

## Site-directed Mutagenesis of Arginine 13 Residue in Human Glutathione S-Transferase P1-1

Jong-Uk Koh, Hyun-Young Cho, and Kwang-Hoon Kong\*

Department of Chemistry, College of Sciences, Chung-Ang University, Seoul 156-756, Korea. \*E-mail: khkong@cau.ac.kr  
Received January 10, 2007

In order to study the role of residue in the active site of glutathione S-transferase (GST), Arg13 residue in human GST P1-1 was replaced with alanine, lysine and leucine by site-directed mutagenesis to obtain mutants R13A, R13K and R13L. These three mutant enzymes were expressed in *Escherichia coli* and purified to electrophoretic homogeneity by affinity chromatography on immobilized GSH. Mutation of Arg13 into Ala caused a substantial reduction of the specific activity by 10-fold.  $K_m^{GSH}$ ,  $K_m^{DCNB}$  and  $K_m^{EPNP}$  values of R13A were approximately 2-3 fold larger than those of the wild type. Mutation of Arg13 into Ala also significantly affected  $I_{50}$  values of S-methyl-GSH that compete with GSH and ethacrynic acid, an electrophilic substrate-like compound. These results appeared that the substitution of Arg13 with Ala resulted in significant structural change of the active site. Mutation of Arg13 into Leu reduced the catalytic activity by approximately 2-fold, whereas substitution by Lys scarcely affected the activity, indicating the significance of a positively charged residue at position 13. Therefore, arginine 13 participates in catalytic activity as mainly involved in the construction of the proper electrostatic field and conformation of the active site in human GST P1-1.

**Key Words** : Arginine 13 residue, Glutathione S-transferase, Site-directed mutagenesis

### Introduction

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of multifunctional enzymes, which consist of homo- or heterodimers of subunits whose molecular weights are about 25 kDa.<sup>1,2</sup> They catalyze the formation of conjugates between reduced glutathione (GSH) and a wide variety of carcinogenic, mutagenic, toxic and pharmacologically active compounds. They are distributed in a wide range of organisms from mammals to *E. coli*.<sup>3</sup> They can be grouped into at least five distinct classes, alpha, mu, pi, sigma, and theta according to based on studies of substrate specificity and primary structures.<sup>1,4</sup> Among them, the pi class hGST P1-1 has attracted attention as reliable preneoplastic or neoplastic marker enzymes and it has been implicated in the development of resistance of tumors towards various anti-cancer drugs in resistant tumor cells.<sup>5,6</sup>

The active sites of GSTs are suggested to consist of a GSH-binding site (G-site) and a nonspecific hydrophobic site (H-site) to accommodate the electrophilic substrates. The catalytic mechanism of GSTs has been the targets of many investigations involving kinetic and structural studies, chemical modification, site-directed mutagenesis and X-ray crystallographic analysis. The extent of the information concerning the precise enzyme-GSH interactions responsible for the catalytic properties has been greatly increased by these studies. The binding of GSH appears to involve ionic bonds,<sup>2</sup> and one of the major candidates for interaction with the carboxyl groups of GSH is arginine residue(s). Arginine residue(s) was implicated also in the catalytic mechanism of GST.<sup>7</sup> Enhancement of the nucleophilicity of the thiol group in GSH by lowering its pKa is considered to be a major role of GSTs in catalyzing the formation of GSH-

conjugate.<sup>8,9</sup> The pKa of the thiol group of GSH bound in hGST P1-1 is about 2.8-pK units lower than that of free GSH in aqueous solution. One of the possible mechanisms for lowering the pKa is positioning of the thiol in a positively charged electrostatic field in the active site, and obvious candidates are the positively charged side chains of arginine, lysine and histidine or secondary structural elements such as  $\alpha$ -helices, which have dipolar character.<sup>7</sup> However, the essentiality of histidine residues to the catalytic mechanism or GSH binding was rejected by the studies using site-directed mutagenesis on the GSTs of class Pi.<sup>10</sup> By chemical

