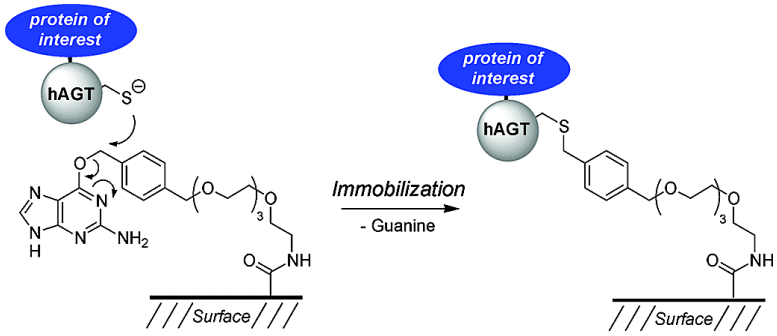
Communication

Covalent and Selective Immobilization of Fusion Proteins

Maik Kindermann, Nathalie George, Nils Johnsson, and Kai Johnsson

J. Am. Chem. Soc., **2003**, 125 (26), 7810-7811• DOI: 10.1021/ja034145s • Publication Date (Web): 10 June 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 14 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/10/2003

Covalent and Selective Immobilization of Fusion Proteins

Maik Kindermann,[†,‡] Nathalie George,[†,‡] Nils Johnsson,[§] and Kai Johnsson*[,†,‡]

Institute of Molecular and Biological Chemistry and Institute of Biomolecular Sciences, Swiss Federal Institute of Technology, CH-1015 Lausanne, Switzerland, and Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, D-76021 Karlsruhe, Germany

Received January 13, 2003; E-mail: kai.johnsson@epfl.ch

Protein microarrays, microbeads, and protein sensor chips play an increasingly important role for characterizing the functions and interactions of proteins.[1] These solid-phase-based assays require the immobilization of proteins in their native state. Current immobilization techniques rely either on adsorption, on direct covalent immobilization of purified proteins to chemically activated surfaces, or on their expression as a fusion to a polypeptide that mediates the immobilization on an appropriately derivatized surface.[2] Here, we report on a general method for immobilization of proteins that are genetically linked to a mutant of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase (hAGT). The high specificity and covalent nature of the approach make it an attractive alternative to all currently used protocols for the immobilization of fusion proteins.

The DNA repair protein hAGT (207 residues) transfers the alkyl group from its substrate, O^6-alkylguanine-DNA, to one of its cysteine residues (Figure 1A).[3] We have recently shown that O^6-benzylguanine (BG) substituted at the 4-position of the benzyl ring can be used to specifically label N- or C-terminal hAGT fusion proteins with different labels in vivo and in vitro.[4] On the basis of these observations, we reasoned that surfaces displaying BG derivatives of the type **1** should result in the selective immobilization of hAGT fusion proteins (Figure 1B, C).

Compound **1** was synthesized from readily available materials (Supporting Information). The primary amino group of **1** should allow its immobilization on a wide variety of different surfaces displaying activated carboxyl groups. A flexible tetraethylene glycol was chosen as a linker between the O^6-benzylguanine and the terminal amino group to maximize the accessibility of immobilized **1** toward hAGT fusion proteins. In all of the following experiments, a recently generated hAGT mutant, ^{GE}hAGT, with nearly 20-fold increased activity against BG was used.[5] The second-order rate constant for the reaction of ^{GE}hAGT with BG derivatives of the type **1** in solution was determined to be 5000 s^{-1} M^{-1} (Supporting Information).

