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Use of a novel affinity tag selected with a bacterial random peptide library for improving activity retention of glutathione *S*-transferase adsorbed on a polystyrene surface

Takaharu Sakiyama[1], Shunsuke Ueno, Koreyoshi Imamura, Kazuhiro Nakanishi*

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Okayama 700-8530, Japan

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Abstract

Aiming at developing a novel affinity tag for site-specific immobilization of functional proteins onto polystyrene (PS) surfaces, *Escherichia coli* random peptide display library was screened for dodecapeptides exhibiting a high affinity toward PS plates. The selected peptides were commonly rich in hydrophobic amino acid residues and had two or three basic amino acid residues. Adsorption and desorption experiments for one of the selected peptide named PS1 (KGLRGWREMISL) showed that it was well and irreversibly adsorbed onto PS latex particles. To study its performance as an affinity tag, PS1 was genetically fused to a model enzyme, glutathione *S*-transferase (GST), in several manners, and the fusion enzymes were compared to the original GST in terms of the adsorption behavior onto the PS latex particles as well as the specific activities before and after the adsorption. The fusion GSTs in solution showed lower specific activities than the original one, and their adsorption behaviors were also altered. In particular, the fusion of PS1 to the N-terminal region of GST resulted in severe losses both in the specific activity and in the adsorptive ability. However, two types of GSTs fused with PS1 at the C-terminal region were well adsorbed onto the PS latex particles, and their specific activities after the adsorption were significantly higher than the original GST adsorbed on the PS latex particles. The fusion of PS1 to the C-terminal region of GST was thus shown to reduce the activity loss upon the adsorption onto the PS latex particles.

Keywords: Affinity tag; Random peptide library; Site-specific adsorption; Polystyrene surface; Glutathione *S*-transferase

1. Introduction

Immobilization of proteins such as enzymes and antibodies is utilized for various purposes including bioreactors, biosensors, affinity chromatography, and immunoassays. Among a variety of methods for immobilization, adsorption is the simplest and is commonly used especially for immunoassays. However, the immobilization onto solid surfaces often causes reduction of biochemical activities of the proteins. For example, it has been reported that most of anti-fluorescein antibodies were functionally inactivated when they were directly adsorbed onto microtiter wells [1]. The inactivation upon adsorption may be a consequence of conformational change of the proteins, as suggested for human lactate dehydrogenase adsorbed on a polystyrene plate in an immunobinding study [2]. Another study suggested that non-random orientation of sperm whale myoglobin adsorbed on a polydimethylsiloxane surface was a cause for diminished binding ability of several antibodies to myoglobin [3]. Thus, inadequate orientation may be another factor for the activity loss of surface-adsorbed proteins.

Site-specific immobilization is considered as an effective approach to cope with the activity loss of proteins upon immobilization [4]. Several techniques are available for the site-specific immobilization of proteins. For example, site-specific immobilization can be achieved by the use

* Corresponding author. Tel.: +81-86-251-8200; fax: +81-86-251-8264.
E-mail addresses: sakiyama@s.kaiyodai.ac.jp (T. Sakiyama), kazuhiro@biotech.okayama-u.ac.jp (K. Nakanishi).
[1] Present address: Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan.

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of affinity tags. Wang et al. [5] genetically fused an octapeptide named FLAG (DYKDDDDK) to subtilisin and immobilized the fusion product onto solid surfaces coated with protein A conjugated with anti-FLAG monoclonal antibody. Their results showed that the immobilization using immunoaffinity of FLAG resulted in a higher activity of subtilisin than random immobilization using glutaraldehyde. Histidine tags, which can form complexes with metal ions such as Zn^{2+}, Cu^{2+}, and Ni^{2+}, are also available for the site-specific immobilization. Carlsson et al. [6] reported that polyhistidine-fused enzymes immobilized on Zn^{2+}- or Cu^{2+}-carrying agarose beads retained comparable activities to the corresponding soluble enzyme. Thus, if we have a peptide exhibiting a high affinity toward a target surface, it might be useful as an affinity tag for the improvement of the functional activity of surface-adsorbed proteins.

Several types of peptides have been known to exhibit a particularly high affinity toward a specific type of solid surface. We previously reported that several types of peptides, obtained by enzymatic proteolysis of β-lactoglobulin and bovine serum albumin, were well and irreversibly adsorbed onto stainless steel surfaces [7,8]. Whaley et al. [9] found peptides with a binding specificity to a semiconductor surface. On the basis of these results, we can expect peptides exhibiting a high affinity toward other solid surfaces.

