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Fluorescent probes designed for detecting human serum albumin on the basis of its pseudo-esterase activity

Sung-Jean Kim [b,†], Hyun-Woo Rhee [a,†], Hyun-Joo Park [a], Hye-Yeon Kim [c], Hyo-Soo Kim [b], Jong-In Hong [a,*]

[a] Department of Chemistry, Seoul National University, Seoul 151-747, Republic of Korea
[b] Department of Biomedical Sciences, Seoul National University, Seoul 110-799, Republic of Korea
[c] Korea Basic Science Institute, Ochang, Republic of Korea

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ABSTRACT

We developed activity-based fluorescent probes for detecting human serum albumin (HSA) on the basis of its pseudo-esterase activity. These probes could also detect HSA in blood-contaminated tissue samples.

Human serum albumin (HSA) is the most abundant protein in human blood plasma, with a concentration of 0.7 mM.[1] It is a 66-kDa protein that is produced in the liver and helps maintain the osmotic pressure of body fluids. HSA works as an extraordinary molecular sponge because it can bind many endogenous and exogenous compounds, including hormones, fatty acids, vitamins, heme, metabolites, and drug molecules at different sites in 3 homologous subdomains.[1] This sponge-like ability of HSA significantly influences the pharmacokinetic and pharmacodynamic effects of many exogenous drugs.[2,3]

Interestingly, HSA exhibits esterase-like activity and hydrolyzes drugs having an ester group, such as acetylsalicylic acid (aspirin).[4–6] Lys199, Lys402, Lys519, and Lys545, which form the pseudo-esterase active site of HSA, are acetylated by aspirin, which allows these acetylated lysine residues to remain unhydrolyzed for over 3 weeks under normal physiological conditions.[6] Drug-induced acetylation of HSA may inhibit the transport of endogenous biomolecules[7] and the natural esterase activity of HSA.[8] Therefore, sensitive monitoring of aspirin-induced acetylation of HSA is crucial in early drug development and clinical drug dosing.

Calorimetric or chromatographic techniques have been developed for analyzing the binding of drugs to HSA,[2] but these techniques are rather laborious to implement and are not adaptable to high-throughput screening. In a recent study, Chang and co-workers used a combinatorial approach to develop fluorescent probes that can non-covalently bind to HSA; these probes proved useful in identifying drugs that bound more strongly to HSA than did the probes.[9,10] However, these non-covalently binding HSA probes cannot be used for detecting HSA in denaturing conditions, such as in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Herein, we report the in-gel fluorescence detection of HSA by using activity-based probes (ABPs) that could covalently bind to HSA on the basis of its esterase-like activity.[11,12] These probes could selectively detect blood contamination in patient-derived protein samples.

ABPs were originally developed using mechanism-based inhibitors (suicide inhibitors)[13] and designed for catalysis by enzymes and covalent binding to the active sites of enzymes. Some researchers have developed ABPs for selective detection of a certain class of enzymes in whole-cell proteomes for proteome profiling[14,15] or to diagnose cancer progression by imaging.[16,17] Some ABPs have been developed on the basis of the formation of a quinone methide as a reactive intermediate by the action of carboxylesterase;[18–21] however, these probes have not been tested until now for detecting HSA.

Because HSA shows pseudo-esterase activity, we attempted to develop ABPs that could be activated by both HSA and normal hydrolases. Our results showed that HSA activated the ABPs (probes **1** and **4**) to a greater degree than normal hydrolases did. HSA hydrolyzes the reactive acyl moiety of the probe, cleaves the carbamate bond to release 4-nitroaniline and carbon dioxide, and eventually produces quinone methide as a temporarily unstable intermediate. Subsequently, a covalent bond is formed between the quinone methide and a nucleophile near the active site of the enzyme, resulting in the formation of a probe–protein complex (HSA-**1**; Scheme 1).

* Corresponding author. Fax: +82 2 889 1568.
E-mail address: jihong@snu.ac.kr (J.-I. Hong).
† These authors contributed equally this work.

