Fluorescent Ag-Nanoclusters for Evaluation of Serum Albumin and Immunoglobulin Content in Protein Mixtures

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Abstract. In this work we designed, synthesized and characterized luminescent metal nanoclusters (NCs) on human serum albumin (HSA) and immunoglobulins (Ig). We demonstrate that the approach developed allows one to determine the relative content of albumins and immunoglobulins in biologically relevant protein mixtures based on the luminescent properties of the NCs. Fast and inexpensive approach which allows to determine concentrations of immunoglobulins (Ig) and serum albumin (HSA) in blood serum might be useful in clinical diagnostics.

1. Introduction

Immunoglobulins and serum albumin are two of the most abundant types of proteins in human blood. Albumin performs transport function for the variety of substances in the blood stream, including metal ions, drugs, hormones and vitamins. Normal concentration of human serum albumin (HSA) ranges from 35 to 50 g/l. Among the different types of globulins, there is a group of proteins known as immunoglobulins, representing one of the key elements of the immune system. There are some types of immunoglobulins: IgG, IgA, IgM, IgE and IgD. Each molecule of IgG, also known as antibodies, is composed of two larger (heavy) polypeptide chains and two smaller (light) chains. IgG comprise approximately 75% of all immunoglobulins in serum, while the rest 25% are mainly IgA and IgM [1]. In case of some blood diseases, such as multiple myeloma, the concentration of Ig may increase significantly, which makes it very important to have cheap and accurate approach for monitoring concentrations of the serum proteins.

The aim of this work was to develop a convenient, selective, cheap and very simple luminescent nanosensor for albumin and immunoglobulin detection. We used silver nanoclusters (AgNCs) as luminescent bio-labels complexed with albumins and immunoglobulins. In the last decade, metal nanoclusters attract a growing interest in chemistry, biology and material science due to their unique properties [2–4]. Fluorescent AgNCs are the brightest ones in the class, exhibiting large absorption
cross-section and high quantum yield \([5,6]\) and have been successfully synthesized on various proteins both intra- and extracellular \([7,8]\).

In our previous studies we have demonstrated that the complexes of AgNCs with different proteins exhibit different spectral and structural properties \([9]\). Here we propose an experimental approach to detect albumin and immunoglobulins in biologically relevant mixtures.

2. Materials and Methods

All human proteins were purchased in form of medical solutions for infusion. Human serum albumin (HSA, 100 g/l) and Human Immunoglobulin G (IgG, 50 g/l) were obtained from Microgen (Russia). Sodium hydroxide (NaOH), silver nitrate (AgNO3) and sodium borohydride (NaBH4) were purchased from Sigma-Aldrich. Rhodamine 6G (R6G) was supplied by Lambda Physik.

The blood samples studied in this work were taken from multiple myeloma patients of the hematology clinic of the Russian Scientific Research Institute of Hematology and Transfusiology (St. Petersburg, Russia). S-Monovette tubes (Sarstedt, Germany) with coagulation activator were used to obtain BS samples. The collected blood samples were left in test tubes for 20-30 minutes at room temperature (18-24 °C). Prior to use all samples were centrifuged at 3 000 rpm for 15 min. on a Heraeus Labofuge 200 centrifuge (Thermo Scientific, United States). Before physicochemical studies, the samples were frozen and stored at a temperature of −30 °C.

Fluorescence emission and excitation spectra were obtained at room temperature using a RF-6000 spectrofluorimeter (Shimadzu). The measurements were carried out in a 0.4 cm quartz cell (Hellma Analytics). Long-wave pass filters were used to remove scattered light. The fluorescence emission spectra were corrected for instrument sensitivity. The fluorescence excitation spectra were corrected for the inner filter effect due to the high absorbance of the samples in the UV range. The bandpass for excitation and emission was set at 5 nm. Absorption spectra were obtained with a Specord 210 Plus double-beam spectrophotometer (Analytik Jena).

3. Results and discussion

In this work, we synthesized AgNCs stabilized by human serum albumin (HSA) and IgG matrices. It should be noted, that different matrices provided growth of clusters with different spectral properties. Thus, AgNCs stabilized by HSA emitted in the red region of the visible spectrum (ca. ~1.85 eV), whereas IgG-stabilized AgNCs had the emission maximum in the near IR region (ca. ~1.6 eV). The luminescence excitation and emission spectra of the obtained complexes are presented in Figure 1.
Figure 1 (a, b). Normalized excitation (a) and emission (b) spectra of AgNCs on HAS and IgG matrices.