GSTs	
Class Pi	13 <sup>o</sup>
1. Human	FPVGR <b>R</b> CAALRMLL
2. Pig	FPVGR <b>R</b> CEAMRMLL
3. Rat	FPVGR <b>R</b> CEATRMLL
4. Mouse	FPVGR <b>R</b> CEAMRMLL
Class Alpha	
5. Rat Ya(a)	FNARG <b>R</b> MECIRWLL
6. Rat Ya(b)	FNARG <b>R</b> MECIRWLL
7. Rat Yc	FDGR <b>R</b> MEPIRWLL
8. Rat 8	FQGR <b>R</b> MEVIRWLL
9. Mouse Ya	FNARG <b>R</b> MECIRWLL
10. Mouse Yc	FDGR <b>R</b> MEPIRWLL
11. Rabbit alpha I	FNGR <b>R</b> MESIRWLL
12. Rabbit alpha II	FNARG <b>R</b> MESIRWLL
13. Human Ha-1	FNARG <b>R</b> MESTRWLL
14. Human Ha-2	SNIR <b>R</b> GMESIRWLL
15. Chick CL3	FNGR <b>R</b> GKMESIRWLL

**Figure 1.** Amino acid sequences of GSTs. Boldfaced letter is amino acid mutated in the present study.

modification study, arginine residues suggested to be present at or near the active site of GSTs and to play essential roles in catalysis.<sup>11,12</sup> Studies on the 3D-structures of porcine GST P1-1 and hGST P1-1 also suggested that Arg13 is located in the active site.<sup>13-15</sup>

Eight arginine residues are present in hGST P1-1. Among them, Arg13 is only conserved in all the known Pi class GSTs and in most Alpha class GSTs (Fig. 1). The highly conserved residues are expected to be important for the structure and/or function of the enzyme. In this study, Arg13 residue was replaced with alanine, lysine, and leucine by oligonucleotide-directed mutagenesis and the effect of the replacements on the enzymatic activity and thermal stability were examined in order to evaluate the role of this residue.

### Experimental Section

**Materials.** 1,2-Dichloro-4-nitrobenzene, ethacrynic acid, GSH and S-hexylGSH were obtained from Sigma (St. Louis, USA). Glutathione Sepharose was purchased from Pharmacia Biotech (Uppsala, Sweden). All other reagents used were of the highest grade commercially available.

**Preparation of mutant enzymes.** Wild-type hGST P1-1 was obtained by expression of a cloned cDNA<sup>16</sup> in *E. coli* as described in the previous paper.<sup>10</sup> The oligonucleotide primers used for site-directed mutagenesis of Arg13 into Ala, Lys, and Leu are shown in Table 1. Mutagenesis was performed according to the procedure of Kunkel<sup>17</sup> using a Mutant<sup>TM</sup>-Super Express Km kit (Takara Shuzo Co.). Construction of single-stranded DNA template for mutagenesis, confirmation of mutation, construction of the expression plasmid, and expression of the mutant enzymes were performed as described in the previous paper.<sup>18</sup> The mutant enzymes were expressed in *E. coli* under the control of the *tac* promoter. Cultured cells were lysed, followed by centrifugation. The dialyzed supernatant of the cell lysate was loaded directly onto a 20 mL column of GSH-Sepharose equilibrated with 20 mM potassium phosphate buffer (pH 7.0) (buffer A). The column was extensively washed with the same buffer. The enzyme was eluted with a 50 mM Tris-HCl buffer (pH 8.5) containing 10 mM GSH and dialyzed against buffer A. The dialyzed purified enzyme was used for next experiment.

**Determination of protein concentration.** Protein concentration of the wild type enzyme was determined by measuring the absorbance at 280 nm as described by Parker<sup>19</sup> and protein concentration of the mutant was determined by using protein assay reagent (Bio-Rad Lab.) and the wild-type enzyme as a standard protein.