Here, we report a novel peptide tag for improving activity retention of proteins adsorbed on polystyrene (PS) surfaces. We employed an *Escherichia coli* random peptide display library called FliTrx to select tag candidates exhibiting a high affinity toward PS surfaces. The FliTrx library, based on the system described by Lu et al. [10], consists of a wide variety of conformationally constrained dodecapeptides displayed as thioredoxin fusions within a non-essential domain of flagellin. Flagellin is the unique component of bacterial fragellar filaments; the fragellar filament of *E. coli* is a helical assembly of flagellin with 11 subunits per two turns and with a pitch of 2.6 nm [11]. Thus, each library clone displays thousands of peptide inserts of a single type on its flagellar filaments. So far, the FliTrx library has been employed to reveal binding motifs for proteins [10,12,13], cells [14], and tissues [15]. We screened the library for dodecapeptides with a high adsorptive ability toward PS plates. Moreover, one of the selected peptides was genetically fused to a model enzyme, glutathione *S*-transferase (GST), to study the performance of the peptide as an affinity tag. GST was employed because various peptides and proteins have been fused to GST without losing its enzyme activity or substrate-binding ability [16–18]. GST is a relatively small protein and its gene is available as pGEX expression vectors [16]. In this study, several types of GST fused with the selected peptide were studied in terms of the adsorption characteristics onto PS latex particles as well as the specific activities before and after the adsorption.

2. Experimental

2.1. Strains and plasmids

The FliTrx random peptide display library, *E. coli* GI826 harboring pFLITRX, was obtained from Invitrogen (Carlsbad, CA, USA). An expression vector pGEX-3X, which carries the GST gene from *Schistosoma japonicum*, and a host strain *E. coli* BL21 were obtained from Amersham Biosciences (Uppsala, Sweden). Oligonucleotides used as primers and inserts were synthesized by Texas Genomics Japan (Tokyo, Japan) and Sigma Genosys Japan (Ishikari, Japan).

2.2. Growth and panning of the random peptide display library

According to the manufacturer's protocol, the FliTrx random peptide display library was grown at 25 °C for 16 h in IMC medium (0.2% casamino acids, 0.5% glucose, 10% 10× M9 salts, and 1 mM $MgCl_2$) containing 100 mg/l ampicillin, where 10× M9 salts contained 60 g/l of Na2HPO4, 30 g/l of KH_2PO_4, 5 g/l of NaCl, and 10 g/l of NH_4Cl, and was adjusted to pH 7.4. Expression of the thioredoxin–flagellin fusion proteins containing random peptide inserts was induced by growth in IMC medium containing 100 mg/l ampicillin and 100 mg/l tryptophan at 25 °C for 6 h.

PS plates (20 mm × 20 mm × 1 mm) were cut out of Iwaki PS culture dishes (Asahi Techno Glass, Tokyo, Japan) and used as a substrate surface in the following 'panning' procedure. A PS plate was brought into contact with 3 ml of a 10^6-fold dilution of the induced *E. coli* culture (approximately 10^4 cells) for 60 min at room temperature. After the culture was decanted, the PS plate was washed 15 times with 3 ml of fresh IMC medium containing 100 mg/l ampicillin. Then, the PS plate was incubated with another 3 ml of IMC medium containing 100 mg/l ampicillin at 30 °C until the library clones attached to the plate grew in the medium. The floating bacteria thus obtained were withdrawn and grown as described above. The culture was then induced by growth with the tryptophan-containing medium, and the cycle of panning was repeated. After five rounds of panning, the resulting culture was plated out. The pFLITRX plasmids were isolated from the individual colonies using S.N.A.P. MiniPrep Kit (Invitrogen) and sequenced with an SQ5500E DNA sequencer (Hitachi, Tokyo, Japan) by the dideoxy-chain termination method [19] using the primer 5′-ATTCACCTGACTGACGAC-3′ to deduce the amino acid sequences of the random peptide insert.

2.3. Synthesis and purification of peptide

One of the peptides selected in the above screening, named PS1, was chemically synthesized to confirm its affinity toward PS surfaces. The synthesis was performed

by a solid-phase method using F-moc chemistry on an automatic peptide synthesizer (Pioneer; Applied Biosystems, Foster City, CA, USA). After the cleavage from support resins by immersion in a trifluoroacetic acid solution, the product was purified by reversed-phase high performance liquid chromatography (RP-HPLC) using an LC10 system (Shimadzu, Kyoto, Japan) equipped with an ODS column (YMC-Pack ODS A-312, 6 mm i.d. × 150 mm, YMC Co. Ltd., Kyoto, Japan) and a D-2500 integrator (Hitachi). The elution was performed at a flow rate of 0.8 ml/min under a linear concentration gradient of acetonitrile from 1 to 40% in the presence of 0.012 N HCl. Monitoring the elution at 210 nm, the peak-top portion corresponding to PS1 was collected at the outlet of the UV detector. The peptide PS1 thus purified was verified in terms of amino acid composition using an L-8500A amino acid analyzer (Hitachi) after hydrolysis with 6 N HCl containing 0.02% phenol in an evacuated and sealed tube at 110 °C for 22 h.

