J. Microbiol. Biotechnol. (2007), **17**(6), 1002–1009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Development of Homogeneous Enzyme Immunoassay for the Organophosphorus Insecticide Fenthion

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Received: January 5, 2007
Accepted: February 9, 2007

Abstract A rapid, convenient homogeneous competitive enzyme immunoassay for estimating the amount of fenthion is described. The assay utilizes glucose-6-phosphate dehydrogenase-hapten conjugates that are inhibited in solution by antibodies obtained from bovine serum albumin-hapten conjugates. In order to investigate the effects of bridging group recognition on the sensitivity of dose response characteristics, the bridging groups of varying alkyl chain length were attached at the phosphate position of fenthion. Among the antibodies used, the one obtained from the use of hapten (fenthion analog) with the same bridging group structure that was used in preparing the enzyme-fenthion conjugates showed maximum inhibition (up to 51.8%) in the absence of fenthion. In the presence of fenthion, the activity of the enzyme-hapten conjugate is regained in an amount proportional to the fenthion concentration. Under the optimized condition, the ED_{50} value for fenthion was 0.809 μg/ml. The assay developed in this study is a rapid effective screening method for fenthion prior to precise analysis.

Keywords: Organophosphorus insecticide, fenthion, homogeneous competitive enzyme immunoassay, protein-hapten bridge structures, glucose-6-phosphate dehydrogenase

Owing to the widespread use of insecticides, there is increasing concern over biological and environmental contamination. Among the insecticides, fenthion [*O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] phosphorothioate] is an organophosphorus insecticide used worldwide in different types of cultivation for the control of many sucking and biting pests [23]. Fenthion has been known to show severe lethal poisoning and be accumulated via contact with skin, inhalation, and ingestion. The proportion of deaths in self-poisoning patients is significantly higher with fenthion than with other organophosphorus insecticides [6]. Therefore, the analysis of fenthion contamination in a wide variety of biological and environmental samples is required. Current methods such as gas chromatography and high-performance liquid chromatography have been used successfully, with high sensitivity and reliability, for analysis of many insecticides [2]. These classical methods, however, require a high cost and skilled analysts, and involve time-consuming sample preparation steps. Therefore, there is a growing demand for more rapid and economical methods for determining insecticide residues. Immunoassays that can meet such demands have recently emerged as an alternative to the traditional methods.

Immunoassays are now routinely used in clinical analysis [15, 16, 22], in environmental measurements [7, 8, 17], and as early warning systems to detect chemical and biological warfare agents [19]. Combining the high specificity of antibodies with the sensitivity of radiotracers, radioimmunoassay enabled to detect a variety of analytes at trace levels [20]. More recently, non-isotopic labels have gained increasing popularity because they avoid inherent disadvantages with the use of radioisotopes. Substituents for radiotracers as labels may include fluorescent molecules, chemiluminescent precursors, electrochemical tags, and enzymes [24]. Each category of labels has specific advantages and limits. Among these labels, enzymes are particularly attractive labels since their activities enable them to act as amplifiers. The main advantages of enzyme immunoassay (EIA) using enzyme labels in devising competitive binding assays is that analytes can be detected at extremely low levels in relatively short periods of time via kinetic methods [4, 5, 12, 24]. EIAs can be classified as either heterogeneous (*e.g.*, enzyme-linked immunosorbent assay, ELISA) or homogeneous (*e.g.*, enzyme-multiplied immunoassay technique, EMIT). Although less sensitive, homogeneous

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methods do not need time-consuming washing steps to separate bound and unbound labels, and therefore are more rapid and amenable to automation [9, 11]. Since Rubenstein *et al.* [21] pioneered an EMIT approach, any EMIT assays for the fenthion-causing severe self-poisoning in humans have not yet been reported.