**Enzyme activity.** The specific activities were determined by measuring the initial rates of the enzyme-catalysed conjugation of GSH with 1,2-dichloro-4-nitrobenzene (DCNB) or 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP) as described by Habig and Jakoby.<sup>20</sup> Assays were performed in a HITACHI U-2000 double-beam spectrophotometer (Hitachi Co., Tokyo, Japan) at 30 °C using cuvettes of 1 cm path-length. Initial rates were measured for 5 min, commencing 10 sec after initial mixing. The reaction was initiated by 20  $\mu$ L of 50 mM DCNB or EPNP to 860  $\mu$ L of 100 mM potassium phosphate (pH 7.5) containing, in order of addition, 100  $\mu$ L of 50 mM GSH and 20  $\mu$ L of the enzyme. Nonenzymatic reaction rates served as controls, and were subtracted from enzymatic rates. DCNB and EPNP were dissolved in ethanol. The concentration of ethanol in the reaction mixture (1 mL) was constant at 2% (v/v). GSH was dissolved in H<sub>2</sub>O immediately before use and kept in an ice-bath to prevent oxidation. The enzymes were diluted in 20 mM potassium phosphate buffer (pH 7.0) containing 3 mM EDTA, 3 mM 2-mercaptoethanol, and 20% (v/v) glycerol to a concentration that the enzymatic reaction rate was linear with time for up to 60 sec after initiation, and up to a  $\Delta A/\text{min}$  of 0.15. All assays were done in the presence of 0.4% (v/v) glycerol. Conditions were: (a) 1 mM DCNB, 5 mM GSH, 345 nm ( $\Delta\epsilon = 8.5 \text{ mM}^{-1}\text{cm}^{-1}$ ) and (b) 0.5 mM EPNP, 5 mM GSH, 360 nm ( $\Delta\epsilon = 0.5 \text{ mM}^{-1}\text{cm}^{-1}$ ). A unit of activity is defined as the amount of enzyme catalyzing the formation of 1  $\mu$ mole of product per min under the conditions of the specific assay. Specific activity is defined as the units of enzyme activity per mg of protein.

**Kinetic studies.** Kinetic studies with GSH and electrophilic substrates were carried out at 30 °C as described by Chen *et al.*<sup>21</sup> Kinetic parameter  $K_m$  values were determined under first order conditions at low substrate concentration with respect to the varied substrate: for GSH with a fixed concentration of 1 mM DCNB, and for DCNB with a fixed concentration of 5 mM GSH. The  $k_{\text{cat}}$  values were calculated on the basis of mol dimeric enzyme using a Mr of 45,000. Other experimental conditions were the same as for determination of specific activities.

**Inhibition studies.** The inhibitory effects on the activity of the enzyme were measured by preincubating the enzyme with 1 mM GSH and the inhibitor for 2 min and initiating the reaction by addition of 20  $\mu$ L of 50 mM 1-chloro-2,4-dinitrobenzene (final concentration, 1 mM). The concentration of inhibitor giving 50% inhibition ( $I_{50}$ ) was determined from plot of residual activity against inhibitor concentration.

**Table 1.** Oligonucleotides used for site-directed mutagenesis

Mutant	Sequence of primer <sup>a</sup>	Sense
R13A	5'-TTCCCAGTTCGAGGCGCCTGCGCGGCCCTGCGC-3'	Forward
R13K	5'-TTCCCAGTTCGAGGCAAAATGCGCGGCCCTGCGC-3'	Forward
R13L	5'-TTCCCAGTTCGAGGCCICTGCGCGGCCCTGCGC-3'	Forward

<sup>a</sup>Changed bases are shown by underlines.

## Results and Discussion

To investigate the role of arginine 13 in hGST P1-1, it was substituted with alanine (R13A), lysine (R13K), and leucine (R13L) by oligonucleotide-directed mutagenesis. The mutant enzymes were expressed in *E. coli* under the control of *tac* promoter. The expressed mutant enzymes were isolated and purified by affinity chromatography on immobilized GSH. The mutants R13K and R13L were isolated in a yield of approximately 3 mg per liter of cultures as in the case of the wild type. However, in the case of R13A, the amount of the isolated enzymes were 0.4 mg per liter of culture. As compared with the total activity in the crude extract of *E. coli* cell lysate, the activity recoveries for the wild type, R13K and R13L were more than 70%, but that for R13A was less than 15%. Thus, the binding abilities R13K and R13L to GSH-Sepharose were not so much different from that of the wild type, but R13A had low affinities for GSH-Sepharose. The purified wild type and mutants give a single band on SDS-PAGE with an apparent *M<sub>r</sub>* of 25 kDa equivalent with that of the wild type (data not shown).