Based on our previous experience [10], we optimized the synthesis conditions, which allowed us to improve the chemical yield of the NCs and, as a consequence, the sensitivity and accuracy of the developed method in this study. Parameters of the synthesis of AgNCs were varied independently for both HSA and Ig. The main varied parameters were \([\text{AgNO}_3]/[\text{protein}]\) and \([\text{NaBH}_4]/[\text{AgNO}_3]\) concentration ratios. The results are presented in Table 1.

| \([\text{NaBH}_4]/[\text{AgNO}_3]\) | \([\text{AgNO}_3]/[\text{HSA}]\) | \([\text{AgNO}_3]/[\text{IgG}]\) |
|---|---|---|
| 0,1 | 0,02 0,73 0,89 0,68 0,50 | 0,12 0,29 0,28 0,12 |
| 0,5 | 0,06 0,80 1,00 0,81 0,64 | 0,37 0,90 0,74 0,44 |
| 1 | 0,02 0,68 0,81 0,78 0,58 | 0,50 1,00 0,61 0,41 |
| 1,5 | 0,11 0,33 0,31 0,27 0,27 | 0,25 0,85 0,33 0,25 |

Afterward, we applied the optimal synthesis protocol to obtain luminescent NCs using human serum (Figure 2).

Figure 2. Normalized emission spectrum of the complexes obtained in the serums.

To perform further analysis, we modified the approach developed earlier for the HAS/Ig mixtures [10], which allowed us to estimate the relative content of HSA and Ig in the samples. We resolved the obtained emission spectra of the serum samples in two bands approximated by asymmetric Gauss-like curves (equation (1) corresponding to the HSA and Ig contributions:}
\[ y(x) = A \left( 1 - \frac{1}{1 + \exp \left( -\frac{x - x_c}{w_3} \right)} \right) + \frac{1}{1 + \exp \left( -\frac{x - x_c + w_1/2}{w_2} \right)} \]  

where \( A \) – the amplitude, \( x_c \) – the maximum position, \( w_{1,2,3} \) – the asymmetry coefficients. We determined the asymmetry coefficients independently for both HSA and Ig, and then fixed them during fitting.

Figure 3(a) shows the emission spectrum of a healthy donor (HD) serum resolved into two bands approximated by equation (1). The asymmetry coefficients of each component were determined from the spectra of the individual components and were fixed, while amplitude and maximum position were varied. The integral percentage for each component was determined (Table 2). In contrast to the spectra of HD, the approximation of the spectrum of a multiple myeloma (MM) patient by two bands (Figure 3(b)) gives a clear preponderance of the Ig fraction compared to the HSA. Decompositions of all studied serum samples are presented in Table 2.

![Figure 3](image)

**Figure 3 (a, b).** Decomposition of the normalized emission spectrum of a HD (a) and of a MM patient (b) serums.

**Table 2 - Data obtained from serum donors.**

| Serum number | HSA component maximum, eV | Ig component maximum, eV | HSA component integral value, % | Ig component integral value, % | Type of the sample |
|--------------|----------------------------|--------------------------|---------------------------------|---------------------------------|-------------------|
| 1            | 1.89                       | 1.62                     | 72                              | 28                              | HD                |
| 2            | 1.88                       | 1.62                     | 72                              | 28                              | HD                |
| 3            | 1.86                       | 1.61                     | 67                              | 33                              | HD                |
| 4            | 1.76                       | 1.61                     | 53                              | 47                              | MM                |
| 5            | 1.79                       | 1.61                     | 58                              | 42                              | MM                |
| 6            | 1.80                       | 1.61                     | 62                              | 38                              | MM                |
4. Summary

We have synthesized the luminescent complexes of AgNCs with human serum proteins, HSA and Ig, in the samples of blood serum. Based on the earlier established fact that the luminescence spectra of the AgNCs complexes with HSA and Ig differ from each other, we have also determined the contributions of HSA and Ig to the emission spectra. The approach developed shows the possibility of measuring the relative content of the two major protein fractions (serum albumin and immunoglobulins) in blood serum. The preliminary results obtained also demonstrate a significant difference in the relative content of the proteins in the serum of MM patients and healthy donors, which might be useful for the preliminary sample discrimination. In general, from our point of view, the approach can be further developed for the fast and also inexpensive HSA/Ig assay of the serum samples in one single experiment.

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