Improvement of the sensitivity of ELISAs can be obtained by enhancing the specificity and affinity of analyte towards antibody. As it has been proven both experimentally and theoretically [3], the higher the affinity constant ratio of free analyte to the enzyme-hapten conjugate, the lower the concentration of free analyte that can be detected. It has been also demonstrated that the detection limits can be affected by antibody recognition of the bridging group that covalently links the hapten to the enzyme [1]. One of the approaches taken to circumvent this bridging group recognition problem in ELISAs is to change the bridging group structure of the enzyme-hapten conjugate (or carrier protein-hapten) so that it will show less affinity towards the antibody. By employing such a less tight binding enzyme-hapten conjugate to antibody, the dose response characteristics of free analyte could be improved. However, the assessment of the effects of bridging group structures on EMIT sensitivity has not yet been reported.

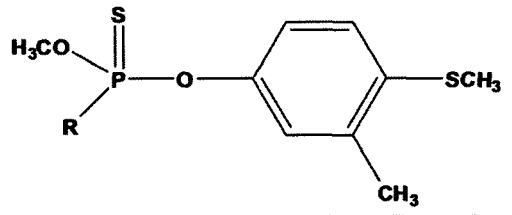
In this work, we describe a new EMIT (*i.e.*, homogeneous competitive enzyme immunoassay) method to rapidly and conveniently determine the amount of fenthion and the effects of bridging structures of hapten, which were used to prepare an enzyme conjugate or immunogen to elicit antibody, on the dose response behavior.

MATERIALS AND METHODS

Instruments and Chemicals

Enzyme activities were measured with a Gilford-Stasaar-III spectrophotometer (Oberlin, OH, U.S.A.) equipped with a vacuum-operated sampling system and temperature-controlled cuvette (maintained at 30°C throughout the experiments). This spectrophotometer was connected to a Syva CP-5000 EMIT Clinical Processor (Mountain View, CA, U.S.A.) for automatically setting the reading intervals and recording the absorbance values. A microdialyzer System 500 from Pierce (Rockford, IL, U.S.A.) was used for dialyzing enzyme-hapten conjugates.

Glucose-6-phosphate dehydrogenase (G6PDH) from *Leuconostoc mensenteroides*, glucose-6-phosphate (G6P), β-nicotinamide adenine dinucleotide (NAD^+), oxaloacetic acid (OAA), and the reduced form (NADH) of NAD^+ were obtained from Sigma (St. Louis, MO, U.S.A.). Fenthion was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The coupling buffer for enzyme-hapten conjugates was a 0.10 M sodium bicarbonate buffer, pH 9.0. The working assay buffer for the G6PDH-hapten conjugate was a 0.05 M



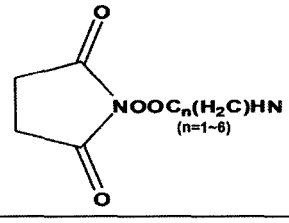
Compound	R residue
Fenthion	H_3CO
Hapten	$HOOC(H_2C)_nHN$ (n=1~6)
Hapten Derivatives (active succinimide ester form)	

Fig. 1. Structures of fenthion and haptens (fenthion analogs for immunization and conjugation).

tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCl) buffer, pH 7.8, containing 0.1 M NaCl, 0.01% (w/v) NaN_3, and 0.3% (w/v) gelatin. Deionized water was used to prepare all buffers. Each experiment was repeated three times and mean values are shown.

Synthesis of Hapten and Production of Antibodies

The structures of fenthion, haptens (fenthion analogs used to prepare immunogens and enzyme tracers), and hapten derivatives (succinimide ester forms of haptens used to prepare conjugates) are presented in Fig. 1. The procedures for the synthesis of haptens (F, 2A–6A), hapten derivatives, and BSA (bovine serum albumin)-hapten conjugates and those for the production of the polyclonal antibodies against fenthion have been described in our previous work [14].