2.4. Construction of plasmids encoding tag-fused GSTs

To construct plasmids encoding GSTs fused with PS1 tag at the C-terminal region, two types of synthetic DNA fragments encoding PS1 tag were prepared: a *Bam*HI–*Eco*RI fragment obtained by annealing: 5′-GATC-AAAGGGTTGCGGGGCTGGAGAGAAATGATAAGCC-TG-3′ and 5′-AATTCAGGCTTATCATTTCTCTCCAGCC-CCGCAACCCTTT-3′, and a *Eco*RI–*Eco*RI fragment obtained by annealing: 5′-AATTCAAAAGGGTTGCGG-GGCTGGAGAGAAATGATAAGCCTC-3′ and 5′-AATT-GAGGCTTATCATTTCTCTCCAGCCCCGCAACCCTTT-TG-3′; the underlined sequences correspond to PS1. The annealing was performed by heating each pair of synthetic single-strand DNAs at 95 °C for 15 min followed by cooling down to 30 °C in 60 min. The *Bam*HI–*Eco*RI fragment thus obtained was ligated into pGEX-3X predigested with *Bam*HI and *Eco*RI by using a DNA Ligation Kit (Takara, Otsu, Japan). The resulting plasmid was named pGEX-PS1. Then the *Eco*RI–*Eco*RI fragment was introduced to pGEX-PS1 predigested with *Eco*RI, the resulting plasmid being named pGEX-PS1x2. To construct a plasmid encoding GST fused with PS1 at the N-terminal region, a unique restriction site, *Sal*I site, was introduced into pGEX-3X at the position just after the initiation codon of the GST gene. The insertion was performed with QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA), which offers a protocol based on the polymerase chain reaction (PCR), using pGEX-3X as a template and the following two primers: 5′-GTCGACTCCCCTATACTAGGTTATTGG-3′ and 5′-GTCGACCATGAATACTGTTTCCTGTGTG-3′; the underline indicates *Sal*I site to be introduced. The PCR conditions were: 95 °C for 30 s to denature the template; 25 cycles at 95 °C for 30 s, 55 °C for 1 min, and 70 °C for 8.5 min; followed by incubation at 70 °C for 6.5 min, then cooling the reaction mixture at 4 °C until use. The plasmid thus obtained was named pGEX-N. A synthetic *Sal*I–*Sal*I fragment encoding PS1 tag was prepared by annealing: 5′-TCGACAAAGGGTTGCGGGGCTGGAGAGAAATGA-TAAGCCTGG-3′ and 5′-TCGACCAGGCTTATCATTTCT-CTCCAGCCCCGCAACCCTTTG-3′ in the same manner as described above, and introduced into pGEX-N predigested with *Sal*I. The resulting plasmid was named pGEX-NPS1. After construction and purification, the plasmids were verified by DNA sequencing.

2.5. Expression and purification of GSTs

E. coli BL21 was transformed with either of the recombinant plasmids, or with pGEX-3X, by electroporation (GTE-10, Shimadzu), and grown on agar plates of Luria–Bertani (LB) medium (0.5% yeast extract, 1% tryptone, 0.5% NaCl) containing 100 mg/l ampicillin. A single colony was picked up and inoculated into 50 ml of LB medium containing 100 mg/l ampicillin, and grown at 37 °C for 15 h with vigorous shaking. Then a 4 ml portion of the culture was diluted into 400 ml of the same medium and incubated at 37 °C with vigorous shaking. When the optical density at 600 nm exceeded 0.5, the expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM, and incubation was further continued for 3 h at 37 °C. Cultured cells were harvested by centrifugation (9000 × *g*, 15 min), washed two times with 400 ml of 10 mM phosphate buffer, pH 7.3, and resuspended in 20 ml of ice-cold PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3). After the addition of 20.9 mg of phenylmethylsulfonylfluoride, a protease inhibitor, dissolved in 1.2 ml of dimethyl sulfoxide, the suspension was sonicated in an ice-cold bath for the lysis of cells and centrifuged at 17,000 × *g* for 15 min to remove insoluble materials. The supernatant was applied to a column of glutathione Sepharose 4B gel (Amersham Biosciences). After the column was washed with PBS, GST was eluted with 50 mM Tris–HCl, pH 8.0, containing 10 mM reduced glutathione (GSH). The purity of GST was confirmed by SDS–polyacrylamide eletrophoresis (SDS–PAGE) on 12% polyacrylamide gels using a mini-PROTEAN 3 system (Biorad, Richmond, CA, USA). LMW Marker Kit (Amersham Biosciences) was used as marker proteins.

