Bioscience Reports, Vol. 22, Nos. 5 and 6, October and December 2002 (© 2002)

Spectroimmunochemistry Using Colloidal Gold Bioconjugates

Alexander A. Kamnev,[1,3] Lev A. Dykman,[1] Petros A. Tarantilis[2] and Moschos G. Polissiou[2]

Received August 14, 2002

Using surface-enhanced infrared absorption (SEIRA) spectroscopy of dry films of colloidal gold (CG) bioconjugates with protein A, it is shown that certain characteristic bands of the protein (e.g., amide I, amide II and some other vibration modes) are essentially affected by the metal surface. Thus, the method may be used for controlling the quality of such bioconjugates. Moreover, it is demonstrated that the biospecific reaction of protein A attached to CG particles with human immunoglobulin G (IgG) results in further essential changes in SEIRA spectra, providing a means for an easy and rapid IR spectroscopic detection of biospecific immunochemical interactions (i.e., spectroimmunochemistry). The results obtained can form a basis for developing test systems for detecting various biospecific interactions.

KEY WORDS: Colloidal gold bioconjugates; protein A; human immunoglobulin G; Fourier transform infrared spectroscopy (FTIR); Surface-enhanced infrared absorption (SEIRA); Spectroimmunochemistry.

ABBREVIATIONS: ATR, attenuated total reflectance; CG, colloidal gold; FT, Fourier transform; FTIR, Fourier transform infrared; IgG, human immunoglobulin G; IR, infrared; SEIRA, surface-enhanced infrared absorption.

INTRODUCTION

Among a variety of modern instrumental methods, vibrational [i.e., infrared (IR) and Raman] spectroscopic techniques, greatly reinforced by using Fourier transform (FT) spectrometers, have proved to be valuable, highly specific and informative tools for characterising diverse biological objects, ranging from small biomolecules to supramolecular structures, tissues and whole cells [1–5]. In both IR and Raman spectroscopies, the effect of a roughened metal surface (mostly of Au or Ag) can result in selective surface-induced enhancement of band intensities for functional groups adjacent to the metal surface [6, 7], which has recently been tested for various

[1]Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prosp. Entuziastov, 410015 Saratov, Russia.
[2]Laboratory of Chemistry, Department of Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.
[3]To whom correspondence should be addressed. E-mail: aakamnev@ibppm.sgu.ru; Fax: 7-8452-970383.

0144-8463/02/1200-0541/0

bioanalytical purposes including immunoassays [8–11]. In particular, the use of colloidal gold (CG) is very promising in surface-enhanced IR absorption (SEIRA) spectroscopy [11]. However, the essential strong IR absorption of water present in wet samples, which were studied using gold films deposited on a Si wafer [10] or colloidal gold bioconjugates collected by filtration on a porous polyethylene membrane [11], was found to obscure some important regions of protein vibrations. Moreover, the presence of water might well have resulted in alterations of absorption maxima owing to hydrogen bonding to polar functional groups of the analytes.

We show herein using SEIRA spectroscopy of dry films of CG bioconjugates with protein A widely used in immunoassay [12] that certain characteristic bands of the protein (e.g., amide I, amide II, and some other vibration modes) are essentially affected by the metal surface. Thus, the method may be used for controlling the quality of such bioconjugates. Moreover, we demonstrate that the biospecific reaction of protein A attached to CG particles with human immunoglobulin G (IgG) results in further essential changes in SEIRA spectra, providing a means for an easy and rapid IR spectroscopic detection of biospecific immunochemical interactions (i.e., what is to be termed spectroimmunochemistry).

