Anal. Chem. **2007,** *79,* 9191−9196

Polyclonal Antibody-Based Noncompetitive Immunoassay for Small Analytes Developed with Short Peptide Loops Isolated from Phage Libraries

A. González-Techera,[†] H. J. Kim,[‡] S. J. Gee,[‡] J. A. Last,[§] B. D. Hammock,[‡] and G. González-Sapienza*,[†]

Cátedra de Inmunología, Facultad de Química, Instituto de Higiene, Universidad de la República (UDELAR), Montevideo, 11600, Uruguay, Department of Entomology and Cancer Research Center, University of California, Davis, California 95616, and Pulmonary/Critical Care Medicine, School of Medicine, University of California, Davis, California 95616

To date, there are a few technologies for the development of noncompetitive immunoassays for small molecules, the most common of which relies on the use of anti-immunocomplex antibodies. This approach is laborious, case specific, and relies upon monoclonal antibody technology for its implementation. We recently demonstrated that, in the case of monoclonal antibody-based immunoassays, short peptide loops isolated from phage display libraries can be used as substitutes of the anti-immunocomplex antibodies for noncompetitive immunodetection of small molecules. The aim of this work was to demonstrate that such phage ligands can be isolated even when the selector antibodies are polyclonal in nature. Using phenoxybenzoic acid (PBA), a major pyrethroid metabolite, as a model system, we isolated the CFNGKDWLYC peptide after panning a cyclic peptide library on the PBA/anti-PBA immunocomplex. The sensitivity of the noncompetitive enzyme-linked immunosorbent assay (ELISA) setup with this peptide was 5-fold (heterologous) or 400-fold (homologous) higher than that of the competitive assay setup with the same antibody. Phage anti-inmunocomplex assay (PHAIA) was also easily adapted into a rapid and highly sensitive dipstick assay. The method not only provides a positive readout but also constitutes a major shortcut in the development of sensitive polyclonal-based assays, avoiding the need of synthesizing heterologous competing haptens.

The need for simple and high-throughput analysis of small molecules such as pesticides, drugs, and hormones in environmental and biomedical analysis is rapidly growing. Immunoassays, which are simple, robust, and low-cost techniques, are widely used for this purpose in a broad range of applications from laboratory analysis to home drug-testing kits.[1] The central component of these assays is the antibody−analyte reaction, which is coupled to a signaling system that converts this reaction into a quantitative reading. In the case of small analytes, upon binding to a specific antibody most of its surface ends up buried in the antigen binding site of the antibody.[2] This limits the possibility of reacting the analyte with a second antibody, preventing the use of two-site assays, which are the standard format for direct noncompetitive measurement of macromolecular analytes. For this reason, small molecules are detected in an indirect way (competitive immunoassays) in which the molecule of interest (analyte) competes for binding to a specific antibody with a structurally related molecule (competing hapten). In this format, either the antibody or the competing hapten molecule must be labeled to produce a signal, and in all cases, the presence of the analyte is registered as a loss in signal (less antibody−competing hapten complex is formed). A key limitation of the competitive format is that in order to achieve significant signal variation at trace amounts of analyte, the amount of antibody and competing hapten have to be minimized. Therefore, minimizing the amount of antibody in turn produces a loss in the assay sensitivity.[3] In addition, noncompetitive immunoassays are preferred for automation or when they need to be adapted into rapid "on-site" formats such as dipstick or immunochromatography.[4,5]

Because of these advantages, there have been many efforts to implement small-molecule noncompetitive immunoassays, but most of these developments are complex, restricted to particular applications, or required analyte labeling.[3,6−8] The most general approach makes use of anti-immunocomplex antibodies to detect the formation of the immunocomplex (IC).[9−11] As mentioned

* Corresponding author. E-mail: ggonzal@fq.edu.uy. Phone: (5982) 4874334.
[†] Universidad de la República.
[‡] Department of Entomology and Cancer Research Center, University of California, Davis.
[§] School of Medicine, University of California, Davis.

