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Homogeneous immunoassay for soy protein determination in food samples using gold nanoparticles as labels and light scattering detection

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ABSTRACT

A homogeneous aggregation immunoassay involving the use of gold nanoparticles (AuNPs) and light scattering detection is described for soy protein determination in food samples. AuNPs act as enhancers of the precipitate that appears when the antigen–antibody complex is formed. The AuNPs-antibody conjugate has been synthesized by physical adsorption of polyclonal anti-soy protein antibodies onto the surface of commercial AuNPs with a nominal diameter of 20 nm. The direct assay is based on the reaction of the conjugate with soy protein, which reaches the equilibrium in about 10 min, and the measurement of the light scattering intensity at 530 nm, which is proportional to the analyte concentration. The dynamic range of the calibration graph is 0.2–20 $\mu g\,mL^{-1}$ and the detection limit value is 65 $ng\,mL^{-1}$. The precision, expressed as relative standard deviation, has been assayed at two different concentrations, 0.2 and 1 $\mu g\,mL^{-1}$, giving values ranging from 4.7 to 5.9%. The interference of other proteins has been assayed. The usefulness of this method has been shown by its application to the analysis of fruit juice and "nonmilk yoghourt" samples. The results obtained with the proposed method are similar to those obtained by using a commercial ELISA kit, but the assay time is significantly shorter and the detection limit was about 10 times lower. A recovery study has been also performed, giving values in the range of 84.0–119.3%.

1. Introduction

The use of materials at the nanometric scale as labels in bioassays has exponentially increased in the last years due to their special features, which are dependant on the size and composition, among other factors. Gold nanoparticles (AuNPs) have been widely used for the development of bioassays with optical and electrochemical detection [1,2]. These nanoparticles present some optical features different from those exhibited by the bulk metal, such as a plasmon absorption band and surface-enhanced Raman scattering (SERS). The absorbing properties of the plasmon band, which are associated with collective electron oscillations, can be used to detect changes in the surrounding medium. An immunosensor based on the changes in the resonance enhanced absorption (REA) of anti-β-lactoglobulin antibodies conjugated to gold clusters by their binding to β-lactoglobulin has been developed to detect this allergen in processed milk matrices [3].

Although AuNPs are coloured and could be used for the development of colorimetric assays, the relatively low sensitivity obtained justifies the use of other detection systems, such as Raman or Rayleigh scattering detection. These particles have been recently used to develop immunoassays with SERS as detection system for the determination of immunoglobulin G (IgG) [4,5] as model analyte. Also, AuNPs have been proposed as enhancers for the development of aggregation-based immunoassays for the determination of antibodies in serum samples [6–8]. The selectivity of these assays is given by the immunochemical reaction. While AuNPs have found a wide application for clinical and biochemical purposes, their use as labels in immunoassays for food or environmental analysis, which often involve samples with complex matrices, has been very limited up to date.

Soy proteins are a group of proteins that are extracted in aqueous solution from soybeans [9]. These proteins are used as additives in some foods, such as baked and meat products, and some beverages, such as fruit juices to raise their protein levels and to improve some mechanical properties. Water binding capacity, the stabilisation of o/w emulsions and the solubility of some beverage ingredients are improved after the addition of soy proteins. Several soy protein products can be obtained from the soybean processing, which usually involves at the first stage the chopping and defatting of soy beans to obtain defatted soy bean flakes. The extraction of soy protein from these flakes can give rise to different products. For instance, protein concentrates are obtained after soaking defatted soybean flakes and then, after centrifuging, the supernatant taken is the soybean concentrate. This concentrate can be further incubated with protease to give rise to modified soybean protein.

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Another product, called soybean protein isolate, is produced after the extraction of defatted soybean flakes in a soft alkaline medium at pH 8.0–9.0, the further centrifugation of the suspension and the acidification of the supernatant at pH 4.0–5.0 to obtain the soy protein. The protein content of concentrate and isolate are 65–90% and >90%, respectively [10].

Soy proteins have been characterized and determined using electrophoretical techniques [10–12], liquid chromatography–mass spectrometry [13] and immunochemical [10,14–19] methods. The latter methods are mainly based on the use of enzyme-linked immunosorbent assays (ELISA) and there are commercial kits available [20]. However, the high number of incubation and washing steps required in these heterogeneous assays, have given rise to the development of faster alternatives. One of them is a surface plasmon resonance biosensor for the determination of nonmilk proteins in milk powder, which involves the immobilization of the antibodies on the sensor surface and the direct detection of the binding of the proteins [18]. Also, an automated fluorescent microsphere-based flow cytometric triplex immunoassay has been described for the detection of soy, pea and soluble wheat proteins as potential fraudulent adulterants in milk powder [19].

The aim of the research presented here was the development of a homogeneous assay to determine soy protein in food samples, alternative to the heterogeneous assays described for this purpose. Homogeneous assays have some desirable features such as expeditiousness, since multiple washing and incubation steps are avoided, and ease of automation, what increases the sample throughput of conventional assays. However, a limitation of these assays is that the detection limits obtained are usually higher than those achieved using the heterogeneous format, owing to the higher background signal and the direct influence of sample matrix. Thus, the sensitivity of the detection systems used has to be enough to compensate this shortcoming.