The specific activities of the mutant enzymes for GSH conjugations of DCNB and EPNP are shown in Table 2. The substitution of Arg13 with alanine resulted in a large decrease of the specific activities to approximately 80-90% of those of the wild type. The substitution of Arg13 with leucine reduced the specific activities by approximately 2-fold. On the other hand, neither the substitution of Arg13 with lysine significantly affected the specific activities. These results suggest that the contribution of Arg13 in

catalytic activity is dependent on both the charge and the size of the side chain in position 13 of hGST P1-1.

The kinetic parameters of the mutants for GSH-DCNB conjugation were determined under first order conditions at low substrate concentration with respect to the varied substrate (Table 3). The substitution of Arg13 with alanine resulted in approximately 2-3 fold increases of  $K_m^{\text{GSH}}$  and  $K_m^{\text{DCNB}}$ , whereas  $k_{\text{cat}}$  values were approximately 72% smaller than that of the wild type. On the other hand, the substitution of Arg13 with lysine resulted in approximately 3-fold increase of  $K_m^{\text{DCNB}}$ , whereas the  $K_m^{\text{GSH}}$  was similar to that of the wild type. This substitution also resulted in approximately 1.8-fold increase of  $k_{\text{cat}}$  toward GSH. The substitution of Arg13 with leucine resulted in approximately 0.3-0.4 fold decrease of  $K_m^{\text{GSH}}$  and  $k_{\text{cat}}$  values, whereas  $K_m^{\text{DCNB}}$  value were approximately 2.4 fold larger than that of the wild type. Table 4 summarizes the kinetic parameters of the mutants for GSH-EPNP conjugation. The substitution of Arg13 with alanine resulted in approximately 1.9 fold increase of  $K_m^{\text{GSH}}$  and  $K_m^{\text{EIA}}$  values, whereas the  $k_{\text{cat}}$  values were approximately 0.5 fold smaller than those of the wild type. On the other hand, the kinetic parameters of R13K and R13L were similar to those of the wild type for GSH-EPNP conjugation. These results demonstrate that the contribution of Arg13 in catalytic activity is very dependent on the size rather than the positive charge of the side chain in position 13 of hGST P1-1.

The inhibition parameters ( $I_{50}$ ) of *S*-methylGSH and ethacrynic acid for GSH-DCNB conjugating activity were determined under the standard assay conditions. The sub-

**Table 2.** Specific activity of the wild type and mutants for GSH-conjugation reaction towards 1,2-dichloro-4-nitrobenzene and 1,2-epoxy-3-(*p*-nitrophenoxy) propane

Enzyme	1,2-Dichloro-4-nitrobenzene		1,2-Epoxy-3-( <i>p</i> -nitrophenoxy)propane	
	Specific activity ( $\times 10^{-2}$ $\mu\text{mol}/\text{min}/\text{mg}$ )	Relative activity (%)	Specific activity ( $\times 10^{-2}$ $\mu\text{mol}/\text{min}/\text{mg}$ )	Relative activity (%)
Wild type	11.8 $\pm$ 1.0	100	36.0 $\pm$ 3.8	100
R13A	1.3 $\pm$ 0.5	11	6.1 $\pm$ 1.2	17
R13K	11.4 $\pm$ 0.8	97	37.1 $\pm$ 0.8	103
R13L	5.1 $\pm$ 0.5	43	19.4 $\pm$ 1.3	54

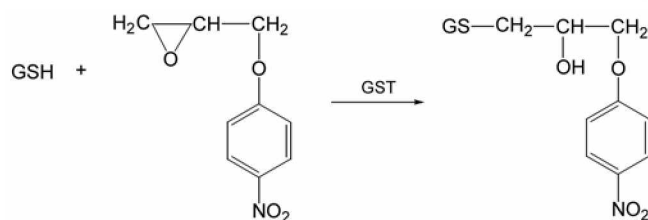
Values are Means  $\pm$  S.D., generally based on  $n \geq 5$ .