EXPERIMENTAL

Materials and Preparation Procedures

CG sol (mean particle diameter 30 nm) was prepared according to Frens [13] using sodium citrate (Fluka, Switzerland) as a reducing agent for tetrachloroauric (III) acid (Aldrich, USA) at 100°C. The size of gold particles was determined spectrophotometrically [14]. To produce a stable bioconjugate, the pH of the gold sol was brought to the value 0.5 units above the isoelectric point (which, in our case, was 6.0) of the protein [15]. Conjugation was performed by mixing the gold sol with staphylococcal protein A (Sigma, USA) taken in amounts exceeding by 20% the minimal amount necessary for stabilizing the gold sol (estimated by changes in the optical properties of the stabilised sol upon addition of NaCl up to 10 mg ml^{-1}). The concentration of protein A in the sol was 6 $\mu g\,ml^{-1}$. To prepare CG/protein A–IgG complex, 200 μl of aqueous solution of 1 mg ml^{-1} IgG (Serva, Germany) or, as a control, of bovine serum albumin (BSA; Serva, Germany) were added to 5 ml of CG/protein A conjugate ($A_{520} = 1.0$) After incubation for 1 hr at ambient temperature the probe was centrifuged for 5 min at 4°C (12000*g*, centrifuge Sigma 3K18, USA). The supernatant was removed, the centrifugate re-dissolved in 5 ml of bidistilled water and centrifuged again.

Spectroscopic Measurements and Data Treatment

For spectroscopic measurements (Nicolet FTIR spectrometer, model Magna IR 750; DTGS detector; Nichrome source; beamsplitter; KBr), a total of 100 scans were applied with a resolution up to 4 cm^{-1}. Spectra were measured in the attenuated total reflectance (ATR) mode using a standard ZnSe 45° flat plate Contact Sampler (12 reflections; Spectra-Tech, USA) on which the centrifuged bioconjugates (or

aqueous solution of pure protein A or IgG) were placed (100 μl) and dried in a thermostatted desiccator (1 hr, 45°C). Spectroscopic data were treated using the standard software (OMNIC 3.1; Nicolet).

RESULTS AND DISCUSSION

We applied FTIR spectroscopy in the attenuated total reflectance (ATR) mode [16, 17], which gives best results for thin films with minimal sample preparation, using standard FTIR-ATR accessories (see above). The spectrum of pure protein A (Fig. 1a, spectrum 1) represents typical IR absorption regions of proteins, including the stretching modes of amide N–B at 3290 and 3077 cm^{-1} (on the background of very broad adsorption of carboxylic and alcoholic O–H groups in side chains centered at about 3400 cm^{-1}); symmetric and antisymmetric vibration bands of CH_2 and CH_3 groups of amino acid side chains (*ca.* 3000–2800 cm^{-1}); strong typical amide I and amide II bands (mainly stretching C=O and bending N–H in peptides, respectively, at 1652 and 1540 cm^{-1}); bending CH_2 modes (1453 cm^{-1}); stretching C–NH_2 of side chain primary amines (near 1400 cm^{-1}, as well as various weaker bands related to C–N, C–O and C–C–O vibrations of the protein backbone and amino acid residues (under 1350 cm^{-1}) [2, 3, 10, 11, 16–19].

The spectrum of protein A conjugated with CG (Fig. 1a, spectrum 2) is markedly different from that of pure protein A. First, it is noteworthy that of the amount of protein A in the sample of its CG conjugate (see spectrum 2) was taken approximately 5-fold less than of pure protein A (see spectrum 1); nevertheless, the 2- to 3-fold higher absorbance values in spectrum 2 as compared to those in spectrum 1 indicate that a SEIRA effect is observed with enhancement factors of the order of 10 to 15. These values correspond to the nature of CG conjugation with a biospecific probe due to the net effect of relatively weak non-covalent interactions (electrostatic and hydrophobic), which allows the nativity of biomacromolecules and their functional activity to be conserved [15]. It is known that stronger chemical (covalent) interactions of adsorbed molecules with the metal surface can result in enhancement factors increased by over an order of magnitude [6]. Second, the SEIRA spectrum 2 shows that the band at 3290 cm^{-1} (see spectrum 1) disappears, and instead of the amide I (1652 cm^{-1} and amide II (1540 cm^{-1}) bands there appears a single intermediate strong peak at 1596 cm^{-1} with a shoulder at about 1650 cm^{1}. These changes may indicate that N–H moieties are directly involved in interaction with the metal surface. Also enhanced are the regions of C–NH_2 vibrations near 1400 cm^{-1} (see spectrum 2), suggesting the involvement of side chain amino groups, and of C–C/C–O vibrations (1150–1000 cm^{-1}). Note that according to the SEIRA theory [6], only those molecular vibrations which appear perpendicular to the metal surface are enhanced, accounting for the selectivity of enhancement.