Preparation of G6PDH-Hapten Conjugates

Enzyme-hapten conjugates were synthesized using hapten derivatives with G6PDH (Fig. 2). The hapten derivatives were dissolved in DMF (dimethyl formamide) and added in small portions to enzyme solutions buffered at pH 9.0 (HCO_3^-/CO_3^{2-}). The coupling reaction was run for 24 h at 4°C under stirring. During the reaction, the catalytic active site of G6PDH was protected by adding an excess of substrates (G6P and NADH). After the reaction, the G6PDH-hapten conjugates were dialyzed against the working assay buffer to remove unreacted hapten derivatives. The conjugates were transferred to a small vial and the final volume adjusted to 2 ml with the working assay buffer. The prepared G6PDH- and BSA-hapten conjugates are shown in Table 1. The resulting conjugates were characterized

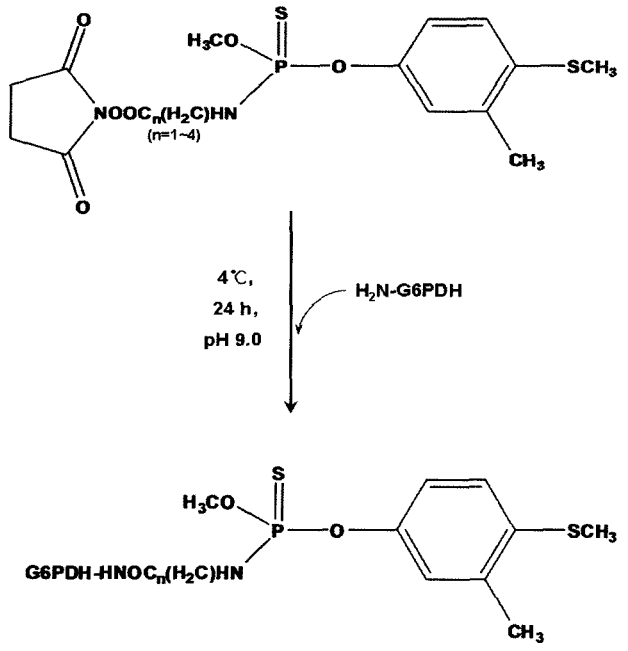


Fig. 2. Reaction sequence used for preparing G6PDH-hapten conjugates.

by their residual activities and percent (%) inhibition induced by an excess amount of antibodies. All the solutions were stored in the refrigerator.

Determination of Enzyme Activity and Maximum Percent Inhibition

The activities of the G6PDH-hapten conjugates were determined by measuring the rate of increase of NADH at 340 nm after the addition of 100 μl of NAD^+ (32.5 mM in the assay buffer, pH 7.8), 100 μl of G6P (50 mM in the assay buffer, pH 7.8), and 100 μl of appropriately diluted conjugate solution to a disposable plastic tube containing 700 μl of the assay buffer. For each assay, after reagents were mixed and subsequently agitated on a vibrator for 2 s, the reaction mixture was aspirated into the thermostated flow cell of the spectrophotometer and the absorbance at 340 nm was measured over a 1-min period after an initial 10-s delay.

In order to determine the maximum percent inhibition value for the conjugate, 100 μl of the assay buffer was replaced by the same volume of antibody solution prepared in the assay buffer. In addition, each G6PDH-hapten conjugate was incubated first with the five different antibodies against haptens (2A, 3A, 4A, 5A, and 6A) for 10 min before the addition of the substrate solutions.

Table 1. Haptens used for preparing G6PDH (glucose-6-phosphate dehydrogenase)-hapten and BSA (bovine serum albumin)-hapten conjugates.

Hapten abbreviation	N (No. of methyl chain of hapten)	G6PDH-hapten conjugate[a]	BSA-hapten conjugate[b]
F	1	○	
2A	2	○	●
3A	3	○	●
4A	4	○	●
5A	5		●
6A	6		●

[a]Number of methyl chain of G6PDH-hapten conjugate varied from 1 to 4.
[b]Number of methyl chain of BSA-hapten conjugate varied from 2 to 6.

