Lanthanide Complex Strategy for Detection and Separation of Histidine-tagged Proteins

Hiroshi Tsukube,*[1] Keiko Yano,[1] Akito Ishida,[2] and Satoshi Shinoda[1]
[1]*Department of Chemistry, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585*
[2]*Department of Intelligence Science, Graduate School of Human and Environmental Science, Kyoto Prefectural University, Kyoto 606-8522*

(Received January 22, 2007; CL-070070; E-mail: tsukube@sci.osaka-cu.ac.jp)

A series of trivalent lanthanide complexes were immobilized on SPR sensory chips and protein separation membranes. They worked as alternatives to transition-metal complexes in detection and separation of histidine-tagged ubiquitin protein. Since the highly coordinated complex between NTA ligand, Eu^{3+} center, and hexahistidine moiety exhibited the enhanced luminescence, the present system provides a further possibility for use in in-situ optical protein imaging process.

Among numerous methodologies for selective detection, separation, purification, and conjugation of biological proteins and enzymes,[1] histidine-tagged protein technology has become a powerful and universal tool for reversible immobilization. In this technology, several transition-metal centers supported by nitrilotriacetic acid (NTA), iminodiacetic acid (IDA), or related ligand bound the oligohistidine array at N- or C-terminus of the targeted protein.[2] Since the histidine tags are readily introduced at protein termini using standard molecular biology procedures and because the protein functions are preserved by the terminal tagging, this is widely employed in surface plasmon resonance (SPR), chromatographic, and electrochemical protein analyses as well as self-assembled membrane and chip protein architectures. We demonstrate here that several lanthanide complexes are promising alternatives as protein-binding and signaling devices effective in the biosensing, surface coating, and protein architecture systems[3] (Figure 1). They provide largely different coordination chemistry from transition-metal complexes and work well in MRI imaging and luminescent labeling.[4] Although some of them further offered specific molecular recognition of bio-substrates,[5] we first apply a series of lanthanide complexes in SPR detection and membrane separation of the histidine-tagged protein.

The lanthanide complexes supported by NTA and IDA ligands have several outstanding properties as effective binding devices for the histidine-tagged proteins:[6] (1) They have high thermodynamic stability enough for use in aqueous media ($\log \beta_1 = 6.73$, $\log \beta_2 = 12.11$ for Eu^{3+}–IDA: $\log \beta_1 = 11.32$, $\log \beta_2 = 20.64$ for Eu^{3+}–NTA); (2) They exhibit large coordination numbers and rapid complex kinetics for effective oligohistidine binding; (3) They selectively form the highly coordinated complexes with external guests; and (4) Some of them give long-lived and line-shaped luminescence signals in response to coordination environments. Thus, they can work as alternative binding devices to transition-metal complexes and also as photosignaling sites for protein conjugation.

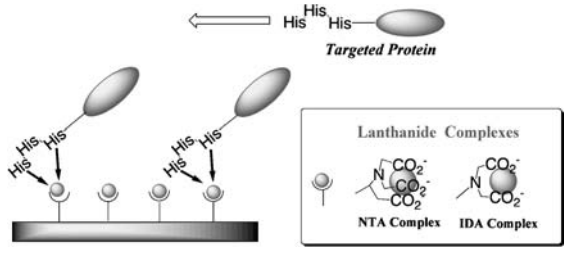

Figure 1. Lanthanide complex with NTA or IDA ligand in histidine-tagged protein detection and separation.

We carried out SPR experiments of histidine-tagged protein with Biacore J apparatus (see Supporting Information).[7] As established by transition-metal complexes,[8] lanthanide cations were loaded onto the NTA-attached dextran chip (Biacore Sensor Chip NTA), and the standard EDTA treatment was available to remove the bound protein and lanthanide cation. Hexahistidine-tagged ubiquitin was chosen as a substrate protein, because this is a relatively small protein (ca. 8500 Dalton), and provides a good indication of practical availability.[9]

Figure 2 illustrates typical SPR binding profiles of the histidine-tagged ubiquitin on the chips coated by NTA–Ni^{2+}, Zn^{2+}, La^{3+}, and Eu^{3+} complexes, which were recorded at pH 5.7. Considering $\log \beta$ values of NTA–Eu^{3+} complexes, such lanthanide complexation was thought to overcome the electrostatic binding between anionic NTA and protonated histidine. Indeed, the employed lanthanide complexes exhibited effective SPR sensorgrams, in which their binding abilities of histidine-tagged ubiquitin were comparable to those of transition-metal complexes. Similar SPR profiles were observed with $EuCl_3$ and $Eu(NO_3)_3$ salts. These observations clearly demonstrate that labile lanthanide complexes were applicable in the SPR detection. As concentration of the sample protein decreased, the recorded SPR amplitude decreased, indicating that the targeted protein