(1) Towt, J.; Tsai, S. C.; Hernandez, M. R.; Klimov, A. D.; Kravec, C. V.; Rouse, S. L.; Subuhi, H. S.; Twarowska, B.; Salamone, S. J. *J. Anal. Toxicol.* **1995**, *19*, 504−510.
(2) Lamminmaki, U.; Kankare, J. A. *J. Biol. Chem.* **2001**, *276*, 36687−36694.
(3) Martens, C. L.; Cwirla, S. E.; Lee, R. Y.; Whitehorn, E.; Chen, E. Y.; Bakker, A.; Martin, E. L.; Wagstrom, C.; Gopalan, P.; Smith, C. W.; Tate, E.; Kooler, K. J.; Schatz, P. J.; Dower, W. J.; Barret, R. W. *J. Biol. Chem.* **1995**, *270*, 21129−21136.
(4) Paddle, B. M. *Biosens. Bioelectron.* **1996**, *11*, 1079−1113.
(5) Luppa, P. B.; Sokoll, L. J.; Chan, D. W. *Clin. Chim. Acta* **2001**, *314*, 1−26.
(6) Shan, G.; Huang, H.; Stoutamire, D. W.; Gee, S. J.; Leng, G.; Hammock, B. D. *Chem. Res. Toxicol.* **2004**, *17*, 218−225.
(7) Pradelles, P.; Grassi, J.; Creminon, C.; Boutten, B.; Mamas, S. *Anal. Chem.* **1994**, *66*, 16−22.
(8) Pulli, T.; Hoyhtya, M.; Soderlund, H.; Takkinen, K. *Anal. Chem.* **2005**, *77*, 2637−2642.
(9) Ullman, E. F.; Milburn, G.; Jelesko, J.; Radika, K.; Pirio, M.; Kempe, T.; Skold, C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1184−1189.

10.1021/ac7016713 CCC: $37.00
Published on Web 11/01/2007

above, the binding site of a hapten-specific antibody buries a large portion of the hapten that does not allow binding of a second antibody. However, the structure of the binding area of the IC presents changes in relation to that of the free binding site of the primary antibody, which can be recognized by anti-IC antibodies, so-called, antimetatype antibodies.[12] These modifications, represented by the solvent-exposed region of the hapten and the antibody conformational changes induced by its binding, are subtle and mostly limited to the binding pocket of the primary antibody. Because of the large surface that is buried in the interface of the ternary complex, this fine discrimination between the unliganded and liganded state of the primary antibody is difficult to obtain, and the cross-reactivity with the uncombined primary antibody is a serious limitation to the preparation of antimetatype antibodies.[13] Another important limitation for the production of these antibodies is that due to the complexity of the approach, all reported examples of noncompetitive assays are based on the use of monoclonal antibodies, which excludes the large number of high-quality polyclonal antihapten antibodies that have been developed for immunodetection of small analytes.

We reasoned that these difficulties could be overcome by reducing the binding surface of the anti-immunocomplex detection molecule by substituting the secondary antibody by a filamentous phage particle bearing a short peptide loop. These peptides can be selected from phage display peptide libraries, using the IC as selector molecule. We have recently successfully applied this concept (phage anti-inmunocomplex assay, PHAIA) to the development of monoclonal antibody-based noncompetitive immunoassays for small analytes.[14] In this work we demonstrate that the huge chemical diversity and power of selection inherent to phage display peptide libraries allow the development of PHAIA, even when the selector molecule is not a single chemical structure but a complex population of polyclonal antibodies. For this we used phenoxybenzoic acid (PBA) because it represents a small analyte of moderate polarity, it is a common metabolite of most commercial pyrethroid insecticides, and thus it is useful for human and environmental monitoring. Also it is important to notice that pyrethroids are now the dominant insecticides worldwide. A phagemid library expressing 8 mer cyclic random peptides fused to pIII phage protein was used as a source of peptide ligands, and affinity-purified polyclonal antibodies were used to form the selector IC.

