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Chip-based analysis of SUMO (small ubiquitin-like modifier) conjugation to a target protein

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Abstract

A chip-based analysis of protein interactions and modifications in cell signaling pathways has been of great potential in drug discovery, diagnostics, and cell biology, because it enables rapid and high-throughput biological assays with a small amount of samples. We report a chip-based analysis of sumoylation, the post-translational modification (PTM) process that involves covalent attachment of the small ubiquitin-like modifier (SUMO) protein to a target protein through multiple enzyme reactions in eukaryotic cells. Substrate proteins were spotted onto a glass surface followed by the addition of the reaction mixture for sumoylation, and the SUMO conjugation was readily detected by using fluorescent dye-labeled antibody. Under the optimized condition, on-chip sumoylation of Ran GTPase-activating protein 1 (RanGAP1) domain resulted in highly specific fluorescence intensity compared to that of its mutant (K524A) irrelevant to SUMO conjugation. The on-chip sumoylation was also verified and quantified by using the surface plasmon resonance(SPR) spectroscopy. As the exemplary study for a parallel analysis of sumoylation, fluorescent detection of sumoylation was conducted in a microarray format on a glass slide. The chip-based analysis developed here is expected to be applicable to assay for screening of target proteins from existing protein pools and proteome arrays in a high throughput manner.

Keywords: SUMO (small ubiquitin-like modifier); Protein microarray; Post translational modification; Chip-based analysis

1. Introduction

The availability of entire genome sequences in humans and eukaryotes and the development of protein expression techniques have greatly prompted a large-scale and high-throughput analysis of biological processes in a chip-based analysis (Zhu and Snyder, 2003). At its early stage, the utilization of protein chips has been primarily focused on verifying interacting partners of known molecules (MacBeath and Schreiber, 2000) and finding out target proteins of post-translational modifications (PTMs) with simple reaction mechanism (Zhu et al., 2000). By exploring a wide range of practical applications up to date, the protein chip technologies have broaden their utility towards a high throughput interrogation of protein interactions (Zhu et al., 2001), functional activity analysis of protein interactions and modifications (Jung and Stephanopoulos, 2004; Nielsen et al., 2003; Zhu and Snyder, 2003), and diagnosis of the proteins related to some diseases (Sreekumar et al., 2001; Wang et al., 2002).

The post-translational modifications of proteins have been of great interest due to their crucial biological functions in modulating the state, location, and turnover of proteins as well as the interactions with other proteins (Mann and Jensen, 2003; Nielsen et al., 2003). Thus, considerable attention has been paid to elucidating the mechanisms and functions in an effort to screen the potent inhibitors and protein substrates of PTM pathways as a potent therapeutic target. Assays of biological processes including PTMs in a chip-based format offer distinct advantages over conventional solution-phase analyses with respect to throughput and quantity of reagents. Several pivotal approaches for a chip-based analysis of PTMs have been explored, but phosphorylation by kinase is unique and well recognized (Zhu et al., 2000), since 300 different PTMs have been

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reported (Nielsen et al., 2003; Zhu et al., 2000). Unlike phosphorylation mediated by single protein kinase, some PTMs have more complicated reaction mechanisms.

With the aim to exploit the potential use of the functional activity screening, we attempted the chip-based analysis of a PTM showing different reaction mechanism from well-studied phosphorylation. For this, we chosen sumoylation which involves the conjugation of SUMO (small ubiquitin-like modifier) protein with its target protein, since it especially joins one protein to another and requires multiple enzyme reactions (Wilson and Rangasamy, 2001). In contrast to a simple PTM process such as phosphorylation by protein kinase, the sumoylation of a target protein is mediated by three-step pathways involving multiple enzymes (Fig. 1A), namely E1(SUMO-activating enzyme) and E2(SUMO-conjugating enzyme), analogous to ubiquitin conjugation (Okuma et al., 1999; Uchimura et al., 2004). The modification of proteins by SUMO is known to be required for viability of most eukaryotic cells, regulating diverse cellular functions of the proteins that participate in transcription, DNA repair, nuclear transport, signal transport, signal transduction, and the cell cycle (Hay, 2001). Since the first elucidation of SUMO function a decade ago, there have been intensive studies concerning the discovery of new target proteins and demonstration of SUMO function using conventional methods. Assay of SUMO conjugation has been usually carried out by conventional methods such as tandem affinity purification, following ms/ms spectrometry analysis (Denison et al., 2005; Rosas-Acosta et al., 2005), which is time-consuming and labor-intensive.

