Colloids and Surfaces B: Biointerfaces 77 (2010) 12–17

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Immunodiagnosis of Chagas disease: Synthesis of three latex–protein complexes containing different antigens of *Trypanosoma cruzi*

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ARTICLE INFO

Article history:
Received 8 July 2009
Received in revised form 1 December 2009
Accepted 22 December 2009
Available online 11 January 2010

Keywords:
Latex–protein complex
Immunoassays
Chagas disease
Recombinant proteins

ABSTRACT

This article describes the physical adsorption and the chemical coupling of 3 antigenic proteins of *Trypanosoma cruzi* onto polystyrene (PS) based latexes to be used as novel immunodiagnosis reagents for detecting the Chagas disease. The coupled proteins were a homogenate of *T. cruzi*, or a recombinant protein (either Ag36 or CP1). With the homogenate, between 30 and 60% of the total-linked protein was chemically coupled, showing a small dependence with the pH. For Ag36 and CP1, around 90% of the total-linked protein was chemically coupled, with a maximum coupling at pH 5 (*i.e.*, close to the isoelectric point). The chemical coupling of CP1 was less affected by the pH than the coupling of Ag36.

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1. Introduction

Immunodiagnosis latex–protein complexes are useful for detecting pregnancies, rheumatoid arthritis, toxoplasmosis, malaria, brucelosis, and leptospirosis. Immunodiagnosis tests involve specific reactions between an antigen (or antibody) contained in a human fluid and an antibody (or antigen) contained in the latex–protein complex. The particles agglutination process can be visualized either directly or *via* instrumental methods [1–8].

Hydrophilic functionalized latexes of uniform particle size and uniform functional group density are the base materials for the chemical coupling of antibodies (or antigens) onto their surface. The hydrophilic nature increases the stability of the latex–protein complexes with respect to complexes of unfunctionalized latexes, and it also prevents nonspecific latex–protein interactions [9]. The uniformity in particle size and functional density is useful for: (a) increasing the colloidal stability; (b) enabling a homogeneous distribution of the diagnosis protein onto the particles surface; and (c) generating a neat agglutination process.

Biomolecules are attached onto latex particles either by simple physical adsorption or by covalent coupling. The latex–protein complexes obtained by physical adsorption are considered of a lower quality, due to the potential desorption and denaturalization of the adsorbed proteins [10]. In fact, when preparing chemically coupled complexes, a fraction of the bound protein is physically adsorbed onto the more hydrophobic regions of the particles surface [11]. However, such non-chemically bound fraction can be desorbed in a final cleaning operation involving an emulsifier [12].

Carboxylated PS latexes have been the basis of several immunoassay kits. Ortega-Vinuesa et al. [13] synthesized and evaluated chemically- and physically coupled complexes of anti-C reactive protein polyclonal IgG onto carboxylated latexes; observing a better immunological response in the chemically coupled complexes. Lee et al. [14] investigated the chemical coupling of bovine serum albumin (BSA) and anti-human IgG onto a core-shell carboxylated latex; observing that the sensitivity of the agglutination test depended on the temperature and on the mass of bound antigen. Menshikova et al. [15] investigated the physical and chemical coupling of BSA onto a carboxylated latex of polystyrene-*graft*-polyvinylpyrrolidone (PS-*g*-PVP). By remaining on the external surface, the pyrrolidone groups stabilized the particles and interacted with BSA. Also, these PS-*g*-PVP particles produced high levels of chemical binding and physical adsorption onto their surface [15].

In our previous work [16], we investigated the synthesis of core-shell latexes with external carboxyl groups through emulsion copolymerizations of styrene and methacrylic acid onto monodisperse PS seeds. In the second part of that article [17], BSA was used as a model protein for adsorption and chemical coupling experiments, and a recombinant protein Ag36 of *Trypanosoma cruzi* was coupled onto two base carboxylated latexes (designed as C1 and C2) that exhibited similar particle diameters but different charge

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doi:10.1016/j.colsurfb.2009.12.021

densities. With BSA, the final density of covalently bound protein was 2.30 mg/m^2 (representing around 55% of the total-linked protein), and the reaction was little affected by the pH. With Ag36, the final density of covalently bound protein was 2.44 mg/m^2, around 80% of the total-linked protein was chemically coupled, and the maximum chemical coupling was observed at pH 5 (*i.e.*, close to the protein isoelectric point) [17].

The chemical linkage of proteins is strongly affected by the medium; and for example its ionic strength can either increase or decrease the amount of bound protein. In addition, the reactions are sensitive to the pH and to the buffer [18]. In principle, the amine groups of proteins and peptides can be directly coupled onto the carboxyl groups of carboxylated latexes. However, this reaction is too slow, and it is convenient to transform the slow carboxyl groups into the faster acilureas by addition of an activator such as the water-soluble N-N-(3-dimethylamine propyl) N′-ethyl carbodiimide (EDC).

Apart from our articles [16,17], two other publications have appeared on the development of an immunoagglutination latex for the detection of the Chagas disease [19,20]. In these last works, the complexes were obtained by physical adsorption of homogenates of *T. cruzi* onto plain PS latex particles, but recombinant proteins were not tested.

With the final aim of producing an immunodiagnosis kit for the Chagas disease, the present article describes the sensitization of PS and core-shell carboxylated latexes by physical adsorption and covalent coupling, respectively, with 3 different antigenic proteins of *T. cruzi*.