Association Kinetic Studies

The rate of binding between the G6PDH-hapten conjugates and anti-hapten antibodies was measured by varying the incubation time periods. The assay solution containing 100-μl portions of appropriately diluted conjugate (1/100 dilution; 8.96×10^{-7} M concentration) and 100 μl of antibody solution (1/1,250 dilution) was mixed and incubated for 0–30 min. After each incubation period, the enzyme activity of the conjugate was determined by adding substrates as described above. Kinetic curves were prepared by plotting percent inhibition *versus* incubation time.

Effects of Different Antibodies and Concentration on the Inhibition of G6PDH-Hapten Conjugates

Aliquots (100 μl) of antibody solutions containing various concentrations of different antibodies were incubated with 100 μl of appropriately diluted (8.96×10^{-7} M concentration) G6PDH-hapten conjugate for 10 min. After incubation, the enzyme activity was measured as described above. Antibody dilution curves were prepared by plotting percent inhibition *versus* dilution factors (1/50–1/2,000) of antibodies added.

Dose-Response Curves of Fenthion

For dose-response curves, 100 μl of standard solution of different concentrations (0.01–100 μg/ml) of fenthion was added to the assay tube containing 100 μl of antibody solution (1/1,250 dilution) for 10 min, and the mixture was incubated for an additional 10-min period. After competitive binding reaction, the enzyme activity was measured as outlined above. Dose-response curves were prepared plotting percent inhibition *versus* the logarithm of the concentration of fenthion in the 100 μl of standard solution added to the assay mixture.

Results and Discussion

Synthesis of G6PDH-Hapten Conjugates and Anti-hapten Antibodies

In the process of developing a successful homogeneous EIA, it is important to prepare appropriate enzyme-hapten (analyte analog) conjugate that must change its catalytic properties in a measurable way upon binding of anti-