In the present study, we demonstrate that SUMO conjugation can be detected in a chip-based format with good specificity and selectivity. A substrate protein was immobilized onto a glass surface followed by the addition of the reaction mixture for sumoylation. SUMO conjugation to a target substrate was

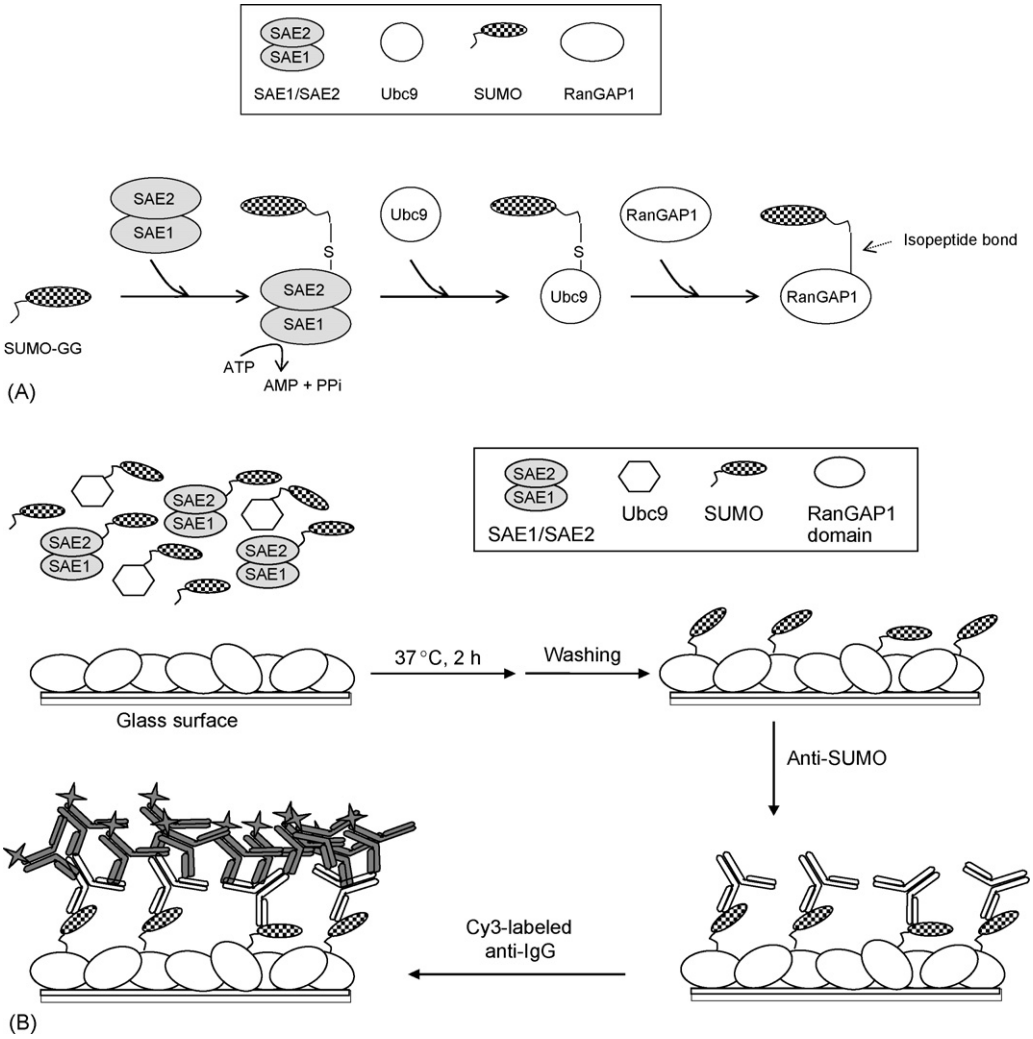

Fig. 1. (A) Conjugation pathway of SUMO to a target protein. SUMO protein is first processed to mature SUMO-1(G) which undergoes ATP-dependent activation by SAE1/SAE2, resulting in formation of a high-energy thioester bond. And then, SUMO-1(G) is transferred to Ubc9, releasing a thioester intermediate. Finally, the carboxyl terminus of SUMO-1(G) is covalently attached to an ε-amino group of a lysine residue in a target protein, generating an isopeptide bond. (B) A schematic representation of on-chip sumoylation reaction and fluorescence detection using antibody reagents. Substrate proteins (RanGAP1 domain, its mutant (K524A), and BSA) were immobilized on a glass surface, respectively. The reaction mixture for sumoylation was added onto the surface for 2 h at 37 °C, and SUMO conjugation was probed by using anti-SUMO and fluorescent dye-labeled anti-IgG.

probed by using anti-SUMO and fluorescent dye-labeled anti-IgG (Fig. 1B). A domain protein of RanGAP1, which is well-known as a target substrate of sumoylation (Bernier-Villamor et al., 2002), was employed. As the negative controls irrelevant to sumoylation, the mutant of RanGAP domain (K526A) and albumin proteins were tested. As the exemplary study for a parallel analysis of sumoylation, fluorescence detection of on-chip sumoylation was performed in a microarray format. Sumoylation reaction was also verified and quantified by using surface plasmon resonance (SPR) spectroscopy. Details are reported herein.