The drastic changes induced by CG in the spectrum of protein A provide evidence that protein molecules are attached directly to the CG surface, so that the method may be used for controlling the bioconjugation process.

Another important effect is observed after the biospecific interaction of CG/protein A complex with human IgG in solution. In consists in the restoration of the

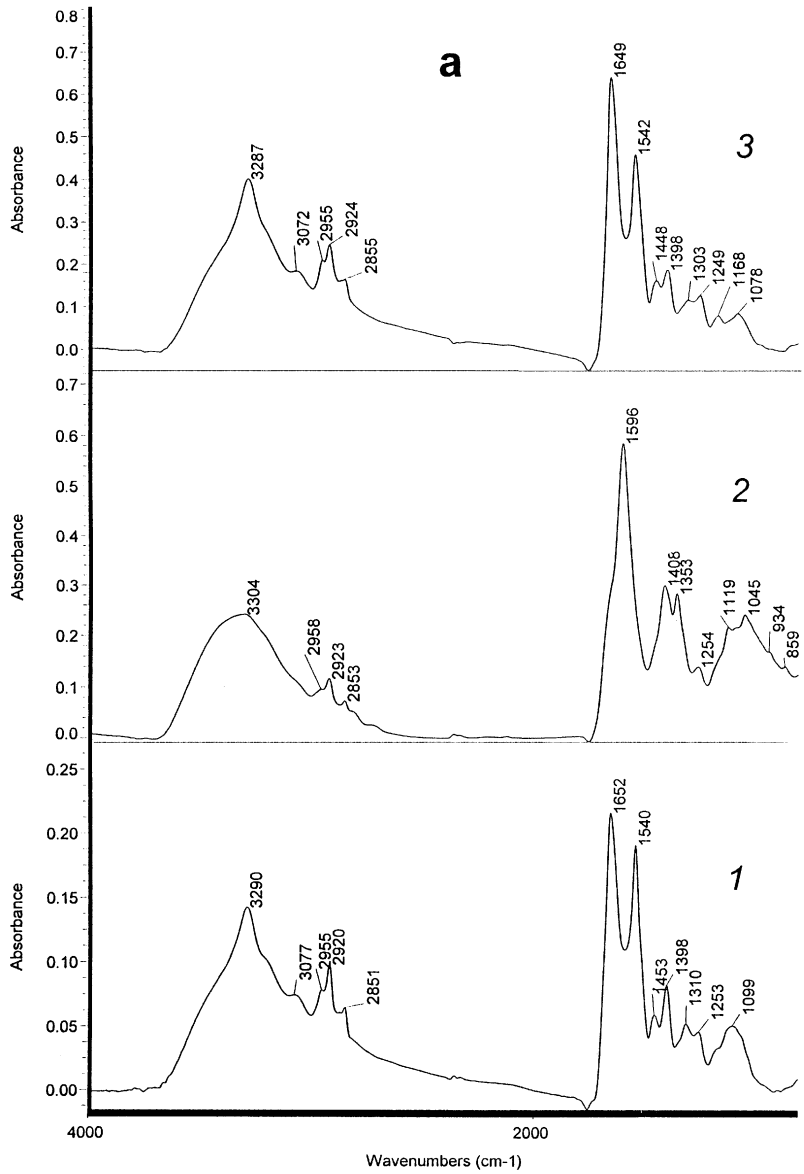

Fig. 1. FTIR-ATR spectra (a) of protein A: (1) pure (100 μg of protein), (2) conjugated with colloidal gold (20 μg of protein), (3) the same as 2 after interaction with human IgG, and (b) of pure human IgG.

main shape of the initial spectrum of protein A with a *ca.* 15-fold enhancement (Fig. 1a, spectrum 3); the latter is somewhat smaller only in the C–C–O spectral region (1150–1000 cm^{-1}. Note that the spectrum of pure human IgG presented for comparison in Fig. 1b, although generally resembling that of protein A (see Fig. 1a,