To demonstrate a selective immobilization of hAGT-X where X can be any protein, **1** was coupled to commercially available carboxymethylated dextran chips suitable for surface plasmon resonance (SPR) experiments.[6] Using SPR, both the immobilization of a protein onto the sensor chip as well as its subsequent interactions with other proteins or compounds can be monitored directly. The carboxyl groups of the carboxymethylated dextran were transiently transformed into *N*-hydroxysuccinimide (NHS) esters and subsequently reacted with **1**. On average, immobilization of **1** led to a SPR signal of 2000 resonance units. Next, the immobilization of a fusion between glutathione S-transferase and ^{GE}hAGT (GST-^{GE}hAGT) was investigated. GST-^{GE}hAGT was flown over a BG-covered sensor chip, and binding was followed by SPR. Binding of GST-^{GE}hAGT led to an increasing SPR-signal over time, and bound protein could not be removed by washing with buffer (Figure 2). Prior incubation of GST-^{GE}hAGT with BG completely prevented its binding to the surface of the sensor (Figure 2). The immobilization of ^{GE}hAGT on chips displaying BG followed pseudo first-order kinetics, and the rate constant for immobilization was measured to be 225 s^{-1} M^{-1} (Supporting Information).

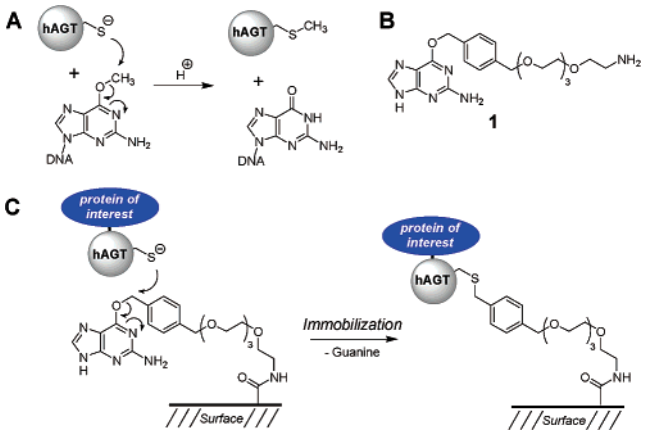


Figure 1. Immobilization of hAGT fusion proteins. (A) hAGT-based DNA repair; (B) structure of **1**; (C) covalent immobilization of hAGT fusion proteins on surfaces displaying **1**.

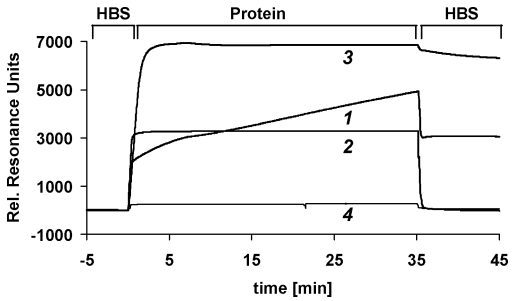


Figure 2. SPR data for the immobilization of GST-^{GE}hAGT on BG-covered sensor chips. The flow rate in all experiments is 1 μL/min. HBS: 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% Tween 20. (1) GST-^{GE}hAGT (0.1 mg/mL); (2) GST-^{GE}hAGT (0.1 mg/mL), preincubated with BG (40 μM); (3) anti-GST antibody (0.1 mg/mL) after incubation of a chip with GST-^{GE}hAGT as in lane 1; (4) anti-GST antibody (0.1 mg/mL) directly on BG-covered sensor chip.

To demonstrate that immobilized ^{GE}hAGT fusion proteins can be used for the analysis of protein−protein interactions, anti-GST antibody was flown over GST-^{GE}hAGT derivatized sensor chips. A strong signal from bound antibody was detected (Figure 2), whereas no binding of the antibody was observed on BG-covered sensor chips lacking the attached GST-^{GE}hAGT (Figure 2). As expected, the ratio of bound antibody to immobilized GST-^{GE}hAGT,

† Institute of Molecular and Biological Chemistry, Swiss Federal Institute of Technology.
‡ Institute of Biomolecular Sciences, Swiss Federal Institute of Technology.
§ Institute of Toxicology and Genetics.