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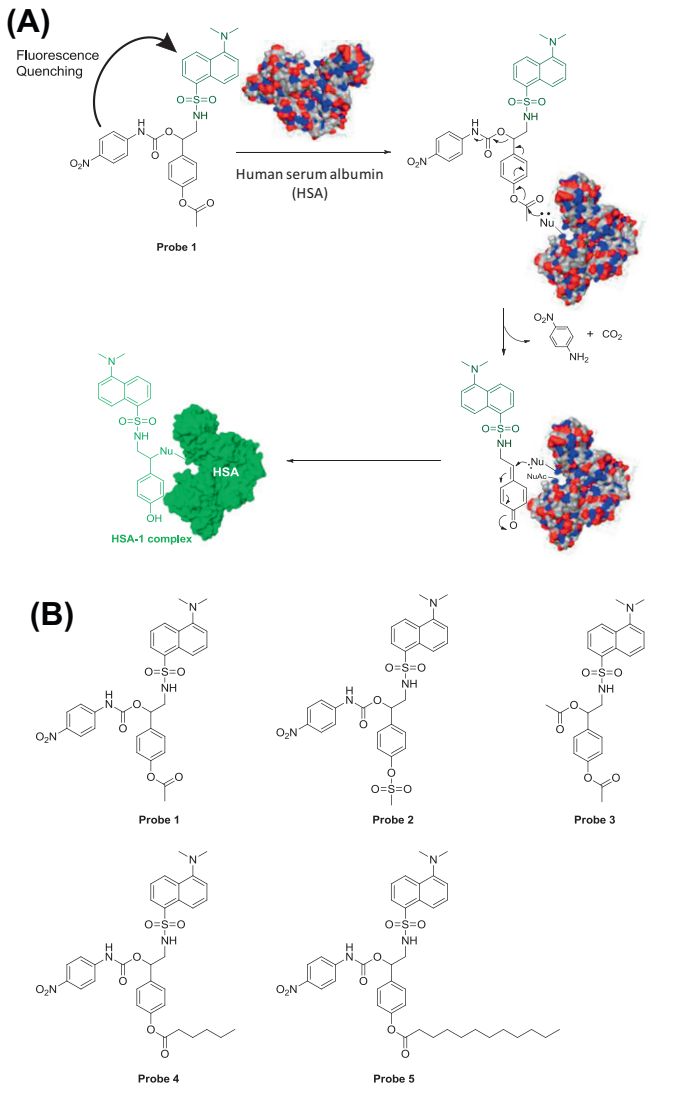

Scheme 1. (A) Mechanism underlying the binding of probes to human serum albumin (HSA). (B) Chemical structures of activity-based probes for HSA.

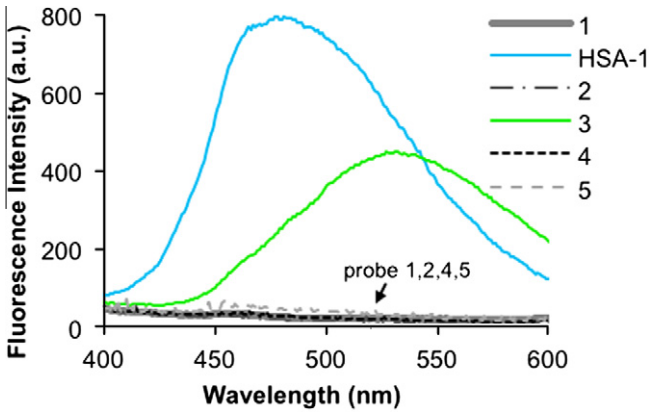

Figure 1. Fluorescence emission spectra (excitation at 340 nm) of each probe (10 μM) and the purified HSA protein–probe **1** complex (HSA-**1**) (10 μM) in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 10 mM) buffer (pH 7.4).