**Table 3.** Specific activity and kinetic parameters for GSH-[1,2-Dichloro-4-nitrobenzene] conjugation (DCNB)



Enzyme	GSH			1,2-Dichloro-4-nitrobenzene		
	$K_m$ (mM)	$k_{\text{cat}}$ ( $\times 10^{-2}$ $\text{s}^{-1}$ )	$k_{\text{cat}}/K_m$ ( $\times 10^{-2}$ $\text{mM}^{-1}\text{s}^{-1}$ )	$K_m$ (mM)	$k_{\text{cat}}$ ( $\times 10^{-2}$ $\text{s}^{-1}$ )	$k_{\text{cat}}/K_m$ ( $\times 10^{-2}$ $\text{mM}^{-1}\text{s}^{-1}$ )
Wild type	0.29 $\pm$ 0.08	4.01 $\pm$ 0.21	13.8	0.65 $\pm$ 0.03	2.02 $\pm$ 0.12	3.1
R13A	0.60 $\pm$ 0.03	1.12 $\pm$ 0.10	1.9	1.98 $\pm$ 0.03	0.55 $\pm$ 0.06	0.3
R13K	0.30 $\pm$ 0.06	7.03 $\pm$ 0.56	23.4	1.92 $\pm$ 0.08	3.21 $\pm$ 0.03	1.7
R13L	0.19 $\pm$ 0.13	2.35 $\pm$ 0.35	12.3	1.57 $\pm$ 0.13	1.13 $\pm$ 0.21	0.7

Values are means  $\pm$  SD, generally based on  $n \geq 3$ .

**Table 4.** Specific activity and kinetic parameters for GSH-[1,2-Epoxy-3-(*p*-nitrophenoxy) propane] conjugation

Enzyme	GSH			1,2-Epoxy-3-( <i>p</i> -nitrophenoxy)propane		
	$K_m$ (mM)	$k_{cat}$ ( $\times 10^{-2} \text{ s}^{-1}$ )	$k_{cat}/K_m$ ( $\times 10^{-2} \text{ mM}^{-1} \text{ s}^{-1}$ )	$K_m$ (mM)	$k_{cat}$ ( $\times 10^{-2} \text{ s}^{-1}$ )	$k_{cat}/K_m$ ( $\times 10^{-2} \text{ mM}^{-1} \text{ s}^{-1}$ )
Wild type	0.40 $\pm$ 0.02	1.08 $\pm$ 0.12	2.7	0.25 $\pm$ 0.03	2.02 $\pm$ 0.08	8.1
R13A	0.75 $\pm$ 0.02	0.49 $\pm$ 0.07	0.7	0.47 $\pm$ 0.02	1.20 $\pm$ 0.04	2.6
R13K	0.43 $\pm$ 0.01	1.38 $\pm$ 0.16	3.2	0.15 $\pm$ 0.01	1.03 $\pm$ 0.03	6.9
R13L	0.45 $\pm$ 0.01	0.78 $\pm$ 0.09	1.7	0.28 $\pm$ 0.04	1.28 $\pm$ 0.13	4.6

Values are means  $\pm$  SD, generally based on  $n \geq 3$ .

stitution of Arg13 with alanine resulted in approximately 2.4 fold increases in the  $I_{50}$  values of *S*-methylGSH that competes with GSH and ethacrynic acid that competes with electrophilic substrate (Table 5). On the other hand, the  $I_{50}$  values for R13K and R13L were similar to those of the wild type.