2. Materials and methods

2.1. Reagents

Anti-glutathione-*S*-transferase (anti-GST, developed in rabbit), Cy3-labeled anti-immunoglobulin G (anti-IgG, developed in sheep, $F/P = \sim 8$), bovine serum albumin (BSA), lysozyme, and 3,3-dithiopropionic acid bis-*N*-hydroxysuccinimide ester were purchased from Sigma. A monoclonal and affinity-purified anti-SUMO was purchased from ABGENT. Cy3 and Cy5 fluorescent dyes were obtained from Amersham Biosciences and used for labeling of antibody according to the manufacturer's instructions. Starburst poly(amidoamine) (PAMAM) dendrimer with fourth generation (G4) was purchased from Aldrich. Bis(sulfosuccinimidyl) suberate (BS^3) and *N*-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Pierce. TopBlock was obtained from Fluka. All other reagents used were of the highest quality available and purchased from the regular source. For the buffer solutions, 50 mM HEPES (pH 7.5) was used as a reaction buffer for sumoylation. Phosphate buffered saline (10 mM phosphate, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) containing 0.05% Tween 20 (PBST) and high salt PBST containing 1 M NaCl were used. All buffer solutions were used after filtration using a membrane (0.2 μm cutoff).

2.2. Plasmids

Human RanGAP1 and Ubc9 cDNAs were obtained by the reverse transcription polymerase chain reaction (RT-PCR) using total RNAs from HeLa cells as the templates. The RanGAP1(418-587) was amplified by PCR and cloned into a pGEX4T-1 vector (Amersham Biosciences) with *BamHI* and *SalI* restriction sites. The Ubc9 gene was also cloned into a pGEX4T-1 vector using *SalI* and *NotI* restriction sites. The cDNA encoding RanGAP1 domain with a single mutation (K524A) was generated by the PCR-directed mutagenesis and subcloned into a pGEX4T-1 vector using *BamHI* and *SalI* restriction sites. SUMO-1(G) cDNA cloned into a pGEX4T-1 vector using *BamHI* and *NotI* restriction sites was kindly provided by the Molecular Genetics Lab in KAIST. The plasmid pGEX4T-3 containing the gene encoding heterodimeric SUMO-activating enzyme (SAE1/SAE2) was a gift from Dr. Hay (University of St. Andrews, UK) (Tatham et al., 2001).

2.3. Protein expression and purification

GST-fused proteins of Ubc9, SUMO, and RanGAP domain were over-expressed in *E. coli* BL21 cells by induction with a final concentration of 0.5 mM isopropyl β-D(-)-thiogalactopyranoside (IPTG). A GST-fused heterodimeric SAE1/SAE2 protein was over-expressed in *E. coli* Rosetta cells by induction with 0.1 mM IPTG at 30 °C. After induction for 3 h, the cells were harvested and resuspended with PBS containing 1 mg/ml lysozyme. After incubation on ice for 30 min, the cells were lysed by sonication. The resulting lysates were centrifuged at 12,000 *g* for 30 min, and the GST-fused proteins were purified by using the packed column with glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer's instructions. The purified RanGAP domain and SAE1/SAE2 proteins were eluted from the beads with 50 mM Tris–HCl (pH 7.5) containing 30 mM glutathione and 120 mM NaCl. The GST-cleaved Ubc9 and SUMO were acquired by on-column digestion with thrombin and removal of thrombin according to the protocols provided by manufacturers. All purified proteins were stored at −70 °C after desalting with 50 mM HEPES.