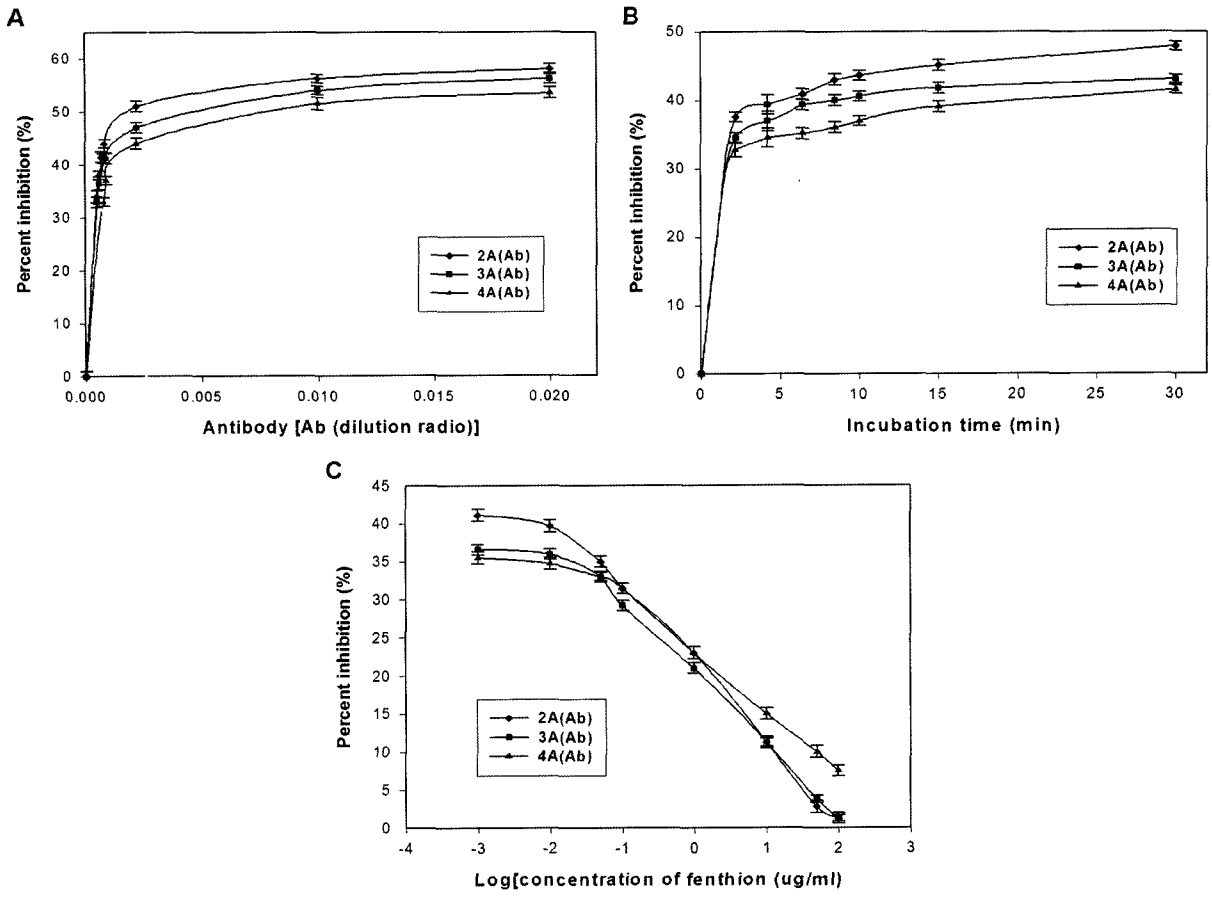


Fig. 3. Antibody dilution curves for 1:100 dilution (8.96×10^{-7} M) of the G6PDH-2AII (**A**), kinetics of association between 1:100 dilution (8.96×10^{-7} M) of the G6PDH-2AII and three different antibodies (1:1,250 dilution) (**B**), and dose-response curves for fenthion with 1:100 dilution (8.96×10^{-7} M) of the G6PDH-2AII and 1:1,250 dilutions of three different antibodies (**C**).
2A(Ab), anti-2A antibody; 3A(Ab), anti-3A antibody; 4A(Ab), anti-4A antibody.

hapten antibody toward the conjugate and that must retain substantial residual activity without the antibody as well. In the presence of free analyte, the modulated catalytic activities of the enzyme must also be restored after competitive binding of both free analyte and enzyme-hapten conjugate to a restricted amount of anti-hapten antibody. Since an enzyme must meet these criteria to be a suitable label, the choice of enzyme is more restricted than that for heterogeneous EIA. In addition, these assays do not require any separation of unbound enzyme-labeled hapten (enzyme-hapten) from sample media before the determination of enzyme activity.

Among the enzymes, G6PDH has been extensively used as a label in homogeneous EMIT type assays [9, 11, 13]. For the proposed work, several G6PDH-hapten conjugates were synthesized as shown in Fig. 2. Substitution of specific residues ($-NH_2$) in the G6PDH molecule with a hapten structure that has the bridging groups of varying alkyl chain length attached at the phosphate position of fenthion was used to synthesize G6PDH-hapten conjugates. The rest of the structure of the hapten moiety was intact to preserve all determinant groups that were recognized by the antibody. Thus, the link apparently makes the G6PDH susceptible to conformational change after interaction with the antibody and leads to inhibition of enzyme activity. In order to get appropriate G6PDH-hapten conjugates, four different hapten derivatives with different initial molar ratios of hapten/G6PDH were employed in preparing the conjugates (Table 1). Since the hapten (fenthion analog) molecule is too small to elicit an immunological response alone, it needs to be attached to a carrier protein (bovine serum albumin, BSA) before being injected into the animal that will produce the anti-hapten antibodies. The resulting antibodies obtained with this immunization procedure could recognize the hapten structure, as it is linked to carrier protein more selectively than unlabeled hapten (free analyte) in solution. This may result in less sensitive dose-response in a competitive type assay due to unequal competition between labeled hapten (BSA-hapten) and free analyte. To investigate the possible bridging group recognition effects in the design of an homogeneous fenthion assay, five different antibodies elicited by different BSA-hapten immunogens, which have

different linking lengths of alkyl chain attached at the phosphate position of fenthion, were utilized (Table 1).