2.6. Adsorption experiments

In the adsorption experiments for the synthetic peptide and GSTs, 100 or 10 mM KNO_3 was used as their solvent (referred to as adsorption medium) after the pH value was adjusted to 6.0 with a small amount of HNO_3 or KOH. PS latex (Immutex S4259, mean diameter: 0.955 mm, specific surface area: 6 m^2/g, JSR Corp., Tokyo, Japan) was used as the substrate surface in all adsorption experiments. The PS latex particles was collected by centrifugation and resuspended in an adsorption medium. The centrifugation and resuspension cycles were repeated several times until the pH

of the supernatant reached 6.0. The latex concentration of the suspension thus obtained was approximately 20 mg/ml, as determined from the optical density at 600 nm. In a 20 ml glass bottle, 1 ml of the suspension was mixed with 1 ml of the adsorption medium containing either of the adsorbates. The bottle was tightly sealed and incubated at 30 °C for 2 h under vigorous shaking. After the suspension was centrifuged, the supernatant was subjected to quantification. The synthetic peptide was quantified by RP-HPLC on the same conditions as those for purification. GSTs were quantified as protein by the method of Lowry et al. [20] using bovine serum albumin as a standard. The amount adsorbed was calculated from the difference between the concentrations before and after the incubation. The latex particles collected by the centrifugation were subjected to desorption experiments or activity measurements described below.

2.7. Desorption experiments

Desorption experiments were performed for the synthetic peptide remaining on the PS latex particles after the adsorption experiment performed in 100 mM KNO_3 solution at pH 6.0. First, the peptide-loaded particles were suspended in 1 ml of 100 mM KNO_3 solution at pH 6.0, incubated at 30 °C for 2 h with vigorous shaking. After the particles were collected by centrifugation, the amount of the peptide desorbed was determined from the peptide concentration in the supernatant. The suspension–incubation–centrifugation cycle was repeated with 99 mM KNO_3–1 mM HNO_3 (pH 3), and then with 90 mM KNO_3–10 mM KOH (pH 12). The amount of the peptide desorbed in each cycle was determined similarly as mentioned above.

2.8. Enzyme activity

The enzyme activities were determined at 25 °C with 1 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) dissolved in 100 mM phosphate buffer, pH 6.5 [21]. The absorbance at 340 nm (A340) of the reaction mixture was measured for 5 min with a UV2500PC spectrophotometer (Shimadzu) equipped with a thermostatted cuvette compartment. The rate of increase in A340 was corrected for the spontaneous reaction by using a complete assay mixture without enzyme as blank. One unit (U) was defined as the amount of enzyme required to produce 1 μmol of GSH–CDNB conjugate per minute. Protein concentration was determined by the method of Lowry et al. [20] using bovine serum albumin as a standard.

The activity of enzyme adsorbed on the PS latex particles was assayed by the following procedure. The PS latex particles collected after the adsorption experiment was resuspended in the adsorption medium and centrifuged. The resuspension and centrifugation cycles were repeated several times until no enzyme activity was detected in the supernatant. A substrate solution (GSH and CDNB dissolved in 100 mM phosphate buffer, pH 6.5) was added to the suspension of the enzyme-loaded particles so that the final concentrations of GSH and CDNB were both 1 mM. After being incubated at 25 °C for 5 min, the reaction mixture was filtered through a 0.2 μm filter and A340 value of the filtrate was measured. The initial A340 value was measured for the substrate solution immediately before the addition to the suspension and corrected for dilution. The activity was determined from the difference between the A340 values before and after the incubation.

3. Results and discussion

3.1. Screening for peptides with high affinities toward PS plates

Screening with the FliTrx system is based on the interaction between the target and the random peptides expressed on the flagellar filaments. Therefore, the attachment to the target through any other exterior parts of the library cells should be eliminated for successful screening. To begin with, using the library cells without induction, we checked whether the panning procedure described above fulfilled this condition. As the result, the growth of the cells was not observed after a round of panning (data not shown), indicating no attachment to the PS plate through cell exterior except flagella.