2.4. On-chip sumoylation on a glass slide

The qualitative evaluation of on-chip sumoylation was performed on slide glasses modified with various functional groups including poly-lysine (Sigma–Aldrich, St, Louis, Mo, USA), aldehyde (Telechem international, Sunnyvale, CA, USA), epoxy (SCHOTT nexterion, Jena, Germany), a nitrocellulose-derived polymer (FAST™ slides, Schleicher & Schuell, Dassel, Germany), and hydrogel polymer (SCHOTT nexterion, Jena, Germany). To make a reaction well for sumoylation on a planar surface, small circles (diameter ∼ 5 mm) were drawn with a hydrophobic pen. A target substrate of sumoylation (RanGAP1 domain) and irrelevant proteins (the mutant of RanGAP1 domain and BSA) (0.8 mg/ml in 50 mM HEPES) were directly immobilized onto the surface inside wells, respectively. After incubation for 1 h, the protein-immobilized surface was blocked with 5% TopBlock solution for 2 h. Following washing the reaction wells with HEPES containing 0.5 M NaCl, the reaction mixture for sumoylation was added to the wells for 2 h at 37 °C. The reaction mixture (50 mM HEPES) was composed of SAE1/SAE2, Ubc9, and SUMO proteins at various concentrations, and also contained additives of 5 mM ATP, 5 mM $MgCl_2$, 50 mM NaCl, and 0.1 mM DTT. After the sumoylated surface was washed with high salt PBST, the SUMO conjugation was probed by serial reactions of anti-SUMO and secondary anti-IgG. The solution of anti-SUMO (10 μg/ml in PBST) was first applied to the reaction wells and incubated for 1 h followed by washing with high salt PBST. Subsequently, the sumoylation signal was amplified by the reaction of Cy3-labeled anti-IgG (2 μg/ml in PBST) for 30 min. After a thorough washing with high salt PBST for 30 min, the surface was rinsed with distilled water and dried with N_2 gas prior to fluorescence analysis. The mean value and standard deviation of the observed fluorescence signals were calculated from 2 × 2 microspots per each well using a fluorescent

scanner (GenePix 4100A, Axon Instruments Inc.) and GenePix Pro 4.1 software.

2.5. Microarray-based assay

Substrate proteins for sumoylation were spotted onto a glass slide by using a robotic arrayer (Microsys, Cartesian Technologies, Irvine, CA) equipped with a CMP3 spotting pin (Telechem International, Sunnyvale, CA). The protein samples were prepared in HEPES supplemented with 0.5% trehalose. The covalent coupling of spotted proteins was processed in a humidity-controlling chamber for 3 h at room temperature. Fluorescence images were recorded following surface blocking, on-chip sumoylation, and detection with antibody reagents as described above. The array format of substrate proteins was confirmed by probing with Cy5-labeled anti-GST (10 μg/ml in PBST).

2.6. SPR spectroscopy

All SPR measurements were conducted with BIAcore-X instrument and a SPR sensor chip with an evaporated gold surface (BIAcore AB) with a flow rate of 3 μl/min. For the immobilization of target substrates on a sensing surface, the monolayer of G4 PAMAM dendrimers, preparation and utility of which were elucidated in our previous study(Seok et al., 2005), was employed. The gold surface was chemisorbed with 3,3-dithiopropionic acid bis-*N*-hydroxysuccinimide esters to give amine-reactive functional groups. The amine-reactive surface was incubated with the solution of a G4 PAMAM dendrimer (22 μM in MeOH, based on the primary amine concentration) for 2 h. The dendrimer-modified surface was immersed in an aqueous solution of NaOH (∼ pH 12) for 30 min to remove residual reactive esters. The dendrimer monolayer presenting a number of amine groups was functionalized with a homo-bifunctional linker (BS^3, 10 mM) for the covalent attachment of substrate proteins. The solutions of RanGAP1 domain and its mutant were immediately injected over the activated surface, respectively. Following the reaction for 30 min, the protein-immobilized surface was blocked with 5% Top Block solution for 30 min. Thereafter, the sumoylation proceeded in a batch mode for 2 h at 37 °C. After the sumoylated surface was washed with high salt PBST, the sumoylation was analyzed by consecutive injections of anti-SUMO (10 μg/ml in PBST) and Cy3-labeled anti-IgG (10 μg/ml in PBST).

3. Results and discussion

3.1. Liquid-phase sumoylation

Prior to investigation of on-chip sumoylation, the reactivity of all purified proteins was validated by conducting in vitro sumoylation. In an effort to provide the chip-based strategy to evaluate sumoylation through unambiguous elucidation of on-chip sumoylation reaction, the RanGAP1 domain and sumoylation-relevant protein components including E1 (SAE1/SAE2), E2 (Ubc9), and matured SUMO-1(G) were first expressed in *E. coli* and purified. Their biological activities were confirmed by a solution-phase sumoylation assay. For this, the reaction conditions used for in vitro sumoylation were acquired from previous reports (Bernier-Villamor et al., 2002; Gong et al., 1999; Tatham et al., 2001; Uchimura et al., 2004). Under this condition, RanGAP1 domain generated a shifted band by sumoylation for 2 h at 37 °C, but no band shift was observed for RanGAP1 domain with a single mutation (K524A) (Fig. 2). The absence of either SAE1/SAE2 or Ubc9 in the reaction mixture led to no sumoylation of RanGAP1 domain. This result indicates that the relevant proteins produced by expression in *E. coli* were biologically active and the SUMO conjugation took place very specifically.