Screening of Optimal G6PDH-Hapten Conjugate and Anti-hapten Antibodies

In order to induce considerable inhibition by the anti-hapten antibodies, a high degree of hapten conjugate (number of hapten molecules per enzyme molecule) has been known to be desirable for homogeneous EIAs [9, 10, 13]. Several G6PDH-hapten conjugates with varying different initial molar ratios of hapten to enzyme were prepared. As per the results summarized in Table 2, the conjugate with the molar ratios of 70 and 100 showed relatively high residual enzyme activities. The conjugates prepared using an initial molar ratio higher than 100 showed little or no residual activities (data not shown), and thus could not be used as G6PDH-hapten conjugates. The active sites of the enzyme were protected during the coupling reaction; however, the conjugate that reacted with a higher initial molar ratio of hapten (or ligand) to enzyme than that of 100 exhibited less residual activity in other reports [9, 10, 13]. From the inhibition studies, it was found that G6PDH-hapten conjugate prepared with the same haptenic linking structure as the original BSA-hapten immunogen showed the highest percent inhibition induced by the antibody (Table 3). The results imply that each antibody can recognize the bridging group of the specific conjugate, and thus binds more tightly and/or more selectively than any other G6PDH-hapten conjugate with different linking structures of hapten. The antibody of these desirable high-inhibition characteristics, however, may cause less tight binding of free analyte (fenthion) than G6PDH-hapten conjugate by the antibody. This behavior will be discussed below in the sections of antibody dilution and competitive dose-response studies. The conjugates prepared using an initial molar ratio of 100 showed greater percent inhibition than those of 70. On the basis of high maximum percent inhibition with substantial residual activity, G6PDH-2AII and three antibodies [2A(Ab), 3A(Ab), and 4A(Ab)] were selected as the enzyme-hapten conjugate and the anti-hapten antibodies for use in all the subsequent homogeneous binding studies.

Table 2. Characteristics of G6PDH-hapten conjugates.

Conjugate	Initial ratio (Hapten/G6PDH)	Percent residual activity (%)[a]
G6PDH-0	0	100
G6PDH-FI	70	40.74
G6PDH-FII	100	36.01
G6PDH-2AI	70	45.86
G6PDH-2AII	100	36.53
G6PDH-3AI	70	26.02
G6PDH-3AII	100	15.51
G6PDH-4AI	70	23.92
G6PDH-4AII	100	8.02

[a]Percent residual activity was determined by using 1:100 dilution (8.96×10^{-7} M) of the G6PDH-hapten conjugate.

Table 3. Percent inhibition of G6PDH-hapten conjugates in the presence of excess amount of the antibodies.

Conjugate	Percent inhibition (%)[a] by the anti-hapten antibodies				
	2A(Ab)	3A(Ab)	4A(Ab)	5A(Ab)	6A(Ab)
G6PDH-0	0	0	0	0	0
G6PDH-FI	17.1	16.8	16.5	11.3	10.7
G6PDH-FII	23.4	27.0	21.2	8.4	17.5
G6PDH-2AI	45.0	38.7	38.4	26.4	26.7
G6PDH-2AII	51.8	47.5	45.3	32.4	36.7
G6PDH-3AI	46.5	47.0	45.5	33.3	37.9
G6PDH-3AII	65.3	60.2	58.8	48.3	50.9
G6PDH-4AI	44.0	45.1	49.5	38.5	39.0
G6PDH-4AII	57.4	52.5	57.4	44.3	47.5

[a]Percent inhibition was determined by using 1:100 dilution (8.96×10^{-7} M) of the G6PDH-hapten conjugate and 1:500 dilutions of the antibodies.