Using the induced library cells, we obtained cells that survived five rounds of panning. Twenty-one clones were picked up, and their plasmids were sequenced. Table 1 lists the deduced amino acid sequences of the peptide insert in the isolated plasmids. We found four sequences and named them PS1, PS2, PS3, and PSstp. PSstp contained stop codons. PS1, PS2, and PS3 were relatively rich in hydrophobic amino acid residues and had two or three basic amino acid residues. Each peptide sequence was identified from two or more clones picked up. The most frequent sequence was PSstp. It was also reported elsewhere that clones containing stop codons like PSstp were found in the clones selected from the FliTrx library [15]. However, owing to the stop codons inserted in the middle of the fused gene, the fusion protein expressed by such clones should lack the conserved C-terminal region of flagellin, which is important for the flagellar assembly [22]. It is thus unclear how the clones with PSstp attached PS plates. Therefore, we chose PS1, which was

Table 1
Deduced amino acid sequences of the random peptides displayed by the library clones selected for PS plates

	Amino acid sequence[a]	Frequency
PS1	KGLRGWREMISL	5
PS2	LDPGAMRTIVRP	3
PS3	QLVEGVRHRIWN	2
PSstp	LGP*AAR*FGDE	11

[a] Hydrophobic and basic amino acid residues are indicated in bold and with underline, respectively. Asterisks represent stop codons.

Table 2
Results of adsorption and desorption experiments for synthetic peptide PS1

Concentration[a] (μM)		Amount adsorbed (nmol/m^2)	Desorption (%)		
Initial	Final		pH 6	pH 3	pH 12
3.4	0.0	32	–[b]	–	–
14.1	0.0	133	0.0	5.1	0.0

Adsorption experiments were performed in 100 mM KNO_3 solution at pH 6.0 using PS latex with a specific surface area of 6 m^2/g as a substrate surface. Desorption experiments were performed first with 100 mM KNO_3 solution at pH 6.0, then at pH 3.0, and finally at pH 12.0.

[a] Peptide concentrations before and after the adsorption experiment.

[b] Not determined.

the most frequent among the clones without stop codons inserted, as an affinity tag candidate for further study.

To confirm the affinity of PS1 towards PS surfaces, we performed the adsorption and desorption experiments for the synthesized PS1 using PS latex particles as a substrate surface. As shown in Table 2, PS1 was completely adsorbed onto the PS latex particles at pH 6.0 for both initial concentrations tested. The desorption experiments showed that PS1 was not removed with 100 mM KNO_3 at pH 6.0, indicating that the adsorption was practically irreversible under the conditions employed. Only 5% of the loaded PS1 was removed from PS latex particles at pH 3.0, and nothing was removed in the following treatment at pH 12.0. Thus, PS1, once adsorbed, was not readily removed from the PS latex particles in a wide range of pH.

The selected peptide PS1 was confirmed to show a high affinity toward the PS latex particles. PS1 was relatively rich in hydrophobic amino acid residues and had three basic amino acid residues. Considering hydrophobic nature of PS surfaces, the abundance in hydrophobic residues is reasonable for their high affinities. PS surfaces have a small negative charge owing to polymerization initiators and others, as shown for PS latex particles by zeta-potential measurements [23] and potentiometric titrations [24]. Thus the presence of basic amino acid residues carrying a positive charge probably contributes to the electrostatic interaction with the negatively charged PS surfaces.

The library subjected to the screening contained approximately 10^4 cells. Because only a limited number of the library clones were used for the peptide selection, it cannot be said that the selected sequences have been optimized in terms of the affinity toward PS surfaces. Nevertheless, one of the selected peptides was confirmed to exhibit a considerably high affinity toward PS surfaces.

3.2. Characteristics of GSTs fused with PS1

For the expression of PS1-fused GSTs, several types of plasmids were constructed from pGEX-3X as illustrated in Fig. 1. First, a synthetic fragment encoding PS1 was ligated into pGEX-3X predigested with *Bam*HI and *Eco*RI, the resulting plasmid being named pGEX-PS1. Then another synthetic fragment encoding PS1 was introduced to pGEX-PS1 predigested with *Eco*RI, the resulting plasmid being named