10.1021/ja034145s CCC: $25.00

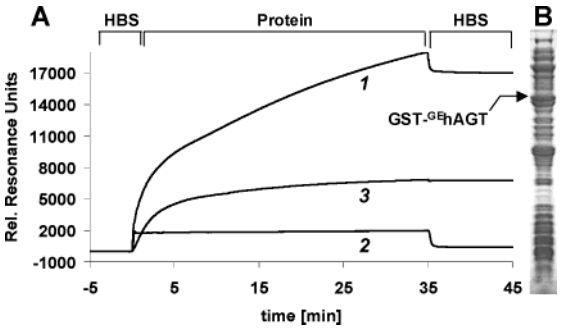


Figure 3. Immobilization of hAGT fusion proteins out of cell extracts. (A) SPR data for the immobilization of GST-^{GE}hAGT on BG-covered sensor chips out of cell extracts. HBS: 10 mM HEPES, pH 7.4, 150 mM NaCl, 1% Tween 20. (1) Lysate (10 mg/mL total protein); (2) lysate (10 mg/mL total protein) preincubated with BG (100 μM); (3) anti-GST antibody (0.1 mg/mL) after incubation of a chip with cell extract as in lane 1. (B) Lane 1: SDS-PAGE of cell extract of Xl1-Blue expressing GST-^{GE}hAGT used in (A) and subsequent staining with Coomassie Blue.

as calculated from the measured resonance units, decreased with increasing surface density of immobilized GST-^{GE}hAGT. GST-^{GE}hAGT densities as low as 0.7 ng/mm^2 gave a 1:1 ratio of antibody bound per GST-^{GE}hAGT, whereas the ratio decreased to 1:6 at densities of 17 ng/mm^2. The density of the immobilized hAGT fusion protein can be readily adjusted by controlling the reaction time on the chip. Furthermore, chips covered with GST-^{GE}hAGT and anti-GST antibody were flushed with 10 mM HCl to remove any noncovalently bound protein. Subsequent application of new anti-GST antibody to this chip resulted again in a specific binding signal, indicating that most of the immobilized GST-^{GE}hAGT is indeed covalently bound (Supporting Information). The intensity of this signal was decreased by 30% as compared to the first incubation with antibody. The observed decrease in the binding of antibody after the HCl washing can be explained in two ways: (i) GST forms a dimer, and not necessarily both halves of a GST-^{GE}hAGT dimer are covalently bound to the sensor chip; (ii) the treatment with 10 mM HCl might denature a fraction of the GST, thereby reducing the subsequent binding of the antibody.

To demonstrate that the hAGT-based immobilization does not interfere with the biological activity of the protein of interest, we measured the GST activity of GST-^{GE}hAGT after its immobilization. GST-^{GE}hAGT was immobilized on agarose beads displaying **1**, and GST activity was measured by incubating the beads with glutathione and 1-chloro-2,4-dinitrobenzene (CDNB). GST catalyzes the addition of glutathione to CDNB, a reaction that can be followed at 345 nm.[7] Incubating agarose beads displaying immobilized GST-^{GE}hAGT with a solution of glutathione and CDNB led to rapid product formation, while agarose beads displaying no GST-^{GE}hAGT showed no such activity, demonstrating that GST-^{GE}hAGT at least partially retains its activity after immobilization (Supporting Information).

hAGT possesses a high activity against a substrate that is otherwise chemically inert. To demonstrate that this feature can be exploited to specifically immobilize hAGT fusion proteins directly from crude protein preparations, GST-^{GE}hAGT was immobilized directly out of cell extracts of *E. coli* XL1-Blue expressing GST-^{GE}hAGT (Figure 3).

E. coli lysates were applied to a BG-covered sensor chip, and a strong signal from irreversibly bound protein was observed that steadily increased over a time period of 35 min (Figure 3A). The immobilized species was identified as GST-^{GE}hAGT as it was recognized by anti-GST antibody on the chip (Figure 3A). Immobilization was specific because preincubation of the *E. coli* extract with BG (100 μM) decreased the SPR signal by a factor of 50 (Figure 3A). Furthermore, no signal was observed from extracts of *E. coli* expressing only GST without the attached hATG (Supporting Information).