EXPERIMENTAL SECTION

Phage Library and Antibodies. A random phage display peptide library with an estimated diversity of 3×10^9 independent clones was constructed on the phagemid vector pAFF/MBP as described.[3] pAFF/MBP vector is a derived vector of Affymax pAFF2 vector[3] and was a kind gift from Dr. Peter Schatz (Affymax Research Institute, Palo Alto, CA). This library, with the general sequence $ASGSACX_8CGP_{6-}$, expresses cyclic 8 mer random peptides flanked by two cysteines and fused to the phage coat protein pIII. Antibodies specific for PBA were purified on 3-((2-oxoethoxy)ethoxy)phenoxybenzoic acid coupled Sepharose, from serum of rabbits immunized with a hapten−KLH conjugate.[6] Alternately, the γ-globulin fraction of the anti-PBA rabbit serum was purified on protein G columns as described by the manufacturer (Amersham-Pharmacia, Uppsala) and was used for the enzyme-linked immunosorbent assay (ELISA) setup.

Panning. Microtiter ELISA plates (Maxisorp, Nunc) were coated with affinity-purified anti-PBA antibodies at a concentration of 5 μg/mL in phosphate-buffered saline (PBS) either 1 h at 37 °C or overnight at 4 °C. After coating, the wells were blocked with 1% bovine serum albumin (BSA) at 37 °C for 1 h and washed three times with PBS, 0.05% Tween 20 (PBST). The peptide library (10×10^{11} transducing units) was mixed with PBA (10 μg/mL final concentration) and BSA (final concentration of 1%) in a final volume of 600 μL of PBS and then added to six microtiter wells coated with the antibody. After incubating for 2 h at 4 °C, the wells were washed five times with PBS, incubated for half an hour in PBS at 4 °C, washed again five times with PBS, after which, the bound phages were eluted with 100 μL per well of 100 mM glycine−HCl, pH 2.2 buffer. After incubation for 10 min at room temperature, the eluted phage was transferred to a 1.5 mL tube, and the pH was neutralized by adding 35 μL of 2 M Tris base.

For amplification of the phage stock, the eluted phage (600 μL) were added to 10 mL of log-phase *E. coli* ARI 292 (Affymax Research Institute, Palo Alto, CA) cells and amplified in SOP medium (Luria broth (LB) media containing 0.25% K_2HPO_4, 0.1% $MgSO_4$) plus 0.1% glucose and 100 μg/mL ampicillin to an $OD_{600} = 0.4$. After that, M13KO7 helper phage (New England Biolabs) at a multiplicity of infection 10:1 was added. After a period of 30 min at 37 °C without shaking, arabinose and kanamycin were added to a final concentration of 0.02% and 40 μg/mL, respectively, and the cultures incubated overnight at 37 °C with vigorous shaking. Phage from liquid cultures were obtained by clearing the supernatants by centrifugation at 12 000*g* for 15 min, precipitated with 0.2 volumes of 20% polyethylene glycol 8000−2.5 M NaCl, (PEG−NaCl), incubated on ice during 1 h, and centrifuged as above. Phage pellets were resuspended in 2 mL of sterile PBS and titrated in ARI 292. A number of 10^{10} transducing units were used for the next round of selection. Additional rounds of panning were performed in a similar way, using 200 μL of the amplified phage stock supplemented with BSA (1% final concentration) and PBA at a final concentration of 10 μg/mL.

Phage ELISA. After three rounds of panning, ARI 292 cells were infected with the eluted phage and grown on LB−agar ampicillin plates. Ten individual clones were picked and used for inoculation of tubes with 5 mL of SOP with ampicillin and glucose as described above; cells were grown with shaking at 37 °C. After cultures reached an $OD_{600} = 0.5$ AU, 1 μL of M13K07 helper phage at a concentration of 1×10^{11} transducing units/mL was added for growing individual recombinant phage supernatants. Cultures were then incubated for 30 min at 37 °C without shaking to allow infection of the cells. Arabinose and kanamycin were then added as described above, and cultures were grown overnight with shaking at 37 °C. The next day, the cells were pelleted by centrifugation at 10 000 rpm for 5 min, and the supernatants were used for screening. ELISA screening for phage that reacted with

(10) Self, C. H.; Dessi, J. L.; Winger, L. A. *Clin. Chem.* **1994**, *40*, 2035−2041.
(11) Nagata, S.; Tsutsumi, T.; Yoshida, F.; Ueno, Y. *Nat. Toxins* **1999**, *7*, 49−55.
(12) Voss, E. W., Jr.; Miklasz, S. D.; Petrossian, A.; Dombrink-Kurtzman, M. A. *Mol. Immunol.* **1988**, *25*, 751−759.
(13) Ban, N.; Escobar, C.; Garcia, R.; Hasel, K.; Day, J.; Greenwood, A.; McPherson, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1604−1608.
(14) Gonzalez-Techera, A.; Vanrell, L.; Last, J. A.; Hammock, B. D.; Gonzalez-Sapienza, G. *Anal. Chem.*, in press.