We synthesized 5 probes having the following acyl groups of various lengths: probe **1**, acetyl group; probe **2**, methanesulfonyl (mesyl) group, which is not normally hydrolyzed by esterases; probe **3**, acetyl group in place of 4-nitrophenylcarbamate in the benzylic position; probe **4**, hexanoyl group; and probe **5**, dodecanoyl group.

All these probes have a dansyl group as the fluorescent reporter and 4-nitrophenylcarbamate as the fluorescent quencher for the dansyl fluorophore.

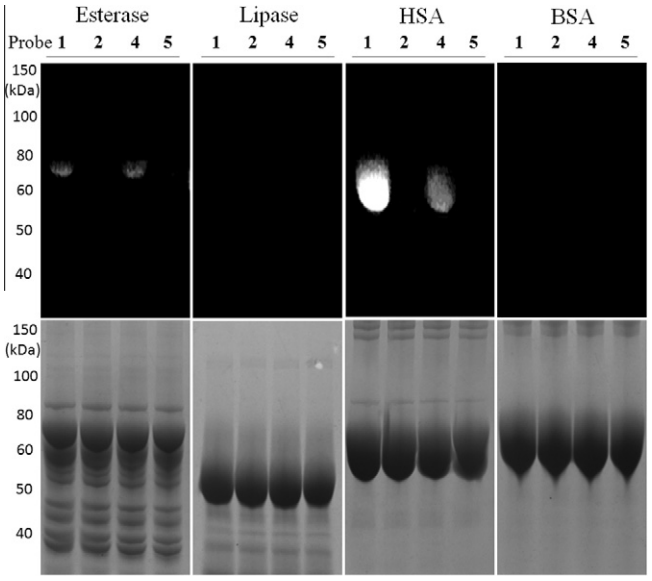

Figure 2. In-gel (8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS–PAGE]) fluorescence (upper) and Coomassie Brilliant Blue stain (below) gel images of esterase (from porcine liver), lipase, HSA and BSA (each 20 μg) after a 12-h incubation with probes **1**, **2**, **4**, and **5** (each 50 μM) at 30 °C.

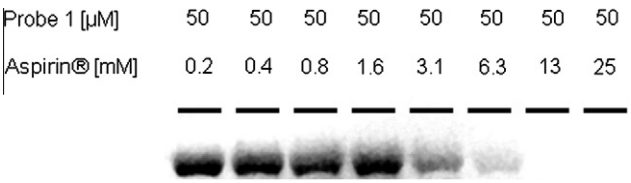

Figure 3. In-gel (8% SDS–PAGE) fluorescence showing loss of esterase activity of HSA (5 mg/mL) after co-incubation with aspirin at various concentrations of aspirin and probe **1** (50 μM).

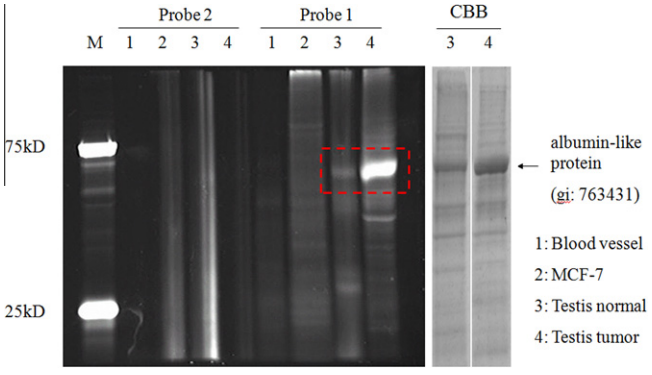

Figure 4. In-gel (12% SDS–PAGE) fluorescence (left) and Coomassie Brilliant Blue (CBB, right) gel staining of lysate samples of blood vessel cells, Michigan Cancer Foundation-7 (MCF-7) breast cancer cells, normal testicular cell lysate, and testicular tumor cell lysate after incubation with probes **1** and **2** (50 μM). A strong fluorescent band was identified as an albumin-like protein (gene identification [GI] number: 763431) by tandem-MS spectrometry (see SI).