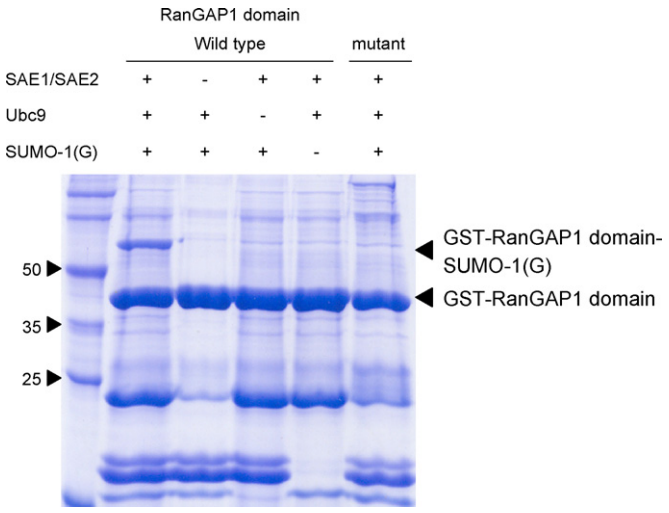

Fig. 2. Solution-phase sumoylation of GST-fused RanGAP1 domain and its mutant (K524A). Molecular weight standard (lane 1); GST-fused RanGAP1 domain (lane 2); Sumoylation in the absence of SAE1/SAE2 (lane 3), Ubc9 (lane 4), and SUMO (lane 5); GST-fused RanGAP1 domain mutant (lane 6). The reaction mixture contained protein components (1.5 μM SAE1/SAE2, 1.5 μM Ubc9, 10 μM SUMO, and 10 μM target substrate) and additives (5 mM ATP, 5 mM $MgCl_2$, 50 mM NaCl, and 0.1 mM DTT) in 50 mM HEPES.

3.2. Reaction conditions for on-chip sumoylation

Unlike the sumoylation in a solution phase, on-chip sumoylation reaction should be optimized in terms of the composition and reducing state of the reaction buffer as well as the concentrations of relevant proteins. In the case of a chip-based assay using a target substrate-immobilized surface, the concentrations of protein components in the reaction mixture for sumoylation are expected to affect sumoylation signal as well as false one caused by nonspecific adsorption of protein on a solid surface. When substrate proteins are confined onto a surface in a monolayer, the total amount of target substrates in a surface area decreases by several orders of magnitude compared to that of a solution-phase reaction.

In this sense, we varied the concentrations of protein components by taking into accounts those used in solution-phase sumoylation (Bernier-Villamor et al., 2002; Gong et al., 1999; Tatham et al., 2001; Uchimura et al., 2004), and investigated the effect on sumolyation on a glass surface. We first optimized the reaction conditions using an epoxy-coated glass. In most