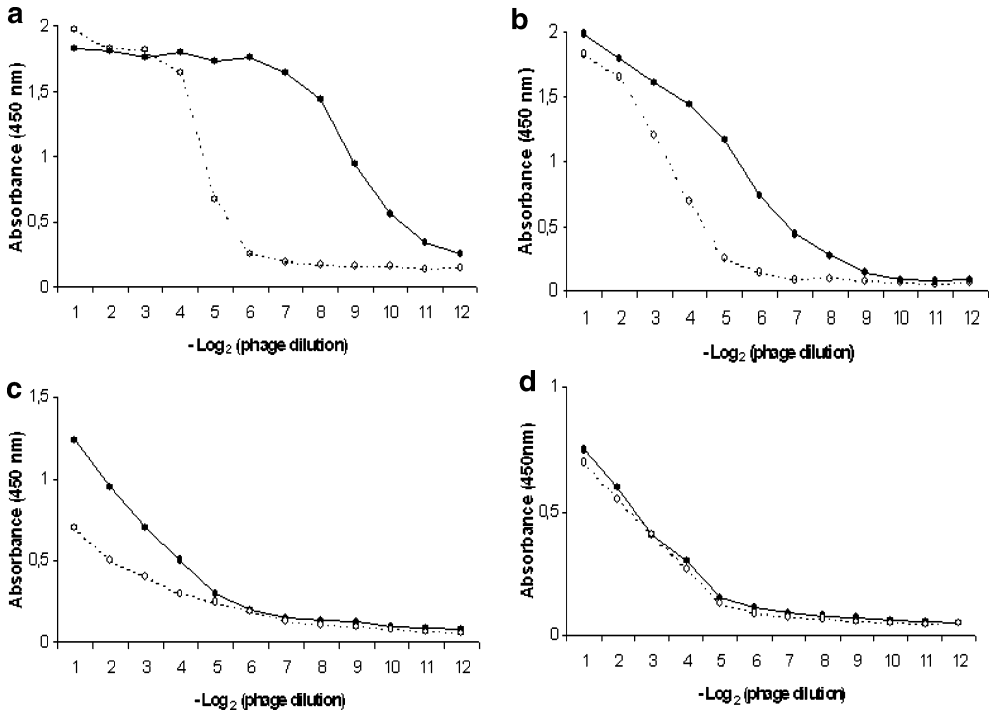


Figure 1. Reactivity of the phage-borne peptide with the PBA IC using different amounts of coating antibody. Plates were coated with 10 μg/mL protein G purified antibodies (a), 5 μg/mL (b), 2.5 μg/mL (c), or 1.25 μg/mL (d) and incubated with serial dilutions of phage-borne peptide in the presence (black circles) or absence (white circles) of 50 ng/mL PBA.

the PBA−antibody complex was performed by direct addition of 50 μL of supernatants to wells coated with 0.5 μg/well of affinity-purified anti-PBA polyclonal antibody, with or without addition of 50 μL of 200 ng/mL PBA per well.

Stabilization of Phage Suspensions. Individual amplified phage clones were obtained as described above. After two steps of precipitation with PEG−NaCl, the phage particles were suspended in 1/50 volume of the original culture volume in PBS, which was supplemented with the complete protease inhibitor cocktail of Roche Diagnostics and sodium azide 0.05%. The preparation was filtered through a 0.22 μm filter and stored in aliquots at 4 and −80 °C.