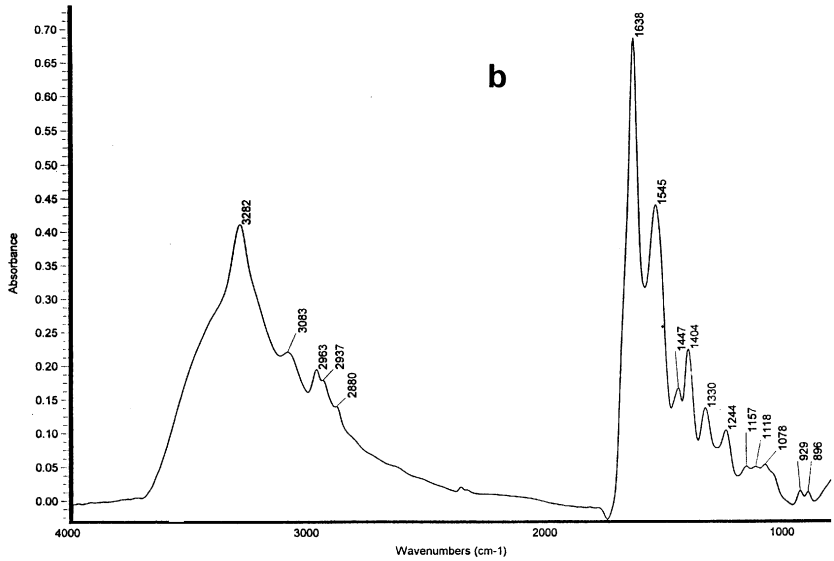

Fig. 1. Continued.

spectrum 3), has some essential differences. The main of these are:

(i) the intensity of the antisymmetric stretching C–H band of *methyl* groups (–CH_3) at 2963 cm^{-1} is higher in IgG, whereas in protein A the corresponding *methylene* (–CH_2–) band at 2920 cm^{-1} dominates, which reflects differences in their amino acid composition;

(ii) the narrow amide I band in IgG has a maximum at 1638 cm^{-1} (with a weak shoulder at *ca.* 1680 cm^{-1}) characteristic for β-structures [18, 19] typical for immunoglobulins [9], whereas in protein A the amide I band is observed at about 1650 cm^{-1} reflecting its dominant secondary structure of a distorted α-helix [19].

The addition of bovine serum albumin (BSA), which does not react with protein A, as a control to the CG/protein A complex had no effect on its spectrum.

The fact that spectrum 3 (for CG/protein A–IgG complex) largely coincides in shape with that of pure protein A (see spectrum 1) and differs from that of CG/protein A complex (see spectrum 2) suggests that upon the reaction of IgG with protein A on CG particles the configuration of protein A is modified. This is in line with the observations obtained using surface-enhanced Raman scattering for another immune reaction on CG [9]. Moreover, it is unlikely that IgG bound to protein A on CG (i.e., separated from the CG surface) would exhibit any SEIRA effect.

In conclusion, the results obtained demonstrate for the first time that SEIRA spectroscopy on dry films of CG bioconjugates is sensitive to interactions of protein molecules with the surface of CG particles and also allows biospecific reactions to

be reliably and easily detected. First, the SEIRA spectroscopic data observed confirm that the biomolecules are attached directly to the CG surface, which is of primary importance for the synthesis of haptens with CG for subsequent immunisation of animals [20], and the method may be used for controlling the quality of such bioconjugates. Moreover, the results obtained can provide a basis for developing test systems for detecting various biospecific interactions including, besides protein A–IgG, also immunochemical (antigen–antibody) and other (e.g., enzyme–substrate, lectin–polysaccharide, etc.) reactions.

ACKNOWLEDGMENTS

This work was supported in part by NATO (Grant LST.CLG.977664), INTAS (Project 96-1015). The Russian Foundation for Basic Research (Grant 01-04-48736) and Russian Academy of Sciences' Commission (Grant 205 under the 6th Competition-Expertise of research projects), as well as by UNESCO short-term fellowships in biotechnology to A.A.K. (in 2000) and to L.A.D. (in 2001).