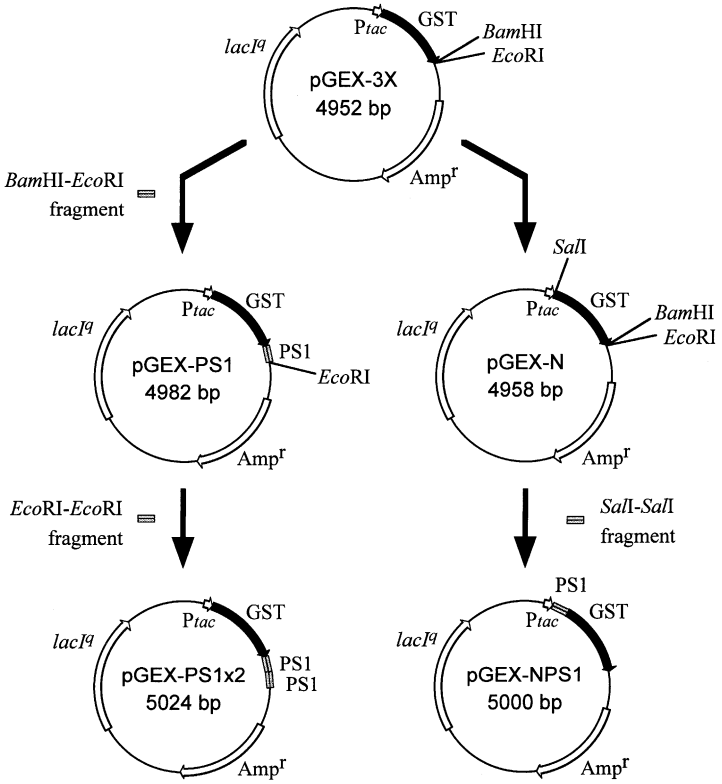

Fig. 1. Plasmids constructed for the expression of GSTs fused with PS1.

Plasmid	Enzyme	Amino acid sequence of *N*- and *C*-terminal regions
pGEX-3X	GST	MSPIL-----GIPGNSS
pGEX-PS1	GST-PS1	MSPIL-----GIKGLRGWREMISLNSS
pGEX-PS1x2	GST-PS1x2	MSPIL-----GIKGLRGWREMISLNSKGLRGWREMISLNSS
pGEX-N	GST-N	MVDSPIL-----GIPGNSS
pGEX-NPS1	GST-NPS1	MVDKGLRGWREMISLVDSPIL-----GIPGNSS

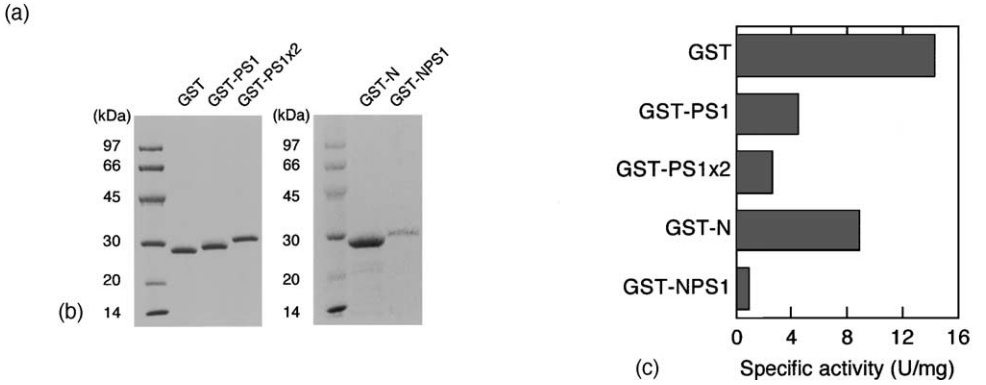

Fig. 2. Results of SDS–PAGE and the specific activity measurements for GSTs prepared in this study. (a) Amino acid sequences of the N- and C-terminal regions are shown with one-letter codes for each type of GST prepared in this study. The omitted region had the same sequence for all types of GST. The boxed sequence represents the PS1 tag. (b) SDS–PAGE. Marker proteins: α-lactalbumin (14.4 kDa), soybean trypsin inhibitor (20 kDa), carbonic anhydrase (30 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase b (97 kDa). (c) Comparison of specific activities.

pGEX-PS1x2. Besides the above, a unique *Sal*I site was introduced to pGEX-3X at the position just after the initiation codon of the GST gene, the plasmid thus obtained being named pGEX-N. A synthetic *Sal*I–*Sal*I fragment encoding PS1 was then introduced to pGEX-N predigested with *Sal*I, the resulting plasmid being named pGEX-NPS1.As shown in Fig. 2a, deduced amino acid sequences of the GSTs expressed by the recombinant plasmids were different only in the N- or C-terminal region from that of GST expressed by the original plasmid, pGEX-3X (referred to as the original GST hereafter). We named the five types of GSTs as indicated in Fig. 2a. Fig. 2b shows the results of SDS–PAGE for the five types of GSTs purified on the glutathione Sepharose 4B column. The molecular mass of the original GST estimated by the band position was in accordance with its deduced value (27 kDa). The molecular mass of GST obviously increased with the number of PS1 fused. The band of GST-NPS1 was very thin owing to a low recovery in the purification. Since the purification on the glutathione Sepharose 4B column was based on the enzyme–substrate affinity, the low recovery of GST-NPS1 probably related with its extremely low specific activity mentioned below.