Noncompetitive Phage ELISA. ELISA plates were coated with the γ-globulin fraction of the anti-PBA rabbit serum purified on Protein G columns (Amersham-Pharmacia, Uppsala) using 100 μL of 10, 5, 2.5, and 1.25 μg/mL in PBS. After incubation for 1 h at 37 °C and blocking 1 h at 37 °C with BSA 1%, the plates were washed three times with PBS−0.05% Tween 20. Serial 2-fold dilutions of phage preparation (starting from a 1/50 dilution) were performed in PBS−0.05% Tween 20 in nontreated polystyrene plates (low binding capacity) in the presence (50 ng/mL) of PBA or in its absence. The phage dilutions were then transferred to wells precoated with the protein G purified antibody. After incubation for 1 h at 37 °C, the plates were washed and 100 μL of the appropriate dilution of an anti-M13 antibody coupled to horseradish peroxidase was added to the wells, incubated for 30 min at 37 °C, and thoroughly washed. The peroxidase activity was then developed by adding 100 μL of peroxidase substrate (25 mL of 0.1 M citrate acetate buffer pH 5.5, 0.4 mL of 6 mg/mL DMSO solution of 3,3′,5,5′-tetramethylbenzidine, and 0.1 mL of 1% H_2O_2) dispensed into each well. The enzymatic reaction was stopped after 15−20 min by the addition of 50 μL of 2 M H_2SO_4, and the absorbance at 450 nm (corrected at 600 nm) was read in a microtiter plate reader (Multiskan MS, Labsystems). Absorbance values were fitted to a four-parameter logistic equation using Genesis Lite 3.03 (Life Sciences (U.K.) Ltd.) package software.

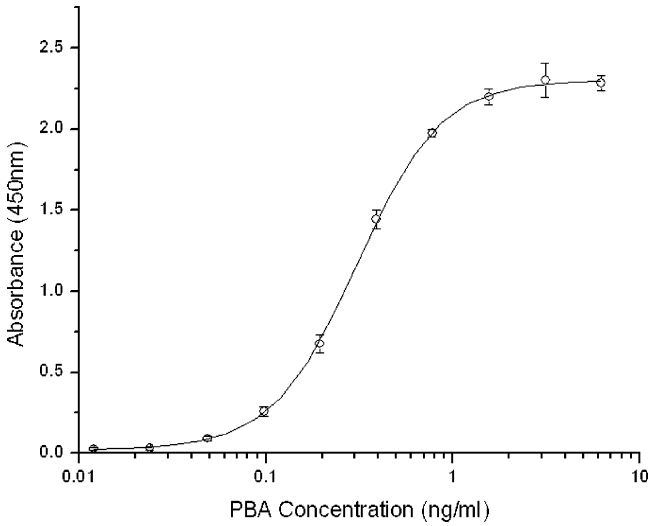
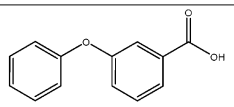
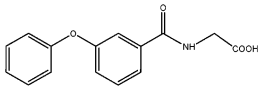
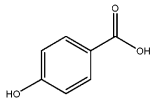
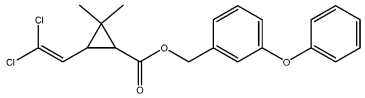
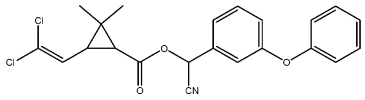
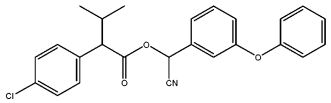


Figure 2. Noncompetitive PHAIA for PBA. The ELISA was performed on plates coated with 100 μL of 10 μg/mL protein G purified antibodies, using 2.5 × 10^9 phage particles/mL expressing the CFNGKDWLYC peptide and anti-M13 peroxidase conjugate for detection.

Cross-Reactivity Assay. The specificity of the noncompetitive assay setup with the phage-borne peptide was characterized by determining its cross-reactivity with structurally related pesticides. PBA concentrations in the 0−2500 ng/mL range were used in the noncompetitive ELISAs. Data were normalized, and the molar compound concentration corresponding to the midpoint of the

Table 1. Cross-Reactivity (CR) of Noncompetitive Phage ELISA for PBA

Chemicals	Structures	SC_{50} (ng/mL)	CR (%)
PBA		0.3	100
PBA-glycine conjugate		107	0.2
4-Hydroxybenzoic acid		>2500	0.01
Permethrin		>2500	0.01
Cypermethrin		>2500	0.01
Esfenvalerate		>2500	0.01

curve (which corresponds to the concentration of analyte producing 50% saturation of the signal (SC_{50}) was used to express the cross-reactivity of the assay according to the following equation:

$$\%\ \text{cross-reactivity} = 100 \times [\{SC_{50}(\text{molinate})\}/\{SC_{50}(\text{cross-reacting compound})\}]$$