Previous studies have suggested that Arg13 in hGST P1-1 is located at or close to the active site of the enzyme and plays an essential role in catalysis.<sup>7,11,12</sup> The Three dimensional structure of pGST P1-1 indicates that the side chain of Arg13 is located in the opposite direction to the sulfonate group of glutathione sulfonate bound in the enzyme.<sup>13</sup> In deed, mutation of Arg13 into Ala caused a substantial reduction of the specific activity by 10-fold (Table 2).  $K_m^{\text{GSH}}$ ,  $K_m^{\text{DCNB}}$  and  $K_m^{\text{EPNP}}$  values of R13A were approximately 2-3 fold larger than those of the wild type (Table 3 and 4). Mutation of Arg13 into Ala also significantly affected  $I_{50}$  values of *S*-methyl-GSH that compete with GSH and ethacrynic acid, an electrophilic substrate-like compound (Table 5). These results appeared that the substitution of Arg13 with Ala resulted in significant structural change of the active site.

Mutation of Arg13 into Leu reduced the catalytic activity and  $k_{cat}$  by approximately 2-fold. However, this substitution scarcely affected  $K_m^{\text{GSH}}$ ,  $K_m^{\text{DCNB}}$ ,  $K_m^{\text{EPNP}}$  and  $I_{50}$  values of *S*-methylGSH and ethacrynic acid. These results suggest that the substitution of Arg13 with Leu scarcely affected the structural change of the active site. Mutation of Arg13 into Lys scarcely affected the activity, indicating the significance of a positively charged residue at position 13. This substitution significantly affected  $K_m^{\text{DCNB}}$ ,  $K_m^{\text{EPNP}}$  and  $I_{50}$  of ethacrynic acid, whereas scarcely affected  $K_m^{\text{GSH}}$ ,  $I_{50}$  of *S*-methylGSH and  $k_{cat}$ . These results appeared that the substitution of Arg13 into Lys might affect the conformation of the electrophilic substrate binding site of the enzyme, but scarcely affected GSH binding site.

The differences in the size and charge of the side chain might lead to differences in the results. We also reported that the mutation of Arg13 into Thr resulted in the significant

**Table 5.** Inhibitory effect of *S*-methylGSH and ethacrynic acid on GSH conjugation reaction

Enzyme	<i>S</i> -MethylGSH		Ethacrynic acid	
	$I_{50}$ ( $\mu\text{M}$ )	%	$I_{50}$ ( $\mu\text{M}$ )	%
Wild type	1.29 $\pm$ 0.08	100	13.5 $\pm$ 0.3	100
R13A	3.16 $\pm$ 0.22	245	32.7 $\pm$ 1.2	242
R13K	1.38 $\pm$ 0.13	107	6.4 $\pm$ 0.5	47
R13L	0.94 $\pm$ 0.08	73	14.1 $\pm$ 1.1	104

Values are Means  $\pm$  S.D., generally based on  $n \geq 3$ .

decrease of the enzyme activities, but the substrate binding was less affected by the replacement.<sup>22</sup> From the plots of  $\log(k_{cat}/K_m^{\text{DCNB}})$ ,  $pK_a$  of the thiol group of GSH bound in R13T was estimated to be the same as that of the wild type, and its contribution of Arg13 residue was supposed not to enhance the nucleophilicity of GSH, although Arg13 was important for the catalytic mechanism. These rather striking differences in the response of the catalytic properties of the mutant enzymes (R13A, R13K, R13L and R13T) are most likely to be correlated with structural differences between their active-sites, and the mutations of Arg13 might affect the interaction between the thiol group and Tyr7 through distorting the orientation of the GSH bound in the enzyme and/or the binding site of electrophilic substrates. Recently, the crystal structure of *S*-nitrosoglutathione bound to hGSTP1-1 showed that the *S*-nitrosoglutathione ligand binds in the active site with the nitrosyl moiety involved in multiple interactions with the enzyme.<sup>23</sup> The crystallographic analysis also revealed three possible conformations of the nitroso group, which could be modeled with  $\sim 33\%$  occupancy for each. In one conformation the nitrosyl moiety was stabilized by water-mediated interactions with Arg13 and Tyr108. In the second conformation the nitrosyl moiety was stabilized by direct interactions with Arg13 (side chain) and Tyr7. In the third conformation the nitrosyl moiety was stabilized by direct interactions with Arg13 (main chain) and Tyr7. These results appeared that the mutation of Arg13 residue might affect the conformation of the GSH binding

