost in the synthesized antigen. In this paper, we chose a commercially available reagent 4,4-bis(4-hydroxyphenyl) valeric acid (BHPVA) to prepare the complete antigen based on a new scheme **(Scheme 1,** conjugate **2)**. The conjugation took place between the amino group of BSA and the carboxyl group of BHPVA, thus the structure of interest was favorably exposed.

N-hydroxysuccunimide(NHS) and 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimi-

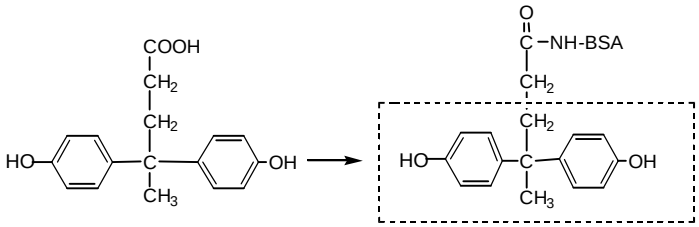
*E-mail: mpzhao@chem.pku.edu.cn

de • HCl (EDC) were used to activate the BHPVA in dimethyl sulfoxide (DMSO) for 2 h. The molar ratios of BHPVA, NHS and EDC were 1:1.25:1.25. BSA solution was prepared in 0.1 mol/L $NaHCO_3$ (pH adjusted to 7.0 by adding HCl). To this solution, the above active ester solution was added slowly in droplets under stirring. The reaction was performed for 2 h and the obtained solution was dialysed against 0.01 mol/L PBS at 4°C for 24 h. After 6 changes of the PBS, the solution was dialysed against pure water for 48 h with 8 changes of water. Finally, the solution was lyophilized and the obtained BHPVA-BSA conjugate was used to immunize rabbits. The molar ratio of BHPVA/BSA was found to be 20:1 based on a Coomassie Brilliant Blue spectrophotometric method[8].

Scheme 1



Bisphenol A Conjugate **1**



4,4-Bis(4-hydroxyphenyl) valeric acid Conjugate **2**

The titre of antiserum was determined with ELISA using BHPVA-OVA (prepared in the same way as BHPVA-BSA) as coating antigen. HRP-conjugated goat anti-rabbit IgG was used as the enzyme tracer. The color reaction of substrate 3,3′,5,5′- tetramethylbenzidine (TMB) was terminated by adding 2 mol/L H_2SO_4. The optical density was read at 450 nm.

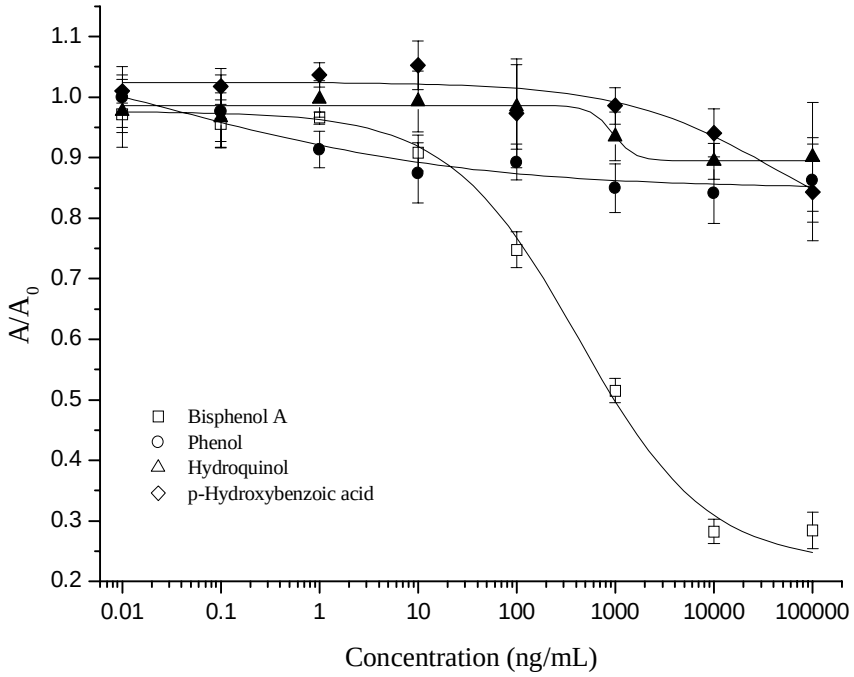
The obtained antibody was purified according to a modified caprylic acid-SAS method[9]. The optimum concentration of BHPVA-OVA and the titers of antiserum and purified antibodies were determined by a chequerboard method. Based on the definition of "titer" as the antiserum dilution required to bind 50% of a small, given amount of labeled antigen[10], the titers of the antiserum and purified antibody were found to be 1:50,000 and 1:10,000, respectively and the optimal concentration for BHPVA-OVA as the coating ligand was 12.0 μg/mL.