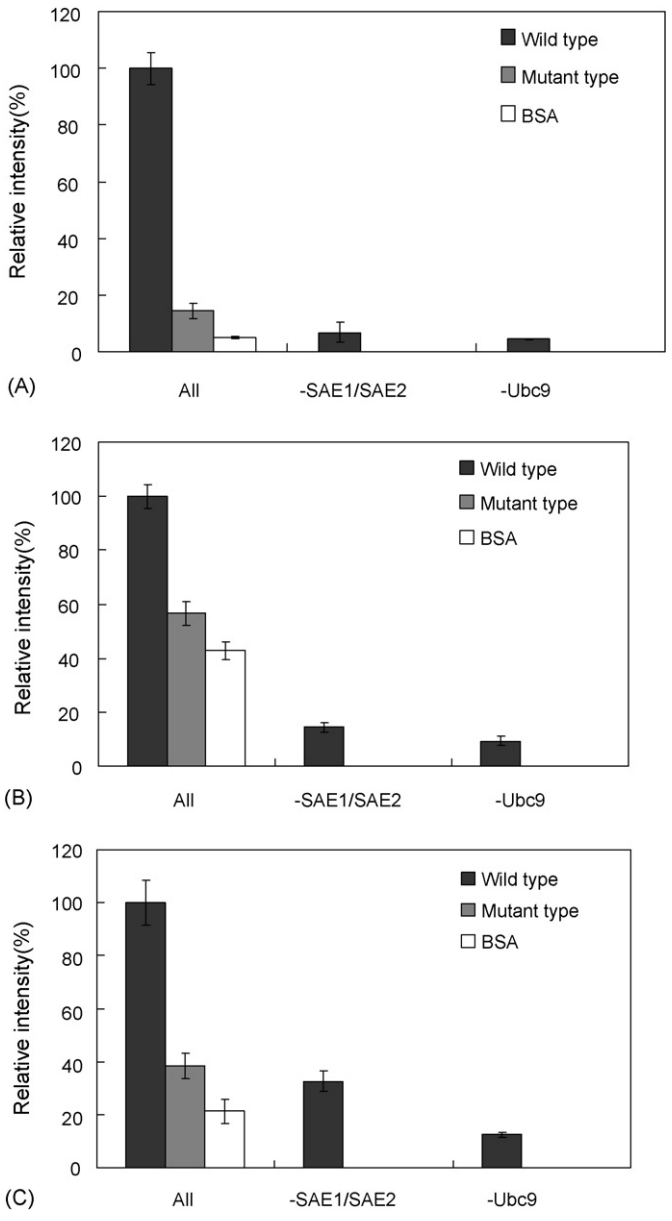

Fig. 3. (A) Sumoylation with wild-type and mutant of RanGAP1 domain under the optimized conditions. Sumoylation was conducted with all componets (column 1), in the absence of SAE1/SAE2 (column 2) or Ubc9 (column 3). Concentrations used are: 0.15 μM SAE1/SAE2, 0.15 μM Ubc9, 0.4 μM SUMO, and additives (5 mM ATP, 5 mM $MgCl_2$, 50 mM NaCl, and 0.1 mM DTT) in 50 mM HEPES. (B) Effect of concentrations of protein components. Concentration of SUMO was five times higher, and E1 and E2 were increased twice, respectively, compared to those in (A). (C) Sumoylation in the absence of DTT in the reaction mixture when other components were same as in (A).

instances of using higher concentrations (Fig. 3B), it was found that most background signal by comparison with the specific signal, when dye-labeled anti-SUMO was applied, was attributed to nonspecific adsorption of SUMO. In a series of tests, the highest signal-to-noise (S/N) ratio was obtained when the concentrations of SAE1/SAE2, Ubc9, and SUMO were fixed at 0.4, 0.15, and 0.15 μM, respectively (Fig. 3A). In this case, the observed efficiency of real sumoylation was preserved at the comparable level to other conditions of using higher concentrations, whereas false signals from all negative controls of omitting SAE1/SAE2 or Ubc9 reduced dramatically at 10% level. Next, we examined the effect of DTT on the efficiency of on-chip sumoylation and the alleviation of false signal caused by nonspecific protein adsorption. It was reported that DTT seems to have somewhat effect on the formation of a thioester bond between SUMO-1(G) and SAE1/SAE2 (or Ubc9) (Okuma et al., 1999; Uchimura et al., 2004). Coincidently, the presence of 0.1 mM DTT in the reaction buffer had a positive effect on the on-chip sumoylation, increasing considerably the sumoylation efficiency but suppressing background signals (Fig. 3C). Based on these results, the composition of the reaction mixture for on-chip sumoylation was determined as follows: 5 mM ATP, 5 mM $MgCl_2$, 50 mM NaCl, and 0.1 mM DTT in 50 mM HEPES (pH 7.5). Finally, the concentrations of antibody reagents were determined by comparing the acquired signal from on-chip sumoylation as a function of their reacted concentrations. The use of 10 μg/ml for anti-SUMO and 2 μg/ml for fluorescent dye-labeled anti-IgG in PBST was observed to exhibit sufficiently maximized intensity from on-chip sumoylation at all tested conditions (Fig. 3A). Besides an epoxy-coated glass, we also tested other slide glasses modified with various functional groups for on-chip sumoylation under the optimized conditions, and found that an epoxy-coated glass gave the best performance in terms of signal-to-noise ratio and non-specific protein binding.

3.3. Analysis of on-chip sumoylation

The target specificity and selectivity of sumoylation on an epoxy-coated glass surface was analyzed under the optimized conditions by employing a target substrate (RanGAP1 domain) and irrelevant proteins (the mutant of RanGAP1 domain and BSA) as a model system. The specificity of SUMO conjuga-

