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Detection of protein by the use of photoluminescence in rare earth-protein-SDBS system

T.Arakawa, Y.Konishi, S.Takoshima
Department of Biological and Environmental Chemistry, Faculty of Humanity-Oriented Science and Engineering, Kinki University, Iizuka, 820-8555, Japan

Abstract

The fluorescence enhancement effect in rare earth-protein-sodium dodecyl benzenesulfonate (SDBS) system has been studied. Tb^{3+}-SDBS-protein system showed a very weak fluorescence, while addition of Gd^{3+}or ethanol to this system significantly enhanced the fluorescence, especially in collagen-containining system. This new method made it possible to determine proteins of ng/ml level.

Keywords: photoluminescence, rare earth, protein

Introduction

Rare earth-fluorescence-probe technique has been applied to the characterization of many inorganic compounds, the availability in the molecular recognition and chirality sensing of biological substances [1, 2]. Recently, many studies have been reported focusing on the detection of proteins [3] that plays an important role in the life science. Since the fluorescence emitted from original protein is weak, the new fluorescence methods is required in order to analyze the protein. A fluorescence enhancement effect in the rare earth-protein system has been studied by Sun *et al.* [4]. In this paper, we present a new co-luminescence system that is effective to detect proteins of ng level.

Experimental

Protein (BSA, elastin and collagen), $TbCl_3$ $6H_2O$,$GdCl_3$ $6H_2O$ and sodium dodecyl benzene sulfonate (SDBS) were purchased from Wako Pure Chem. Ind., Ltd. *Tris*-HCl buffer of 5.00mol/l was prepared with 0.606g of Tris reagent and 100ml of deionized water by adjusting pH to 7.0 with HCl. *Tris*-HCl, SDBS, Tb^{3+}, Gd^{3+}, protein solutions were added in a 10ml test tube. The mixture was diluted to 7.74ml with deionized water and was shaken for 10 seconds. Emission spectra were measured with a HITACHI recording absolute spectrofluorophotometer (F-4500) at room temperature. Ultraviolet absorption spectra were recorded on a JASCO V-550 UV spectrophotometer.

Results and discussion

The emission spectra under the excitation at 290 nm of Tb-SDBS (1), Tb-protein (2), Tb-SDBS-protein (3), and Tb-Gd-SDBS-protein (4) systems have shown in Fig. 1. The emission peak at 545 nm is attributed to the $^5D_4$-$^7F_5$ transition of Tb^{3+}. It was found that the fluorescence of Tb-SDBS-protein system was weak. However, the fluorescence intensity of the system is greatly enhanced by the addition of Gd^{3+}.The optimum conditions for the determination of protein are as follows; 1.0 x 10^{-6} g/ml protein, 5.0 x 10^{-3} M *Tris*-HCl, 1.0 x 10^{-4}M SDBS, 1.0 x 10^{-5} M Tb^{3+} and 1.5 x 10^{-4} M Gd^{3+}.

UV spectra showed that the peak of BSA observed at 200 nm decreased and shifted to higher wavelength (ca.215nm) when SDBS was added in succession. Since the peak observed at 200 nm reflects the framework conformation of the protein[5], the change of UV spectra observed after addition of SDBS indicates that the binding between SDBS and protein molecules would lead to substantial changes of the protein conformation. Although both BSA and SDBS are negatively charged in the

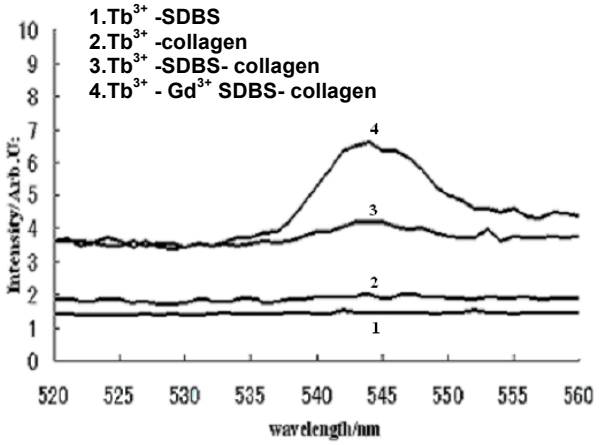

Fig. 1 Fluorescence spectra of Tb-SDBS-protein system.

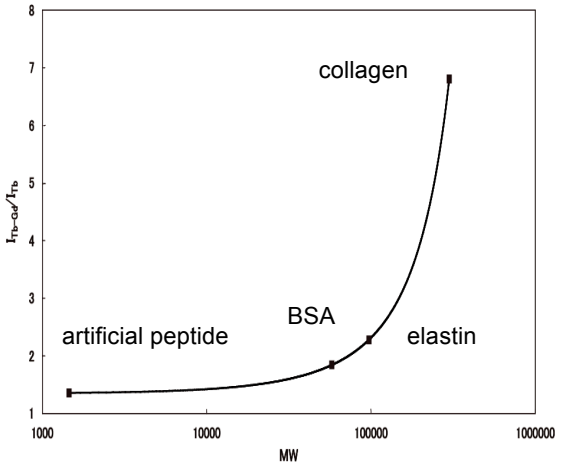

Fig.2 Dependence of the relative emission intensity of Tb^{3+} on the molecular weight of protein. The emission intensity for Tb-SDBS-protein system is 1.

solution of pH 7.0, there will be a variety of physicochemical interaction. Previous studies[6] showed that the one of the interaction would be the results of aromatic ring stacking between SDBS and residues of tryptophan, tyrosine or phenylalanine residues in BSA. There is a difference in the enhancement of the fluorescence among four proteins as shown in Fig.2 This effect was increased along the molecular weight of the protein and was the most remarkable in the case of collagen. Tb^{3+} can bind with the negatively charged amino acid residue in the protein and form the BSA-Tb^{3+} associate. That is, it is clear that the number of amino acid residue is dependent on the kind of the proteins.