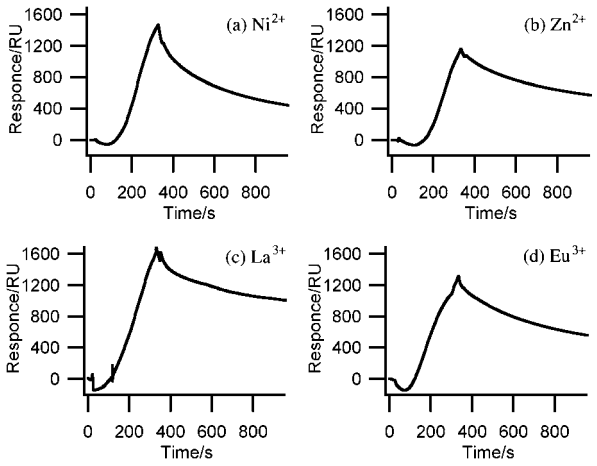

Figure 2. SPR sensorgrams with NTA–metal complexes: (a) Ni^{2+}, (b) Zn^{2+}, (c) La^{3+}, and (d) Eu^{3+}.

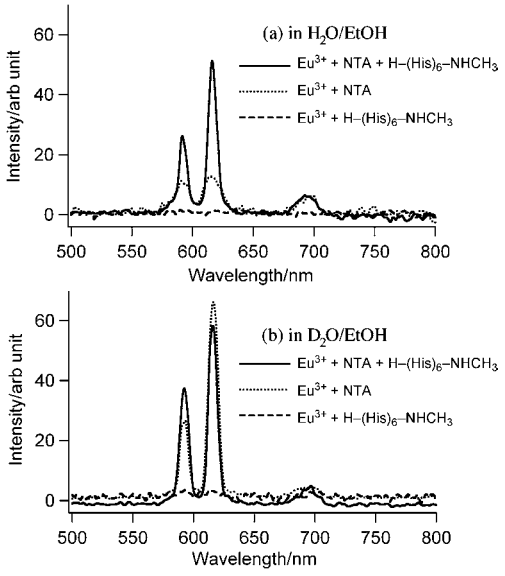

Figure 3. Enhanced europium luminescence upon ternary complexation with hexahistidine methylamide. (a) in H_2O/EtOH (1:4 v/v) and (b) in D_2O/EtOH (1:4 v/v). Conditions: $[NTA] = [Eu(OTf)_3] = 1.0 \times 10^{-4}$ M, $[H(His)_6NHCH_3] = 5.0 \times 10^{-4}$ M, 25 °C, $\lambda_{ex} = 230$ nm, delay 0.05 ms, gate 3.0 ms, slit widths 10 nm.

was detected at a μM level (see Figure S1).[7] When Na^+ and Ca^{2+} cations having similar ion sizes to the lanthanide cations were employed, no effective SPR detection was observed. Since non-tagged ubiquitin was rarely detected, the lanthanide complexes worked well in the histidine-tagged protein detection.

As reported earlier,[10] NTA–transition-metal complexes exhibited low affinities for hexahistidine derivatives ($K_d >$ 10 μM), in which two histidine units were thought to coordinate one NTA–metal center. The employed NTA–Eu^{3+} complex still had two or more coordination sites for external substrates.[6] Since the introduced histidine moiety was supported to give slight influence on the structure and function of the parent protein, the ternary complexation between NTA, Eu^{3+}, and hexahistidine methylamide was characterized (Figure 3). When hexahistidine methylamide was added to an equimolar mixture of NTA and $Eu(CF_3SO_3)_3$ in the H_2O/EtOH (pH 4.5, 1/4), irradiation at 230 nm gave larger luminescence signals around 600 nm than those observed with NTA–$Eu(CF_3SO_3)_3$ or hexahistidine methylamide–$Eu(CF_3SO_3)_3$ combination. Since the ternary system further gave different intensity ratio of luminescence signals at 595 and 613 nm from binary ones, these observations indicated the highly coordinated complexation between hexahistidine and NTA–Eu^{3+} complex. The luminescence experiments were also carried out in D_2O/EtOH (1/4) solutions. Although the NTA–$Eu(CF_3SO_3)_3$ complex exhibited the enhanced luminescence due to the coordinated D_2O, the ternary complex system containing hexahistidine guest exhibited similar luminescence intensities in both solvents. Thus, the vacant sites of the NTA–Eu^{3+} center were completely occupied by hexahistidine guest.

We further characterized the IDA–lanthanide complexes immobilized on the filter membrane (Vivascience, Vivapure Metal Chelate Mini Spin Column) in the separation of histidine-tagged ubiquitin. After an aqueous solution of the histidine-tagged ubiquitin (50 μM) was passed through the metal complex-immobilized membrane, the targeted protein in the filtrate was rarely detected by SPR method (<0.3 μM). Although the IDA–La^{3+}, Eu^{3+}, and Tb^{3+} complexes were less stable than NTA complexes, they exhibited similar abilities to that with the IDA–Zn^{2+} complex system. Therefore, the lanthanide complex provides the selective recognition of histidine-tagged protein and protein fabrication based on unique complexation and luminescence characteristics.

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7 Supporting Information includes details of SPR and membrane experiments: see CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
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