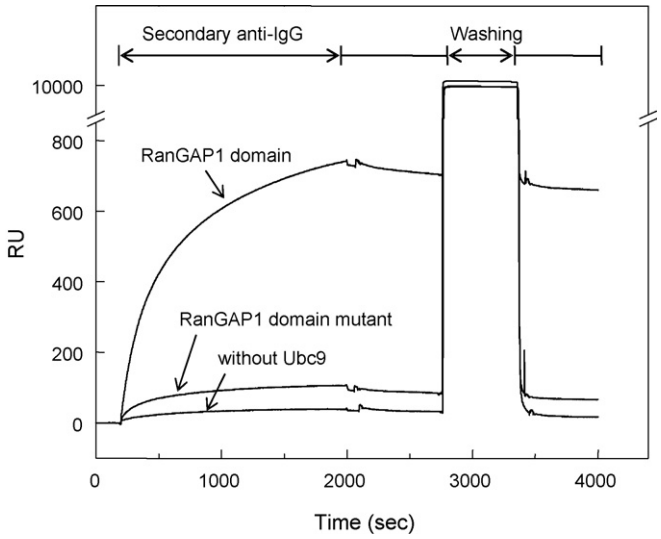

Fig. 4. SPR measurements. Sensorgrams show the changes in a SPR response when secondary anti-IgG (10 μg/ml in PBST) was injected over an anti-SUMO-reacted surface following on-chip sumoylation reaction. The target specificity of SUMO conjugation was tested at RanGAP1 domain- (up) and its mutant-immobilized surfaces (middle). A below sensorgram indicates a negative control of omitting Ubc9 in the reaction mixture for sumoylation.

tion was also verified by conducting the reaction in the absence of either SAE1/SAE2 or Ubc9. From a series of tests using three different glass slides, fluorescence signals were recorded from 2×2 microspots per reaction well and then, the mean value and standard deviation were calculated as displayed in graphs (Fig. 3A–C). As a result, the specific on-chip sumoylation of target substrate was distinguished from the false one of irrelevant proteins. The fluorescence intensity from RanGAP1 domain was about seven-fold higher than that from RanGAP1 domain with a single mutation (K526A). The BSA-immobilized surface revealed the weakest signal among all tested sets. From on-chip sumoylation and fluorescence detection, a SUMO target protein was clearly distinguished from irrelevant proteins. These observations indicate that the conjugation of SUMO to the lysine residue of a target protein takes place very specifically, which is known to be conferred by the presence of a consensus ψKXE motif (where ψ is typically a large hydrophobic residue, K the target lysine, X any residue, and E a glutamic acid) in a target substrate (Wilson and Rangasamy, 2001) It should be noted that there are clearly other determinants involved in substrate selection as well. Moreover, the real sumoylation signal was quite higher by over one order of magnitude when compared with the negative ones in the absence of either SAE1/SAE2 or Ubc9. This also shows that no sumoylation proceeds in the absence of SUMO-activating or conjugating enzymes which are essential to the conjugation of SUMO to a target protein.

3.4. Verification of sumoylation using SPR

In order to verify on-chip sumoylation quantitatively, SPR measurements were carried out. In this case, a substrate protein was covalently attached to an amine-reactive surface via an amide linkage as previously described (Seok et al., 2005), employing the same coupling strategy used in the epoxy-coated glass surface. It was confirmed that the substrate protein was immobilized onto a sensing surface at sufficiently high spatial density (data not shown). Like the fluorescence detection method, the relative magnitude of sumoylation was estimated by the reaction of anti-SUMO and secondary anti-IgG. Fig. 4 shows the sensorgrams for on-chip sumoylation of RanGAP1 domain and its mutant and for the negative control without Ubc9 in the reaction mixture. Collectively, the obtained results were almost identical to those from fluorescence detection on a