Under the experimental conditions, Tb^{3+}-BSA-SDBS solution remained transparent. However, when Gd^{3+} was added into the system, the solution became opaque like diluted milk. This means that the larger complex particles were formed. Tb^{3+}or Gd^{3+} could bind with the negatively charged amino acid residues in protein and formed the complex with BSA-SDBS as described in elsewhere[4].

Figure 3 shows a Gd^{3+} concentration dependence of the fluorescence enhancement effect. Gd^{3+} concentrations is expressed by the relative value to the Tb^{3+} concentartion of 1.0 x 10^{-5} M. The enhancement effect was maximum when Gd^{3+}/ Tb^{3+}=10 and decreased with an increase of Gd^{3+} concentration. Since the concentration of Gd^{3+} was higher than that of Tb^{3+} we can consider that the self-aggregation of SDBS shortened the distance between these two ternary complexes, *i.e*, there exist large numbers of Gd^{3+} complexes around Tb^{3+}complexes. However, the fluorescence intensity of Tb^{3+} showed a decrease with the concentration of Gd^{3+} when the Gd^{3+}/Tb^{3+} ratio exceeded 10. Since the characteristic emission of in the GD-SDBS-BSA system was not observed under the present experimental condition, we can consider that excess Gd-SDBS-BSA complex might inhibit the efficincy of energy transfer of Gd-Tb-SDBS-BSA complex, as also described above.

When BSA or SDBS was excited at 290 nm, the emission peak of BSA and SDBS shifted to *ca.* 340 nm. Next, when Tb^{3+} was added to BSA or SDBS, the fluorescence of 340 nm was decreased. On the other hand, when Tb^{3+} was added to the binary system of BSA-SDBS, the fluorescence peak of Tb^{3+} was observed as shown in Fig.1. In the binary complexes of BSA-Tb and SDBS-Tb , the energy transfer efficiency will be low and, while in the ternary

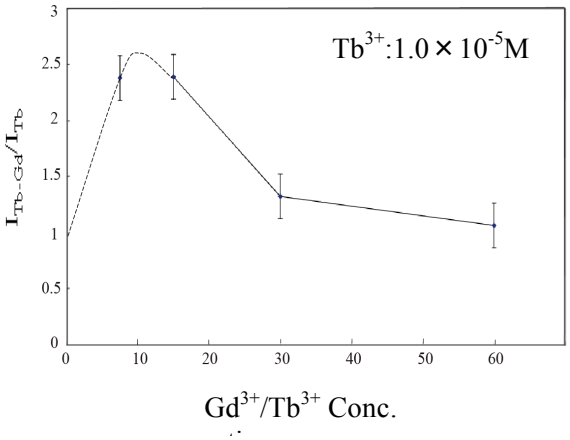

Fig.3 Dependence of the relative intensity of Tb^{3+} for Tb-Gd-SDBS-BSA system on the concentration ratio of Gd^{3+}/Tb^{3+}. The concenntration of Tb^{3+} is 1.0 x 10^{-5} M.

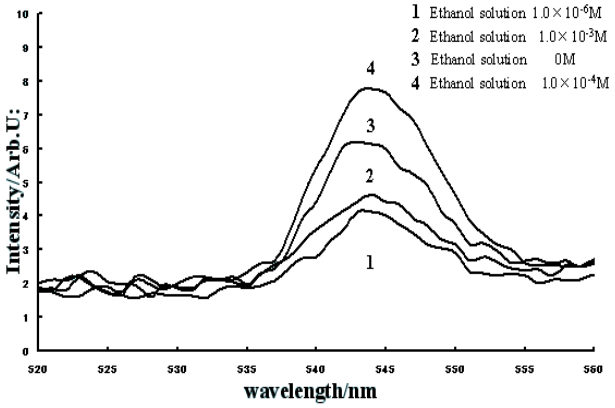

Fig.4 Fluorescence spectra of Tb-Gd-ethanol-SDBS-protein system.

complex of Tb-SDBS-BSA, the energy transfer between any two substances will be more efficient than that in the relevant binary complex. Hence, we can consider that Tb^{3+} would combine with SDBS and that Tb^{3+} would be bound to BSA through SDBS.

The fluorescence intensity in Tb-Gd-SDBS-protein is enhanced by adding a small anount($1.0x10^{-4}$ M) of ethanol, as illustrated in Fig.4. Many proteins are labile and readily modified by changing pH, heating, *etc*. The fluorescence was first observed in Tb-SDBS-protein system (Fig.1 (2)). This will be due to a denatured conformation of protein That is, the polarity of the microenvironment around the protein is changed and the addition of SDBS can stabilize the unfolded structure of protein.

Conclusions

In this work, it was found that Tb^{3+} could combine with protein and SDBS, and emitted the characteristic fluorescence of Tb^{3+}. When Gd^{3+} or ethanol was added into this system, the fluorescence is significantly enhanced. These phenomena would be correlated to the denatured conformation of protein and hence the present method will be applied to the detection of protein at nanogram level.

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