Fig. 2c shows specific activities of the purified enzymes. Fusion of PS1 more or less lowered the specific activity of GST. In particular, the specific activity of GST-NPS1 was <10% of that of the original GST. Even GST-N, with only two extra amino acid residues in the N-terminal region, showed 38% loss in the specific activity. Thus the N-terminal fusion had a greater influence on the specific activity than the C-terminal fusion. This is probably related with the fact that the N-terminal region is essentially important for the catalytic activity of GST [18,25]. The fusion of PS1 to the C-terminal region reduced the specific activity of GST, though the reduction was less than that caused by the N-terminal fusion. However, Chen and Chen [18] reported that the specific activity of pGEX-expressed GST was not affected by the fusion of a $(His)_6$ tag to the C-terminal end. They employed an expression vector pGEX-5X-2, which by itself expresses GST with a C-terminal tail (-GRGIPGIPGSTRAAAS) longer than that expressed by pGEX-3X (-GRGIPGNSS). Thus, the activity loss by the C-terminal fusion observed in this study does not seem the matter of the length of fused tail. Hydrophobic nature of the fused peptide might be a cause for the decrease in the specific activity.

Fig. 3 compares adsorption isotherms of GSTs onto the PS latex particles in the presence of 100 or 10 mM KNO_3. The original GST was well adsorbed onto the PS latex particles in the presence of 100 mM KNO_3. A steep increase in the amount adsorbed at negligibly low equilibrium concentrations suggested its high affinity toward the PS latex particles. In fact, as described later, the adsorption of the original GST was practically irreversible in this region. However, the decrease in KNO_3 concentration from 100 to 10 mM significantly suppressed the adsorption of GST. This suggests that the hydrophobic interaction mainly controls the adsorption of GST onto the PS latex particles. The fusion of PS1 to the C-terminal region of GST suppressed the adsorption at a KNO_3 concentration of 100 mM, but promoted it at a KNO_3 concentration of 10 mM. Consequently, the adsorption isotherms of GST-PS1 and GST-PS1x2 were far less affected by KNO_3 concentration. The amount of GST-PS1x2 adsorbed rather increased, though slightly, with decreasing KNO_3 concentration. Although the reason

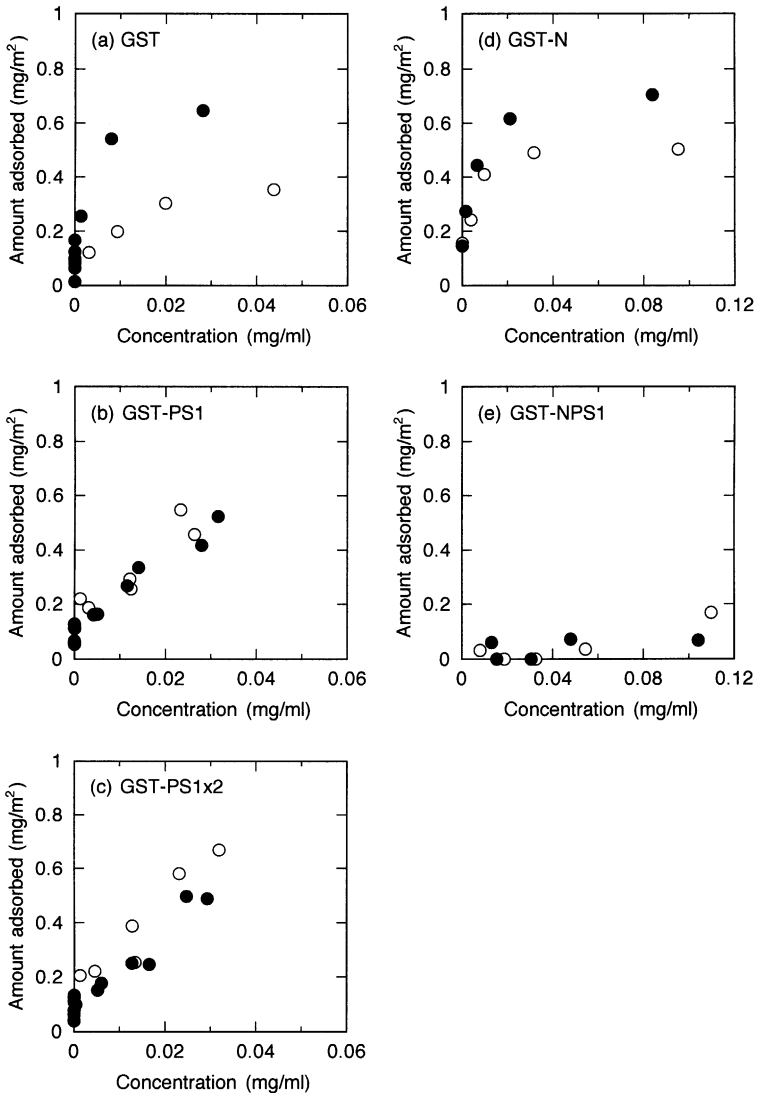

Fig. 3. Adsorption isotherms of GSTs onto PS latex particles at 30 °C and pH 6 in the presence of: (●) 100 mM or (○) 10 mM KNO_3. For each adsorption experiment, the amount of GST adsorbed onto PS latex particles was plotted against the equilibrium concentration of GST in the supernatant.