As it is known, macromolecular antigens form insoluble complexes in their binding to their specific antibodies. This phenomenon has been used traditionally in agglutination assays, which usually involve the use of latex particles as enhancers of the aggregation. However, the micrometer size of these particles gives rise to a relatively high background signal. As indicated above, AuNPs have been also used as aggregation enhancers for the determination of antibodies by measuring the change in absorbance [6,7] or in light scattering [8].

The method presented here is the first homogeneous immunoassay described for the determination of soy proteins in food samples. It is based on the increase in the light scattering intensity of a conjugate formed by anti-soy protein and AuNPs in the presence of soy proteins after an incubation time of 10 min. The measurements are obtained at 530 nm, which is close to the absorption band of AuNPs, with the aim of monitoring the changes in the surrounding environment of these particles by the formation of the antibody–antigen complex. The proposed analytical method has been applied to the analysis of fruit juice and "nonmilk yoghourt" samples and the results obtained have been compared to those provided by a commercial ELISA kit, giving both similar values, but obtaining the analytical results in a shorter time with the proposed method. Also, the detection limit using the proposed method is about 10 times lower than that afforded by the commercial ELISA kit.

2. Experimental

2.1. Instrumentation

An SLM Aminco Bowman (Urbana, IL, USA) Model 8100 photon-counting spectrofluorimeter, equipped with a 450 xenon arc source and a R928 photomultiplier tube, was used to perform light scattering intensity measurements. Excitation and emission wavelengths were set at 530 nm and the excitation and emission slits were adjusted to provide 4-nm band-passes. A 1-cm path-light quartz cell and a conventional cell compartment were used. All the measurements were carried out at room temperature.

2.2. Reagents

All chemicals used were of analytical grade. Polyclonal anti-soy protein antibodies raised in rabbits (Sigma–Aldrich, St. Louis, MO, USA) were used. Colloidal AuNPs (20 nm, ca. 0.01% $HAuCl_4$) used to synthesize the tracer were also supplied by Sigma. A carbonate buffer solution (0.05 M, pH 9.55) was prepared in distilled water and used to prepare the solutions involved in synthesis of antibody-AuNP conjugate and to perform the extraction of soy protein from food samples. An antibody stock solution of 1000 $\mu g\,mL^{-1}$ was prepared in this buffer solution. The soy protein isolate, PROTEINA SUPRO 500E, was kindly gifted by DOSCADESA (Murcia, Spain). A stock solution of 140 $\mu g\,mL^{-1}$ soy protein was prepared by dissolving the commercial product in carbonate buffer (0.05 M, pH 9.55) by heating at 60 °C. This solution was stable for at least a month when stored at 4 °C. Intermediate and working solutions were prepared by dilution of the stock solution in carbonate buffer (0.05 M, pH 8.8). A commercial ELISA kit (ELISA SYSTEMS, Windsor, Australia) for the determination of soy protein was used for comparative purposes.

2.3. Procedures

2.3.1. Synthesis of gold nanoparticle conjugated antibody

The colloidal gold labelled antibody was obtained by mixing 0.5 mL of colloidal AuNPs with 0.6 mL of polyclonal anti-soy protein antibody stock solution and diluting to 10 mL with carbonate buffer (0.05 M, pH 9.55). The solution was allowed to stand at room temperature for 1 h to achieve the adsorption of the antibody onto the AuNPs through a combination of ionic and hydrophobic interactions. The mixture was then centrifuged at 9000 rpm for 30 min, obtaining two phases: a clear supernatant and a pellet of the conjugate. The supernatant solution was discarded and the soft sediment was resuspended in 10 mL of carbonate buffer (0.05 M, pH 9.55) solution and stored at 4° C.

The conjugate concentration was determined by measuring the absorbance at 280 nm, which is the maximum absorption wavelength of IgG. The same synthesis procedure performed in the absence of the antibody was used to subtract the blank signal.

2.3.2. Determination of soy protein

Solutions containing the conjugate of anti-soy protein antibodies (0.3 nM) and soy protein standards or sample extracts, at final concentrations in the range of 0.2–20 $\mu g\,mL^{-1}$, were prepared in a final volume of 2 mL in carbonate buffer (0.05 M, pH 8.8) and incubated at room temperature for 10 min. Then, the light scattering intensity of the solutions was measured at 530 nm. The relative light scattering intensity (B) was used as the analytical parameter.

2.3.3. Analysis of fruit juice and soy yoghourt samples

Samples were mixed up to achieve a higher degree of uniformity. Fruit juice (5.0 g) and yoghourt (2.0 g) samples were extracted for 15 min with 40 mL of carbonate buffer (0.05 M, pH 9.55) solution at 60 °C, centrifuged at 3000 rpm for 15 min and the supernatant was taken. The solid residue was resuspended and extracted again with the same volume of this buffer solution, being the two supernatants combined and diluted to 100 mL with the same buffer. A volume (15 μL) of this solution was treated following the procedure above indicated for the determination of soy protein.