site and the electrophilic substrate binding site of the enzyme in various ways. Taken together, we conclude that Arg13 in human GST P1-1 participates in catalytic activity as mainly involved in the construction of the proper electrostatic field and conformation of the active site. Further accurate elucidation on the relationship between the structure and catalytic activity of this residue requires three-dimensional structure analysis of these mutant enzymes.

### References

- Mannervik, B. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1985**, *57*, 357.
- Mannervik, B.; Danielson, U. H. *CRC Crit. Rev. Biochem.* **1988**, *23*, 283.
- Fahey, R. C.; Sundquist, A. R. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1991**, *64*, 1.
- Mannervik, B.; Awasthi, Y. C.; Board, P. G.; Hayes, J. D.; Ilio, C.; Ketterer, B.; Listowsky, I.; Morgenstern, R.; Muramatsu, M.; Pearson, W. R.; Pickett, C. B.; Sato, K.; Widersten, M.; Wolf, C. R. *Biochem. J.* **1992**, *282*, 305.
- Morgan, A. S.; Ciaccio, P. J.; Tew, K. D.; Kauvar, L. M. *Cancer Chemother. Pharmacol.* **1996**, *37*, 363.
- Tsuchida, S.; Sato, K. *CRC Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 337.
- Zhang, P.; Graminski, G. F.; Armstrong, R. N. *J. Biol. Chem.* **1991**, *266*, 19475.
- Kong, K.-H.; Nishida, M.; Inoue, H.; Takahashi, K. *Biochem. Biophys. Res. Commun.* **1992**, *182*, 1122.
- Kong, K.-H.; Takasu, K.; Inoue, H.; Takahashi, K. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 194.
- Kong, K.-H.; Inoue, H.; Takahashi, K. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 748.
- Asaoka, K.; Takahashi, K. *J. Enzyme Inhibit.* **1989**, *2*, 77.
- Andersson, C. B.; Morgenstern, R. *Biochem. J.* **1990**, *272*, 479.
- Reinemer, P.; Dirr, H. W.; Ladenstein, R.; Schäffer, J.; Gallay, O.; Huber, R. *EMBO J.* **1991**, *10*, 1997.
- Reinemer, P.; Dirr, H. W.; Ladenstein, R.; Huber, R. *J. Mol. Biol.* **1992**, *217*, 214.
- Oakley, A. J.; Rossjohn, J.; Bello, M. L.; Caccuri, A. M.; Federici, G.; Parker, M. W. *Biochemistry* **1997**, *36*, 576.
- Kano, T.; Sakai, M.; Muramatsu, M. *Cancer Res.* **1987**, *47*, 5626.
- Kunkel, T. A. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 488.
- Park, H. J.; Koh, J. U.; Ahn, S. Y.; Kong, K. H. *Bull. Korean Chem. Soc.* **2005**, *26*, 433.
- Parker, M. W.; Bello, M. L.; Federici, G. *J. Mol. Biol.* **1990**, *213*, 221.
- Habig, W. H.; Jakoby, W. B. *Methods Enzymol.* **1981**, *77*, 398.
- Chen, W.-L.; Haieh, J.-C.; Hong, J.-L.; Tsai, S.-P.; Tam, M. F. *Biochem. J.* **1992**, *286*, 205.
- Kong, K.-H.; Inoue, H.; Takahashi, K. *J. Biochem.* **1992**, *112*, 725.
- Tellez-Sanz, R.; Cesareo, E.; Nuccetelli, M.; Aguilera, A. M.; Baron, C.; Parker, L. J.; Adams, J. J.; Morton, C. J.; Lo Bello, M.; Parker, M. W.; Garcia-Fuentes, L. *Protein Sci.* **2006**, *15*, 1093.