is not clear why the fusion of PS1 decreased the amount of GST adsorbed at a KNO_3 concentration of 100 mM, the hydrophobic interaction between the original GST and the PS surface is suggested to be too strong at high ionic strengths in comparison with the interaction between PS1 and the PS surface. Nevertheless, the C-terminal fusion of PS1 was revealed to increase the amount of GST adsorbed at a low ionic strength. Moreover, the different dependencies on KNO_3 concentration suggest different modes of adsorption. The fused PS1 segment possibly controlled the adsorption behaviors of GST-PS1 and GST-PS1x2. The adsorption isotherm of GST-N in the presence of 100 mM KNO_3 was very similar to that of the original GST. The decrease in KNO_3 concentration from 100 mM to 10 mM suppressed the adsorption, though the decrease was not so large as in the case of the original GST. After PS1 was fused to the N-terminal region of GST-N, however, adsorption was scarcely observed regardless of KNO_3 concentration. Although this may be interesting from a scientific aspect of protein adsorption, the reason is not clear at present.

Table 3 compares the specific activities of GSTs, with and without PS1 fused to the C-terminal region, before and after the adsorption onto the PS latex particles. The adsorption of each enzyme was performed using a rather dilute solution to give a negligible equilibrium concentration. The activity measurements for the supernatants obtained after rinsing the GST-loaded particles showed that the adsorption was irreversible except for the case of the original GST adsorbed at 10 mM KNO_3. The amount of each GST immobilized was also listed in Table 3. In all activity measurements, no GST activity was detected in the filtrate of the reaction mixture. When the original GST was adsorbed on the PS latex particles in the presence of 100 mM KNO_3, the residual activity was only 2.3%. However, the residual activities of GST-PS1 and GST-PS1x2 after the adsorption onto the PS latex particles were significantly higher, both in absolute values and in

Table 3
Comparison of specific activities of GSTs immobilized on PS latex particles

	Amount immobilized (mg/m^2)	Specific activity (U/mg)		Activity retention (%)
		In solution	Immobilized	
(a) GSTs adsorbed in the presence of 100 mM KNO_3				
GST	0.16	14.2	0.33	2.3
GST-PS1	0.15	4.5	0.77	17.1
GST-PS1x2	0.13	2.6	0.55	21.3
(b) GSTs adsorbed in the presence of 10 mM KNO_3				
GST	–[a]	14.2	–	–
GST-PS1	0.11	4.5	1.10	24.4
GST-PS1x2	0.11	2.6	0.91	35.0

[a] Not determined because the adsorption was reversible.

percentages, than that of the original GST. When GST-PS1 and GST-PS1x2 were adsorbed at a lower ionic strength (10 mM KNO_3), the residual activity became still higher. The maximum specific activity on the PS latex particles was given by GST-PS1 adsorbed at a KNO_3 concentration of 10 mM. Note that the specific activity was not determined for the original GST adsorbed at 10 mM KNO_3 because its leak from the PS latex particles was detected during the rinsing step. This also indicates the contribution of fused PS1 segment to the increase in the affinity of GST toward the PS latex particles.

The fusion of PS1 to the C-terminal region of GST was revealed to improve the activity retention on the surface of PS latex particles. Considering that the results of adsorption experiments suggested the importance of the fused PS1 segment in the adsorption of GST-PS1 and GST-PS1x2, the improved activity retention on the PS surface possibly arise from direct contact of the fused PS1 segment, rather than any other segments of GST, with the surface. On the other hand, the severe loss of specific activity observed for the original GST upon adsorption is probably ascribed to conformational changes and inadequate orientations of the adsorbed enzymes. In the cases of GST-PS1 and GST-PS1x2, direct and exclusive contact of the fused PS1 segment with the PS surface may suppress the conformational changes and improve the orientation.

In this study, improved activity retention on the PS surface was shown for GSTs fused with an affinity tag, PS1, at the C-terminal region, though the tag was not necessarily optimized in terms of the affinity toward PS surfaces. For further improvement of the activity retention, it might be contributory to optimize the tag sequence by screening a wider variety of the library clones.

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