Dipstick Assay. Amounts of 1.5 μL of serial dilutions of protein G purified anti-PBA antibodies in PBS were spotted onto a 0.45 μm nitrocellulose membrane (Biorad Laboratories Inc., CA). Immediately after drying, the membranes were quenched by soaking into 5% skimmed milk in PBS for 30 min; after rinsing in PBST 5 mm strips were cut, dried, and kept at 4 °C until used. The assay was performed by dipping the membrane strips for 15 min into water spiked with different amounts of PBA and containing the appropriate dilution of phage, washed under tap water incubated for 10 min into a diluted solution of anti-M13 peroxidase conjugated, washed under tap water, and developed using the diaminobenzidine−nickel chloride substrate mix.

RESULTS

Panning a Peptide Library with Polyclonal Antibodies against PBA. A phage display peptide library expressing random octapeptides flanked by a disulfide bridge and fused to the phage coat protein pIII was panned as described above. In order to favor the isolation of peptides that recognize the PBA−antibody IC, an excess of PBA was included in the panning experiments. After three rounds of panning, 10 individual clones were tested for binding to the affinity-purified anti-PBA antibody coated wells in the presence or absence of PBA as described in the Experimental Section. Nine out of 10 clones exhibited stronger binding to the polyclonal antibodies in the presence of PBA (100 ng/mL) than in its absence. Sequencing of the nine reactive clones revealed that all of them coded for the same peptide sequence, CFNGKDWLYC. The phage-borne peptide did not bind to the analyte−KLH conjugate, which demonstrated that the peptide is specific for the PBA−antibody immunocomplex.

Table 2. Recovery of PBA in Urine Measured by Noncompetitive PHAIA

final concn of urine (%)	spiked PBA (ng/mL)	measured PBA (ng/mL)	mean recovery (%, $n = 3$)
0	1	1.1 ± 0.1	115
	2	1.6 ± 0.4	82
1	1	1.2 ± 0.2	124
	2	2.3 ± 0.1	115
2.5	1	1.2 ± 0.2	88
	2	2.0 ± 0.2	92
5	1	1.1 ± 0.2	106
	2	2.2 ± 0.1	108
10	1	1.1 ± 0.2	109
	2	1.7 ± 0.2	85

Noncompetitive ELISA. One of these clones was amplified and prepared as a stabilized phage suspension as described above (8×10^{12} transducing units/mL). This phage solution was used to determine the antibody coating conditions that allowed us to maximize the differential signal in the presence or absence of PBA. As shown in Figure 1, both the amount of protein G purified total IgG used for coating and the concentrations of phage particles are critical to observe differential binding to the IC. The best result was obtained when 100 μL of 10 μg/mL protein G purified total IgG was used for coating, in combination with 2.5×10^9 phage transducing units/mL. These conditions were used to setup the noncompetitive PHAIA shown in Figure 2. The binding

