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Evaluation of two lanthanide complexes for qualitative and quantitative analysis of target proteins via partial least squares analysis

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Abstract

Two lanthanide complexes, namely 5-aminosalicylic acid ethylenediaminetetraacetate europium(III) (5As-EDTA-Eu^{3+}) and 4-aminosalicylic acid ethylenediaminetetraacetate terbium(III), were evaluated for the analysis of carbonic anhydrase, human serum albumin (HSA), and γ-globulin. Quantitative analysis is based on their luminescence enhancement upon protein binding and qualitative analysis on their lifetime capability to recognize the binding protein. Analytical figures of merit are presented for the three proteins. The limits of detection with 5As-EDTA-Eu^{3+} are at the parts per billion level. Partial least square regression analysis is used to determine HSA and γ-globulin in binary mixtures without previous separation at the concentration ranges typically found in clinical tests of human blood serum.

Keywords: Luminescence; Lanthanide ions; Carbonic anhydrase; γ-Globulin; Human serum albumin; Partial least squares regression; Proteins

The development of methodology for protein recognition and quantification in complex biological matrices has long been an analytical challenge. The limitations of popular clinical and laboratory tests have been extensively discussed in the literature [1]. The Lowry [2] and the Bradford [3,4] methods, which determine total protein content, lack selectivity for specific protein determination. Spectrophotometric [5,6], chemiluminescence [7,8], fluorimetric [9,10] and resonance light scattering [11,12] methods have shown distinct improvement over classical assays. Their main advantages include accuracy of analysis and better limits of detection. Their selectivity, however, still falls short for the problem at hand. Other trends, which combine mass spectrometry to liquid chromatography [13,14] or electrophoresis [15,16], provide excellent selectivity and sensitivity but require elaborate sample separation prior to protein determination.

Selectivity improvements for direct protein determination have been recently reported with synchronous fluorescence [1] and near-infrared [17,18] spectroscopy. The fluorescence assay [1] relies on chemical interactions among targeted proteins and functionalized nanoparticles. The spectral response of a fluorescence tag chemically attached to nanoparticles is monitored via synchronous excitation to extract both qualitative and quantitative information. The near-infrared approach [17,18] takes advantage of vibrationally resolved spectra with fingerprint information for protein identification.

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Because near-infrared absorption bands are inherently weak, spectral assignment is made possible with the aid of chemometric methods that minimize spectral interference from instrumental noise and sample concomitants.

Our approach focuses on chemical receptors with the potential to recognize specific proteins in complex samples. We incorporate lanthanide ions into polymerized liposomes that offer a lipophilic platform for protein interaction with the lanthanide ion [19,20]. The specificity of our approach relies on the capability of the polymerized liposome to recognize a specific protein in the complex sample. The expectation from the lanthanide ion is to report qualitative and quantitative information on the interacting protein(s), even if the target protein(s) is at much lower concentration than sample concomitants. In this article, we present a thorough investigation of two lanthanide complexes and their analytical potential as luminescence reporters for liposome–protein interaction. We first describe their synthetic preparation and then evaluate their quantitative and qualitative performance for three targeted proteins, namely carbonic anhydrase (CA),[2] human serum albumin (HSA), and γ-globulin. With the aid of partial least squares (PLS) regression, we demonstrate the feasibility to determine HSA and γ-globulin in binary mixtures at the concentration levels typically found in human blood tests.

Experimental

Instrumentation

Preliminary collection of excitation and emission spectra was carried out with a commercial spectrofluorimeter (Photon Technology International). For steady state (SS) measurements, the excitation source was a continuous-wave 75-W xenon lamp with broadband illumination from 200 to 2000 nm. Detection was made with a photomultiplier tube (PMT; Model 1527) with wavelength range from 185 to 650 nm. The method of detection was analog for high signal levels or photon counting for low signal levels. In analog mode, the inherent peak to peak noise was 50×10^{-12} A with a 0.05-ms time constant. In photon counting mode, the maximum count rate was 4 MHz, pulse pair resolution was 250 ns, the rise time was 20 ns, and the fall time was 100 ns with a 220-ns pulse width. For time-resolved (TR) measurements, the excitation source was a pulsed 75-W xenon lamp (wavelength range from 200 to 2000 nm) with a variable repetition rate from 0 to 100 pulses per second and a pulse width of approximately 3 μs. Detection was by means of a gated analog PMT (Model R928) with extended wavelength range from 185 to 900 nm. SS and TR spectra were collected with excitation and emission monochromators having the same reciprocal linear dispersion (4 nm mm^{-1}) and accuracy (±1 nm with 0.25-nm resolution). Their 1200-grooves/mm gratings were blazed at 300 and 400 nm, respectively. The instrument was computer controlled using commercial software (Felix32) specifically designed for the system. Spectra were not corrected for instrumental response. Wavelength reproducibility was approximately ±2 nm.

Luminescence lifetimes were measured with an instrumental setup mounted in our laboratory [21]. Samples were excited by directing the output of a Northern Lights tunable dye laser (Dakota Technologies) through a KDP frequency-doubling crystal. The dye laser was operated on LDS 698 (Exiton) and pumped with the second harmonic of a 10-Hz Nd:YAG Q-switched solid-state laser (Big Sky Laser Technologies). Luminescence was detected with a multichannel detector consisting of a front-illuminated intensified-charge fiber-coupled device (ICCD; Andor Technology). The minimum gate time (full width at half maximum) of the intensifier was 2 ns. The CCD had the following specifications: active area = 690 × 256 pixels (26 mm^2 pixel size photocathode), dark current = 0.002 electrons/pixel/s, and readout noise = 4 electrons at 20 kHz. The ICCD was mounted at the exit focal plane of a spectrograph (SPEX 270M) equipped with a 1200-grooves/mm grating blazed at 500 nm. The system was used in the external trigger mode. The gating parameters (gate delay, gate width, and the gate step) were controlled with a digital delay generator (DG535; Stanford Research Systems) via a GPIB interface. Custom LabView software (National Instruments) was developed in-house for complete instrumental control and data collection. This system was also used to collect wavelength–time matrices for the analysis of target proteins in binary mixtures.

Reagents

All reagents and solvents were purchased from commercial suppliers and used without further purification. Nanopure water was used throughout. The organic solvents used in the synthesis were of HPLC grade. Anhydrous solvents were obtained by distillation of the HPLC-grade solvents over CaH_2.

Synthesis of the complexes

The syntheses of the complexes use the selectively hydrolyzed EDTA ester **1** [22]. The carboxylic acid

[2] *Abbreviations used:* CA, carbonic anhydrase; HSA, human serum albumin; PLS, partial least squares; SS, steady state; PMT, photomultiplier tube; TR, time-resolved; ICCD, intensified-charge fiber-coupled device; CCD, charge-coupled device; DCC, dicyclohexyl carbodiimide; NHS, *N*-hydroxysuccinimide; DMF, dimethylformamide; THF, tetrahydrofuran; As, aminosalicyclic acid; LOD, limit of detection; LDR, liner dynamic range; RSD, relative standard deviation.