The results demonstrate a number of major advantages of the hAGT-mediated immobilization procedure. (1) The attachment to the surface is very gentle, and the fusion protein is exclusively coupled via the hAGT moiety, leaving the protein of interest accessible for interactions with other molecules. (2) The attachment to the surface is covalent. This ensures that the protein remains linked to the solid phase under a variety of different conditions. (3) The immobilization on BG-covered surfaces is specific, enabling the immobilization of hAGT fusion proteins without time-consuming purification steps. BG shows no significant reactivity against proteins other than hAGT. This makes it superior to the suicide inhibitor 4-nitrophenyl phosphonate as an immobilization device, because 4-nitrophenyl phosphonates are known to react with a variety of proteases, lipases, and esterases.[2d,8] (4) hAGT as a fusion tag can be used both for the covalent immobilization and the attachment of a variety of chemical or spectroscopic probes.[4] This versatility makes possible the use of once constructed hAGT fusion proteins for very different investigations. All features combined should establish hAGT fusions as unique tools in functional proteomics.

Acknowledgment. This work was financed by the Swiss National Science Foundation, the Bundesamt fuer Berufsbildung und Technologie (CTI project 6255.1), and the Bundesamt fuer Bildung und Wissenschaft (project X-TB). We thank Christoph Bieri, Kirstin Leufgen, and Horst Vogel for advice and support.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews: (a) Wilson, D. S.; Nock, S. *Curr. Opin. Chem. Biol.* **2002**, *6*, 81−85. (b) MacBeath, G. *Nat. Genet.* **2002**, *32 Suppl 2*, 526−532.
(2) For recent examples: (a) Lee, K. B.; Park, S. J.; Mirkin, C. A.; Smith, J. C.; Mrksich, M. *Science* **2002**, *295*, 1702−1705. (b) MacBeath, G.; Schreiber, S. L. *Science (Washington, D. C.)* **2000**, *289*, 1760−1763. (c) Zhu, H.; Bilgin, M.; Bangham, R.; Hall, D.; Casamayor, A.; Bertone, P.; Lan, N.; Jansen, R.; Bidlingmaier, S.; Houfek, T.; Mitchell, T.; Miller, P.; Dean, R. A.; Gerstein, M.; Snyder, M. *Science* **2001**, *293*, 2101−2105. (d) Hodneland, C. D.; Lee, Y. S.; Min, D. H.; Mrksich, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5048−5052. (e) Lesaicherre, M. L.; Lue, R. Y.; Chen, G. Y.; Zhu, Q.; Yao, S. Q. *J. Am. Chem. Soc.* **2002**, *124*, 8768−8769.
(3) Pegg, A. E. *Mutat. Res.* **2000**, *462*, 83−100.
(4) Keppler, A.; Gendreizig, S.; Gronemeyer, T.; Pick, H.; Vogel, H.; Johnsson, K. *Nat. Biotechnol.* **2003**, *21*, 86−89.
(5) ^{GE}hAGT possesses the mutations Asn157Gly and Ser159Glu: Juillerat, A.; Gronemeyer, T.; Keppler, A.; Gendreizig, S.; Pick, H.; Vogel, H.; Johnsson, K. *Chem. Biol.* **2003**, *10*, 313−317.
(6) Chips were purchased from Biacore (CM5-research grade).
(7) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. *J. Biol. Chem.* **1974**, *249*, 7130−7139.
(8) (a) Becker, E. L. *Biochim. Biophys. Acta* **1967**, *147*, 289−296. (b) Bjorkling, F.; Dahl, A.; Patkar, S.; Zundel, M. *Bioorg. Med. Chem.* **1994**, *2*, 697−702.

JA034145S