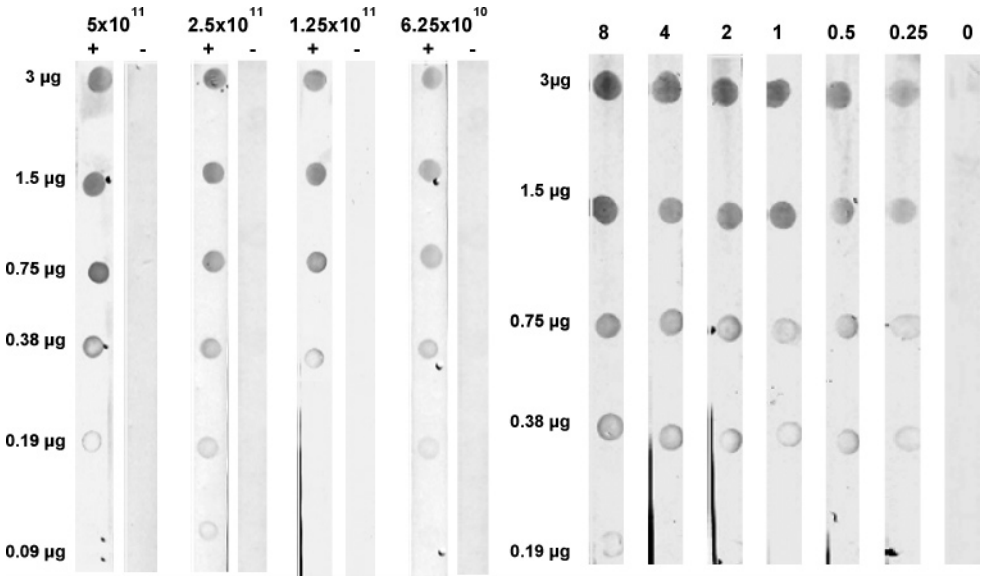


Figure 3. Dipstick PHAIA for PBA. Left panel: protein G purified anti-PBA antibodies (ranging from 3 to 0.09 μg) spotted onto nitrocellulose strips were incubated with different concentrations of phage particles (as indicated on the top) in the presence (+) or absence (−) of 50 ng/mL PBA. Right panel: dipstick assay set with various amounts of protein G purified anti-PBA antibodies (3 to 0.19 μg/spot) and using 5×10^{11} phage particles/mL for detection. The concentration of PBA ranged from 8 to 0 ng/mL as shown in the top of the right panel.

dose−response curve had a typical sigmoid shape with signal saturation at high concentration of analyte. The detection limit of the assay, estimated from the reading of zero analyte concentration plus four standard deviations, was 0.05 ng/mL, and the working range was 0.05−2.0 ng/mL. The midpoint of the curve, corresponding to the concentration of analyte at which ELISA readings are 50% of the maximal signal (SC_{50}) was $SC_{50} = 0.31 \pm 0.03$ ng/mL. This represents a 5-fold improvement in assay sensitivity as compared to the midpoint of the heterologous competitive ELISA setup with the same polyclonal antibodies reported by Shan et al.,[6] which exhibited a 50% inhibition concentration of 1.65 ng/mL.

PBA PHAIA Cross-Reactivity. The cross-reactivity of the PHAIA for PBA was assessed using PBA-related compounds including several common pyrethroids, Table 1. In all cases the cross-reactivity was negligible, in agreement with what has been previously observed for the competitive assay.[6] Although this result was expected because we were using the same polyclonal antibodies, it was not necessarily obvious. The peptide could stabilize binding of the PBA-related compounds to the antibody, consequently lowering the specificity of the assay. This does not seem to be the case, and the ternary complex was only formed when PBA was present.

Matrix Effect. PBA is a common metabolite of many major pyrethroid pesticides, and its presence in urine is a valuable biomarker of human exposure to these compounds.[15,16] For this reason we examined the urine matrix effect in the performance of the PBA PHAIA. This effect was evaluated by monitoring the recovery of spiked PBA in urine measured by the noncompetitive phage ELISA (Table 2). Similar results were obtained for different urine samples (not shown). These experiments showed a good correlation and almost null effect when up to 10% of urine final concentration was used.

Adaptation of PHAIA into a Dipstick Format. One of the contexts in which direct readings are preferred over indirect ones is when the assay needs to be adapted into an "on-site" rapid test such as dipsticks or immunochromatography. In these formats, the assay sensitivity is favored by the fact that the visual reading produced by trace amounts of the analyte can be easily discriminated from the zero signal. For this reason, we examined the feasibility of adaptation of the PHAIA into a dipstick format using protein G isolated antibodies immobilized onto nitrocellulose strips, as described above. As shown in the left panel of Figure 3, there was no background reactivity in a wide range of antibody coating densities for different phage concentrations. Similarly, there was no detectable reactivity of the phage-borne peptide with the unliganded antibody in all the examined concentrations of phage. This facilitates the setup of the test, and the working dilutions can be chosen on the basis of the desired sensitivity, economy of the reagents and intensity of the signal. In our case we used 5×10^{11} phage particles/mL for detection, to explore the sensitivity of the assay in a dipstick format as shown in the right panel of Figure 3. Detection of as low as 0.25 ng/mL PBA was possible using 3μg/dot of coating antibody.