2. Experimental

2.1. Materials

Two base latexes were employed, and their characteristics are presented in Table 1. The uniform PS latex was synthesized through an emulsifier-free and unseeded emulsion polymerization of styrene (St); with recipe and procedure similar to those described in [16]. The carboxylated latex (indicated by C2) was synthesized through a semibatch copolymerization of St and methacrylic acid (MAA) onto a uniform PS latex seed [16]. Most of the polymer molecules contained sulphate groups at their chain ends, corresponding to remnants of the persulphate initiator that was employed in the latex syntheses.

The *T. cruzi* homogenate was obtained as follows. The epimastigotes were harvested, washed in phosphate-buffered saline (PBS), and resuspended in 10 volumes of water. The suspension was sonicated, and the lysate containing the parasite antigen was cleared by centrifugation at 10,000 × *g* for 15 min. The supernatant was aliquoted and kept at −20 °C until use. The antigen molar masses ranged from 20 to 90 kDa, with a main fraction at 40–50 kDa. Their isoelectric point (Ip) was in the range 5.9–6.3 [21].

The employed recombinant antigens of *T. cruzi* were Ag36 and CP1. The Ag36 protein was produced by Wiener Laboratory (Argentina); and exhibited a molar mass of 26.4 kDa and an Ip ≅ 5.0 [22]. The CP1 protein was synthesized by us from the tandem expression of 2 highly antigenic peptides (RP1 and RP2). It exhibited a molar mass of 18.6 kDa and Ip ≅ 5.4 [23]. The proteins were purified by Nickel Affinity Chromatography. The purity of the recombinant proteins was analyzed by 15% polyacrylamide gel electrophoresis (PAGE), and stained with Coomassie brilliant blue. The proteins were not lyophilized.

The following buffers were used. The PBS solution (pH 7.4) contained: NaCl (Cicarelli, Argentina, 0.16 M), KCl (Cicarelli, 0.0027 M), Na_2HPO_4 (Cicarelli, 0.0100 M), and KH_2PO_4 (Cicarelli, 0.0018 M). The 0.1 M glycine buffer (pH 3) contained $C_2H_5NO_2$ (Sigma) and HCl. The 0.1 M acetate buffer (pH 5) contained $CH_3COONa{\cdot}3H_2O$ (Cicarelli) and CH_3COOH (Anedra, Argentina). The 0.1 M phosphate buffer (pH 7) contained KH_2PO_4 (Cicarelli) and KOH. The 0.002 M borate buffer (pH 8) contained $Na_2B_4O_7{\cdot}10H_2O$ (Anedra) and HCl. The 0.1 M carbonate buffer (pH 9) contained Na_2CO_3 (Cicarelli) and $NaHCO_3$ (Cicarelli).

Table 1
Characteristics of the employed base latexes.

	PS latex	Carboxylated latex (C2)
Particle morphology	Homogeneous	Core-shell[a]
$\bar{D}_{DLS}$ (nm)[b] at 90°	300 ± 6	418 ± 8
PDI[c]	1.06	1.05
Shell-thickness (nm) by DLS at 90°	–	35
h[d] (nm) by DLS at 90°	4	11
σ[e] (μC/cm^2)	14.9 ± 1.8	78.4 ± 3.1
$\delta_{SO_4^=}$[f] (×10^7 mEq/cm^2)	1.54 ± 0.23	1.76 ± 0.25
δ_{COOH}[g] (×10^7 mEq/cm^2)	–	7.95 ± 0.36
c.c.c.[h] (mM KBr) by visual method	250	450
c.c.c.[h] (mM KBr) by DLS at 90°	75	200

[a] With a PS core and a PS-MAA shell.
[b] Average particle diameter determined by dynamic light scattering (DLS).
[c] Polydispersity index by scanning electron microscopy.
[d] Hairy layer thickness.
[e] Surface density of total charge by conductimetric titration.
[f] Surface density of sulphate groups.
[g] Surface density of carboxyl groups.
[h] Critical coagulation concentration at pH 6.

Table 2
Physical adsorption of homogenate of *Trypanosoma cruzi* onto the PS latex: recipes and surface densities.

		Sample no.						
		1	2	3	4	5	6	7
	Common recipe:							
	PS latex (mL)	0.500[a]	0.500[a]	0.500[a]	0.500[a]	0.500[a]	0.500[a]	0.500[a]
	Buffer sol.[b] (mL)	0.030	0.030	0.030	0.030	0.030	0.030	0.030
	H_2O (mL)	0.870	0.829	0.747	0.665	0.583	0.500	0.377
	Homogenate sol.[c] (mL)	–	0.041	0.123	0.205	0.287	0.370	0.493
	C^o_{Hom} (mg/mL)[d]	–	0.1	0.3	0.5	0.7	0.9	1.2
Experiment:								
A (pH 3)	Γ_{Hom} (mg/m^2)	–	0.44	1.31	2.15	2.88	3.26	3.26
B (pH 5)	Γ_{Hom} (mg/m^2)	–	0.49	1.50	2.42	3.17	3.76	3.77
C (pH 7)	Γ_{Hom} (mg/m^2)	–	0.49	1.36	2.20	2.94	3.26	3.29
D (pH 9)	Γ_{Hom} (mg/m^2)	–	0.53	1.60	2.63	3.35	–	3.35

[a] Or total surface area = 0.20 m^2.
[b] Containing a 0.1 M solution of the appropriate buffer.
[c] 3.41 mg/mL.
[d] Initial protein concentration.

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