Optimal Concentration of Anti-hapten Antibodies

The effects of varying dilutions of different anti-hapten antibodies [2A(Ab), 3A(Ab), and 4A(Ab)] on the percent inhibition observed with the G6PDH-2AII conjugate were also examined. As demonstrated above (see Table 3), differences in maximum values of percent inhibition were observed when three different antibodies were used (Fig. 3A). Relatively small amount of each antibody is required to inhibit a given concentration of the G6PDH-2AII conjugate (1:100 dilution of original G6PDH-2AII conjugate preparation; 8.96×10^{-7} M), and the optimum amount of each antibody was a 1:1,250 dilution of the original antibody preparation (Fig. 3A). The characteristics of antibody dilution curves for different antibodies with the same G6PDH-2AII conjugate of fixed concentration depend on several factors. The binding of antigen with antibody may involve electrostatic interactions, hydrophobic interactions, hydrogen bonds, and van der Waals' forces [18]. These interactions are inversely proportional to the distances between antigen and antibody, where the closer the antigenic determinant area to the antibody binding site, the higher is the affinity constant. The antibody must have a reasonably high association constant for hapten structure appended to labeling enzyme in order to achieve a high degree of inhibition at relatively low levels of antibodies. Since homogeneous inhibition of the enzyme-hapten conjugate in the presence of anti-hapten antibodies has been attributed to steric exclusion of substrates and/or conformational changes in the vicinity of the active site structure of labeling enzyme induced by the binding reaction, the specific three-dimensional structure and size of the antibodies will affect the maximum degree of inhibition in the excess amount of the

antibodies. Thus, the shape and steepness of the antibody dilution curves shown in Fig. 3A provide some information about the affinity constants between the enzyme-hapten conjugate and anti-hapten antibody, whereas the maximum value of percent inhibition in the presence of an excess amount of each antibody depends on the size and three-dimensional structure of each antibody. In this regard, the different linking structure of each hapten is critically important to achieve substantial binding affinity for each antibody.

Association Kinetic Studies

The association kinetics between the antibodies and the G6PDH-2AII conjugate were studied in order to optimize the incubation time required for the competitive homogeneous binding assay. Three different sets of optimally selected G6PDH-2AII conjugate/antibody systems were employed in these studies. As shown in Fig. 3B, fast association kinetics were observed for each of the systems. Indeed, more than 75% of the maximum value of percent inhibition was obtained within a 2-min incubation period for all systems and only 30 s of incubation were required to achieve a comparable extent of inhibition for each system. This fast binding kinetics between antibodies and G6PDH-2AII conjugate requires a more reduced assay time than heterogeneous type EIAs (*e.g.*, ELISAs) [4, 5, 12, 24]. In the subsequent experiment including dose-response studies, 10-min incubation periods were employed. As demonstrated above (see Table 2 and Fig. 3A), differences of maximum values of percent inhibition were also observed when three different antibodies were used (Fig. 3B).

Dose-Response Studies and Assessment of the Effects of G6PDH-Hapten Bridge Structures on Assay Sensitivity