DISCUSSION

A general technique is described here for specific and sensitive noncompetitive detection of small-size analytes based on the use of phage-borne peptides isolated from phage libraries and polyclonal antibodies. It is expected that the trivalent detection of the analyte will increase the assay sensitivity as observed in this study and in our previous development of PHAIAs with monoclonal antibodies. The ternary complex is anticipated to have a higher overall affinity, as has been observed for antimetatype/IC complexes.[9] In addition, this method may be an advantageous alternative to the preparation of antimetatype antibodies for various reasons: (a) Antimetatype antibodies are difficult to prepare,

(15) Leng, G.; Leng, A.; Kuhn, K. H.; Lewalter, J.; Pauluhn, J. *Xenobiotica* **1997**, *27*, 1273−1283.
(16) Saieva, C.; Aprea, C.; Tumino, R.; Masala, G.; Salvini, S.; Frasca, G.; Giurdanella, M. C.; Zanna, I.; Decarli, A.; Sciarra, G.; Palli, D. *Sci. Total Environ.* **2004**, *332*, 71−80.

which is reflected by the limited number of applications that have been reported after the principle was first described.[9,10] The main drawback appears to be the strong cross-reactivity with the empty binding site of the capture antibody, which makes it an arduous trial and error process.[9] PHAIA technology avoids the immunization protocols and monoclonal antibody preparation that are necessary to obtain these antimetatype antibodies, substituting them by a rapid selection process from phage display peptide libraries, which can be prepared in house or bought from commercial suppliers. (b) The rational behind this approach supports its general applicability. This is so because phage display peptide libraries offer a huge chemical diversity as a starting point that surpasses in many orders of magnitude the number of hybridoma clones that could be practically tested in the search for antimetatype antibodies. But also, the selected short peptide loops present a smaller binding surface than anti-IC antibodies, which allows focusing the recognition of the IC to the changes produced upon binding of the analyte and, therefore, having a higher control over the background cross-reactivity with the unliganded antibody. (c) The strategy can be applied when the capture antibody is polyclonal in nature. Polyclonal antibodies are easy to prepare and are often the first choice in the development of immunoassays for small analytes. However, due to their heterogeneity, they cannot be used as immunizing IC for the preparation of antimetatype antibodies. PHAIA technology may help to remove this limitation and facilitate the development of polyclonal antibody-based noncompetitive immunoassays. Although this requires affinity purification of the polyclonal antibodies for the panning experiments, it can be easily accomplished by immobilization of the immunizing hapten used to raise them.

Finally, it has been shown that, in general, competitive polyclonal-based immunoassays attain maximum sensitivity when the immunizing and competing haptens are different (heterologous assays). This requires extensive chemical synthesis work in order to develop a proper panel of candidate haptens, which must afterward be tested to examine whether the desired sensitivity can be reached. This trial and error process can be completely avoided by application of the PHAIA technology, which thus not only allows a more sensitive and convenient positive readout but also provides a convenient shortcut in the development of polyclonal-based immunoassays.

CONCLUSIONS

This work shows that despite the heterogeneity of antihapten polyclonal antibodies, it is possible to isolate peptides that specifically react with the analyte/antibody IC. These phage-borne peptides can be used to set up noncompetitive assays that surpass the performance of the best heterologous assay setup with the same antibodies. In the particular case of polyclonal antibodies, this is a major advantage of the PHAIA approach because it avoids the laborious synthesis of a large panel of competing antigens, which is required for the development of highly sensitive heterologous immunoassays.

ACKNOWLEDGMENT

This work was supported in part by the following: NIH Fogarty International Center Grant TW05718; PDT54-41 DINACYT, Uruguay; the NIEHS Superfund Basic Research Program, P42 ES04699; the NIEHS Center, P30 ES05707; the National Institutes of Environmental Health Sciences R37 ES02710. The support of the Western Centre for Agricultural Health and Safety at the University of California Davis is also acknowledged.

Received for review August 6, 2007. Accepted September 24, 2007.

AC7016713