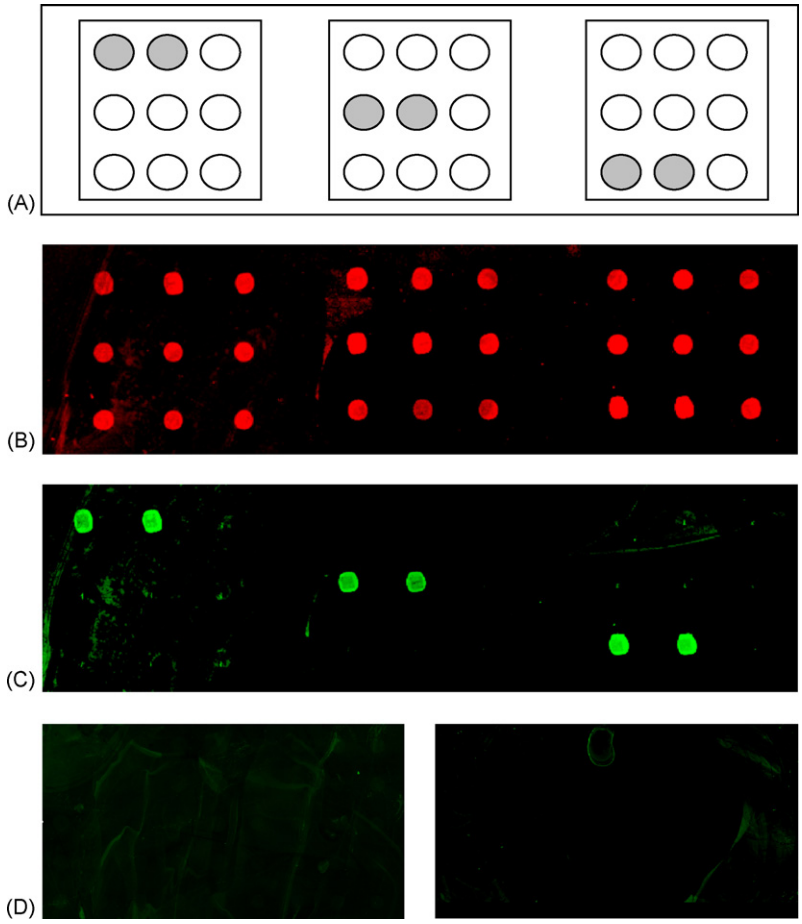

Fig. 5. Analysis of sumoylation in a microarray format. (A) The array layout of spotted RanGAP1 domain (gray) and its mutant (white) onto a glass slide. (B) Fluorescence image of substrate protein arrays traced by Cy5-labeled anti-GST (10 μg/ml in PBST). (C) Identification of SUMO-conjugated target proteins by the reaction of anti-SUMO (10 μg/ml in PBST) and Cy3-labeled anti-IgG (10 μg/ml in PBST) following on-chip sumoylation. (D) Negative controls using the reaction mixture without SAE1/SAE2 (left) or Ubc9 (right) for sumoylation.

glass slide. The RanGAP1 domain-immobilized surface exhibited the greatest signal shift of 630 ± 30 RU when treated with secondary anti-IgG (10 μg/ml in PBST). On the other hand, on-chip sumoylation of RanGAP1 domain mutant and the removal of Ubc9 in the reaction mixture resulted in a negligible shift, accounting for less than 10% level of the real sumoylation signal.

3.5. Sumoylation in a microarray format

From the qualitative and quantitative validation of on-chip sumoylation using a glass slide and a SPR sensor chip, it is evident that the conjugation of SUMO to its bona fide target protein, locally immobilized onto a surface, can be detected by using SUMO-specific antibody and secondary antibody. To develop an approach to a fast screening of SUMO target proteins, a microarray-based assay of sumoylation was carried out as displayed in the layout of 3 × 3 array format (Fig. 5A). The array format of spotted substrate proteins was traced by using Cy5-labeled anti-GST (F/P = ∼ 4, 10 μg/ml in PBST) recognizing a GST fusion partner of the substrates (Fig. 5B). Through on-chip sumoylation and fluorescence detection, green fluorescence was clearly visible from spots of RanGAP1 domain (Fig. 5C). The average S/N ratios were greater than 7, reflecting the efficacy of a chip-based assay. Each pair of spots generated mutually similar intensities in a correct position per 3 × 3 array format, which is identical to the layout (Fig. 5A). Except for green-colored spots of RanGAP1 domain, the rest of spotted RanGAP1 domain mutant released negligible signals due to no sumoylation. The absence of SAE1/SAE2 or Ubc9 in the reaction mixture also led to no fluorescent signal (Fig. 5D). These results indicate that the SUMO conjugation occurs specifically for individually spotted target proteins in a microarray format.

Recently, it has been reported that sumoylation plays an important role in the regulation of cellular stresses and immune regulatory mechanism (Guo et al., 2005; Li et al., 2005). In addition, sumoylation has been revealed to be linked with the pathogenesis of a variety of disorders including Alzhemer's disease(AD) (Li et al., 2003), Huntington's disease(HD) (Steffan et al., 2004). In this context, screening and identification of target proteins or inhibitors for sumoylation can be of great significance in development of potential therapeutics. With the development of proteome scale expression and purification techniques for functional proteomics (Braun et al., 2002; Braun and LaBaer, 2003) and the self-assembling protein microarrays using in vitro transcription and translation system (Ramachandran et al., 2004), it is anticipated that the chip-based analysis developed here can provide a versatile tool for a high throughput screening of SUMO target proteins and inhibitors.

4. Conclusion

We have demonstrated that SUMO conjugation to a target protein, which is one of the typical cell-signaling processes in eukaryotes and mediated by multiple enzymes, can be detected on a glass surface. A target substrate (RanGAP1 domain) was immobilized on a glass surface followed by addition of the reaction mixture containing the protein components such as SUMO-activating and conjugating enzymes. Sumoylation of target substrate was detected by anti-SUMO and dye-labeled anti-IgG antibodies with high sensitivity. Sumoylation was validated and quantiated by SPR spectroscopy. Assay of sumoylation in a microarray format was also conducted by spotting the target substrate on a glass surface and by analyzing sumoylation using a fluorescence tag. Based on the results, we expect that the chip-based analysis developed here can be applied to assay for screening of target proteins that requires complicated reactions from existing protein pools and proteome arrays in a high throughput manner.

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