Competitive dose-response curves for three different antibodies [2A(Ab), 3A(Ab), and 4A(Ab)] were constructed by plotting percent inhibition *versus* the concentration of fenthion. As shown in Fig. 3C, the activity of the G6PDH-2AII conjugate was regained in an amount proportional to the fenthion concentration. Among the antibodies examined, 2A(Ab) showed the best antibody characteristics for performing the proposed competitive homogeneous EIA, although the dose-response curve shifted slightly to the right position. In general, the concentration (ED_{50}) of fenthion required to achieve a 50% reduction in the maximum values of percent inhibition can be related to the relative affinity of the given antibody toward fenthion [9]. The ED_{50} values, calculated from the data of Fig. 3C, for 2A(Ab), 3A(Ab), and 4A(Ab) were 0.809, 1.042, and 1.194 μg/ml, respectively. As mentioned above, 2A(Ab) antibody could recognize labeled hapten (G6PDH-hapeten) structure more selectively than free hapten (fenthion) in solution. This may result in a less sensitive dose-response in heterogeneous competitive type assays (*e.g.*, ELISAs) because of unequal competition between labeled hapten and fenthion toward antibodies. However, the new proposed EMIT assay is based on the homogeneous inhibition of the enzyme-hapten conjugate by anti-hapten antibodies, which has been attributed to steric exclusion of substrates and/or conformational changes in the vicinity of the active site structure of the labeling enzyme induced by the binding reaction between the enzyme-hapten conjugate and anti-hapten antibodies. Furthermore, in the presence of free hapten (fenthion) recognized by the antibodies, the enzyme activity was regained in an amount of proportional to the concentration of fenthion. Thus, one of the most important characteristics of the enzyme-hapten conjugate is the significant inhibition of catalytic activities by the anti-hapten antibodies. Naturally, ED_{50} values that have been obtained by a homogeneous competitive assay approach will be highly dependent on the maximum percent inhibition. That is, a higher maximum percent inhibition is advantageous to achieve a lower ED_{50} value among the dose-response curves with similar dynamic ranges. On the consideration of a high percent inhibition value regarding this point, the G6PDH-2AII/2A(Ab) set presents a more sensitive dose-response than any other sets. Unlike heterogeneous competitive enzyme immunoassays, unequal competition between enzyme-labeled hapten and fenthion toward anti-(BSA-hapten) antibody might not be favorable to get a lower ED_{50} value in the design of the new proposed EMIT assay. That is, we found that the theoretically simulated presumption, the less sensitive dose-response in heterogeneous competitive type assays due to the unequal competition, is not essentially adaptable to the EMIT type assay. Since the new proposed EMIT is the first developed method for fenthion analysis, the comparison between sensitivities of the method and other EMIT methods for fenthion cannot be performed. The sensitivity of the new proposed EMIT method in terms of ED_{50} value is better than those of other EMIT methods for D-mannose (ED_{50} values of 0.809 *versus* 6.7 μg/ml) [10] and for *p*-aminophenyl α-D-galactopyranoside (ED_{50} values of 0.809 *versus* 8.68 μg/ml) [9]. The percent residual activity and percent inhibition values are reproducible to ±1.4% (standard deviations for n=3). This reproducibility of the proposed homogeneous competitive enzyme immunoassay is comparable to that observed in previously homogeneous EMIT methods involving natural binding proteins [9, 10, 13].

We have described the performance of a new homogeneous competitive enzyme immunoassay that is useful to rapidly and conveniently determine the amount of fenthion, and have investigated the effects of bridging group recognition for studying the interactions of various anti-hapten antibodies with enzyme-hapten conjugates having different linking structures. When the assay is utilized in a homogeneous competitive binding mode with an appropriate enzyme-hapten conjugate, the changes in the haptenic linking structures of immunogen to elicit anti-hapten antibodies might result in a more or less sensitive dose-

response depending on the degree of bridging group recognition (*i.e.*, changes in the ED_{50} value for fenthion). In the proposed arrangement, unlike heterogeneous competitive EIAs, antibodies elicited from the immunogen of the same linking structures [2A(Ab)] as those of enzyme-hapten conjugates (G6PDH-2AII) presented lower ED_{50} values than other sets. Indeed, in EMIT type assays, the concentration (ED_{50}) of free hapten required to achieve 50% reduction in the maximum enzyme inhibition values can be related to not only the relative affinity of a given antibody toward hapten structure, but also the maximum percent inhibition value of catalytic activities of enzyme-hapten conjugates by antibodies, which may be partially resulted from the recognition of the specific linking structure of the enzyme-hapten conjugate.

With the appropriately prepared enzyme-hapten (fenthion analog) conjugates and anti-hapten antibodies, the proposed EMIT method may offer a simple and rapid screening of fenthion prior to precise analysis.

Acknowledgments

This study was supported by research grants from the National Institute of Health (NIH) of KCDC in Republic of Korea, BioGreen 21 Program of the Rural Development Administration in Republic of Korea, the Driving Force Project for the Next Generation of the Gyeonggi Provincial Government in Republic of Korea, and the Second-Phase of the Brain Korea 21 Project. Kim, Park, Lee and J. H. Lee contributed equally in this work.

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