REFERENCES

1. Naumann, D., Helm, D., and Labischinski, H. (1991) Microbiological characterizations by FT-IR spectroscopy. *Nature* **351:**81–82.
2. Naumann, D., Keller, S., Helm, D., Schultz, Ch., and Schrader, B. (1995) FT-IR spectroscopy and FT-Raman spectroscopy are powerful analytical tools for the non-invasive characterization of intact microbial cells. *J. Mol. Struct.* **347:**399–405.
3. Mantsch, H. H. and Chapman, D. (eds) (1996) *Infrared Spectroscopy of Biomolecules*. Wiley, New York.
4. Brandenburg, K. and Seydel, U. (1998) Infrared spectroscopy of glycolipids. *Chem. Phys. Lipids* **96:**23–40.
5. Schrader, B. *et al.* (1999) NIR Raman spectroscopy in medicine and biology: results and aspects. *J. Mol. Struct.* **480–481:**21–32.
6. Osawa, M. (2001) Surface-enhanced infrared absorption. In: *Near-Field Optics and Surface Plasmon Polaritons. Topics in Applied Physics* (Kawata, S. ed.), Vol. 81, Springer, Berlin, pp. 163–187.
7. Campion, A. and Kambhampati, P. (1998) Surface-enhanced Raman scattering. *Chem. Soc. Rev.* **27:**241–250.
8. Kneipp, K., Kneipp, H., Itzkan, I., Dasari, R. R., and Feld, M. S. (1999) Ultrasensitive chemical analysis by Raman spectroscopy. *Chem. Rev.* **99:**2957–2975.
9. Doux, X., Yamaguchi, Y., Yamamoto, H., Doi, S., and Ozaki, Y. (1998) NIR SERS detection of immune reaction on gold colloid particles without bound/free antigen separation. *J. Raman Spectrosc.* **29:**739–742.
10. Brown, C. W. *et al.* (1998) Immunoassays based in surface-enhanced infrared absorption spectroscopy. *Anal. Chem.* **70:**2991–2996.
11. Seelenbinder, J. A., Brown, C. W., Pivarnik, P., and Rand, A. G. (1999) Colloidal gold filtrates as metal substrates for surface-enhanced infrared absorption spectroscopy. *Anal. Chem.* **71:**1963–1966.
12. Dykman, L. A. and Bogatyrev, V. A. (1997) Colloidal gold in solid-phase assays. A review. *Biochemistry* (Moscow) **62:**350–356.
13. Frens, G. (1973) Controlled nucleation for the particle size in monodisperse gold suspensions. *Nature Phys. Sci.* **241:**20–22.
14. Khlebtsov, N. G., Bogatyrev, V. A., Dykman, L. A., and Melnikov, A. G. (1996) Spectral extinction of colloidal gold and its biospecific conjugates. *J. Colloid Interface Sci.* **180:**436–445.
15. Roth, J. (1983) The colloidal gold marker system for light and electron microscopic cytochemistry. In: *Techniques in Immunocytochemistry*. Vol. 2. (G. R. Bullock, and P. Petrusz, eds.), Academic Press, London, pp. 217–284.

16. Wanzenbok, H. D., Mizaikoff, B., Weissenbacher, N., and Kellner, R. (1997) Multiple internal reflection in surface enhanced infrared absorption spectroscopy (SEIRA) and its significance for various analyte groups. *J. Mol. Struct*. **410–411:**535–538.
17. Schmitt, J. and Flemming, H.-C. (1998) FTIR-spectroscopy in microbial and material analysis. *Int. Biodeterior. Biodegrad*. **41:**1–11.
18. Bonnin, S., Besson, F., Gelhausen, M., Chierici, S., and Roux, B. (1999) A FTIR spectroscopy evidence of the interactions between wheat germ agglutinin and *N*-acetylglucosamine residues. *FEBS Lett*. **456:**361–364.
19. Naumann, D., Schultz, Ch., Görne-Tschelnokow, U., and Hucho, F. (1993) Secondary structure and temperature behavior of the acetylcholine receptor by Fourier transform infrared spectroscopy. *Biochemistry* **32:**3162–3168.
20. Dykman, L. A., Matora, L. Yu., and Bogatyrev, V. A. (1996) Use of colloidal gold to obtain antibiotin antibodies. *J. Microbiol. Meth*. **24:**247–248.