The cross reactions were tested with several commonly existing substances containing phenol groups, including phenol, hydroquinol and *p*-hydroxybenzoic acid. Standard solutions of one of the competitive compounds (BPA, phenol, hydroquinol and *p*-hydroxybenzoic acid) were five-fold diluted in serial and then mixed with the 5000-times diluted purified antibody solution (volume ratio 1:1) and then added to the coated wells. The dose-response curves of the selected compounds were shown in

Figure 1. It can be clearly seen that with the increasing of the concentration of the competitive compounds, the antibody-antigen reaction was significantly inhibited by BPA, while the other three simple compounds have little influence on the reaction. According to the 50% displacement method[10], the cross reactions of phenol, hydroxyquinol and *p*-hydroxybenzoic acid were all much lower than 1%. Thus it was demonstrated that the produced polyclonal antibody specifically recognizes the bisphenolic group, *i.e.* two phenolic rings bridged through a carbon atom. The linear range for quantitative determination of BPA was found to be 1.0 – 10000 ng/mL, with the lower limit of detection of 0.1 ng/mL of BPA.

An immunoaffinity column (IAC) was also generated by coupling the polyclonal antibodies to Sepharose 4B. The IAC showed high affinity to BPA while other compounds with similar structures were barely retained. Further application of the polyclonal antibody is under study now in our group.

Figure 1 Dose-response curves of the selected compounds based on competitive ELISA.



Acknowledgments

This work was financially supported by the National Natural Science Foundation of China(Grant No. 20075001).

References

1. A. V. Krishnan, P. Stathis, S. F. Permuth, L. Tokes, D. Feldman, *Endocrinology*, **1993**, *132*, 2279.
2. P. Perez, R. Pulgar, F. Olea-Serrano, M. Villalobos, A. Rivas, M. Metzler, V. Pedraza, N. Olea, *Environmental Health Perspectives*, **1998**, *106*, 167.
3. H. G. J. Mol, S. Sunarto, O. M. Steijger, *J. Chromatogr. A*, **2000**, *879*, 97.
4. J. Sajiki, K. Takahashi, J. Yonekubo, *J. Chromatogr. B*, **1999**, *736*, 255.
5. S. N. Pedersen, C. Lindholst. *J. Chromatogr. A*, **1999**, *864*, 17.
6. Y. Goda, A. Kobayashi, K. Fukuda, S. Fujimoto, M. Ikc, M. Fujita, *Water Science and Technology*, **2000**, *42*, 81.
7. S. Nishii, Y. Soya, K. Matsui, T. Ishibashi, Y. Kawamura, *Bunsei Kagaku*, **2000**, *49*, 969.
8. Y. Xu, *Immunoassay Techniques*, 2nd Edition, Science Press, Beijing, **1997**, 393.
9. D. Tang, Y. C. Wang, W. B. Chang, Y. X. Ci, Z. Q. Guo, *Chin. J. Anal. Chem*., **1999**, *27*, 899.
10. J. J. Pratt, *Clin. Chem*., **1978**, *24*, 1869.

Received 19 December, 2001