Talanta 81 (2010) 314–319

Contents lists available at ScienceDirect

Talanta

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

A combinatorial strategy of a new monoclonal ELISA and immunoaffinity chromatography using sodium deoxycholate to increase the recovery of multimeric proteins like r-HBsAg

Alberto Leyva [a,*,1], Julio C. Sánchez [a,1], Rodolfo Valdes [b], Milagros Font [a], Lissette López [a], Neyda Hernández [a], Williams Ferro [b], Yenisley Medina [b]

[a] Process Control Department, Center for Genetic Engineering and Biotechnology. 31st Ave /158 and 190, P.O. Box 6162, Havana 10600, Cuba
[b] Monoclonal Antibody Production Department, Center for Genetic Engineering and Biotechnology. 31st Ave /158 and 190, P.O. Box 6162, Havana 10600, Cuba

ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form 5 December 2009
Accepted 7 December 2009
Available online 16 December 2009

Keywords:
ELISA
Hepatitis B surface antigen
Immunoaffinity chromatography
Sodium deoxycholate

ABSTRACT

In this work, a sandwich monoclonal-based ELISA for quantifying the HBsAg obtained from yeast cells was standardized and validated. The monoclonal antibody employed in this assay reacts uniformly with different molecular isoforms of r-HBsAg. Immunoassay allowed the r-HBsAg quantification in an analytical range 11.9–191.7 ng/mL. Inter- and intra-assay precision variation coefficients were between 0.77–3.43% and 1.95–8.89%, respectively, and the recovery ranged 98.2–100.8%; which confirms its reliability. r-HBsAg is a complex of carbohydrates, proteins and lipids assembled into spherical particles with an average diameter of 24 nm. Many host contaminants accompany this protein during purification process, which can interfere the antigen recognition by the immunoaffinity matrix. To solve this problem, the effect of several detergents in the quantification and purification of r-HBsAg were studied. The addition of the surfactant sodium deoxycholate (NaDoc) at 0.1% in this ELISA improved the recognition and quantification of r-HBsAg by 2.4-fold higher than untreated samples. Similar results were observed in the immunoaffinity chromatography where a 1.5-fold increasing recovery values was shown. The application of NaDoc allows to reduce the inhibitory effect upon the antigen–antibody recognition, increasing the quantification and immunoaffinity chromatography efficiency. This analytical combination could be applied to multimeric proteins like r-HBsAg of HB vaccine.

1. Introduction

The hepatitis B surface antigen (HBsAg) virus is a complex macromolecular particle with a molecular weight estimated of 24 nm, composed by glycoproteins and host lipids [1–4]. The development of genetic engineering has allowed the use of eukaryotic hosts such as various yeast strains [5,6] and mammalian cell lines [7,8] for producing heterologous proteins such as recombinant HBsAg (r-HBsAg). In the assembly process of the r-HBsAg particle, many host contaminants accompany this protein [9]. Therefore, these contaminants could interfere in its recognition at the immunoaffinity matrix affecting the recovery of this stage. Thus, the removal of these contaminants would allow obtaining a purified molecule with high quality, purity and recovery.

Detergents are molecules whose unique properties enable the disruption or formation of hydrophobic and hydrophilic interactions among molecules in biological samples [10]. Sodium deoxycholate (NaDoc) is originally a native strong ionic detergent found in mammalian biles at high concentrations [11]. This detergent is also used for supplementing cell culture media and for preventing non-specific binding in affinity chromatography [12].

Immunoaffinity chromatography (IAC) has been widely used for the purification of proteins. It has proved to be a powerful tool in several purification procedures, mainly because of its high selectivity. CB.Hep-1 monoclonal antibody (mAb) has successfully been immobilized to sepharose for specific recognition and adsorption of the r-HBsAg as an efficient protein purification method widely used not only in the laboratory but also at industrial scale [13].

Many strategies have been carried out to optimized and reduce the high cost of mAb used in this purification step [14–17]. One of the most significant factors in this chromatography is the ligand density chemically bound to a resin. However, the integrity and quality of antigen is essential to the recognition and specificity of the mAb couple to a matrix.

In this paper, we report the standardization and validation of a sensitivity sandwich monoclonal-based ELISA (m-ELISA) using the CB.Hep-1 mAb for the efficient quantification of the r-HBsAg

* Corresponding author. Tel.: +53 7 2716022x5113; fax: +53 7 2714764.
E-mail address: alberto.leyva@cigb.edu.cu (A. Leyva).
[1] These authors have contributed equally to this work.

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.12.002

during the manufacturing process. The treatment of the sample with NaDoc 0.1% eliminated inhibitory elements that prevent the coupling between CB.Hep-1 mAb and the antigen. Therefore, the application of this detergent in the IAC increased the recovery of r-HBsAg and the effectiveness of the hepatitis B vaccine manufacturing process.

2. Materials and methods

2.1. Materials

All chemicals were of analytical-reagent grade. NaDoc, Triton X-100 (TX-100), Tween 20 (T20), sodium dodecyl sulfate (SDS) and other chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Source of the r-HBsAg

r-HBsAg was produced by fermentation of a recombinant strain of Pichia pastoris (C-226) in saline medium supplemented with glycerol and its expression was induced with methanol. r-HBsAg was recovered and submitted to initial purification steps as previously described [18]. A material of up to 25% purity was used as a starting material in the IAC experiments.

2.3. Mouse CB.Hep-1 mAb production

CB.Hep-1 mAb was generated according to Fontirrochi et al. [19]. The antibody was raised against the HBsAg and produced in specific pathogen free BALB/c mice. CB.Hep-1 mab was purified from ascitic fluid by protein A-Sepharose fast flow affinity chromatography [20]. The purity of the final antibody preparation was >98% assessed by SDS-PAGE under reducing conditions.

2.4. Buffers and solutions used in the manufacturing process of r-HBsAg

All buffers were made in injection grade water. The disruption (SCD) and precipitation buffers (SAP) (20 mM Tris, 5 mM EDTA, 300 mM NaCl, pH 7 and pH 8, respectively) were supplemented with potassium thiocyanate (KSCN). The semipurification (SSD) and negative ion-exchange chromatography (NIEC) buffers were prepared as follows: 20 mM Tris, 3 mM EDTA, 250 mM NaCl, pH 7.2. The elution fractions of IAC, contains the following buffer: 20 mM Tris, 3 mM EDTA, 3 M KSCN, pH 7.2.

2.5. Determination of protein concentrations

Protein concentration was determined according to Bradford's method with slight modifications [21]. Briefly, 96-well microplate was applied as the reaction well and absorbance was measured by microplate reader at 620/450 nm (Labsystem, Helsinki, Finland). The amount of protein was calibrated against bovine serum albumin used as reference standard. Several samples were measured in the same microplate and results were reliable (the determination coefficient of the calibration curve was above 0.99).

2.6. Immunoaffinity chromatography procedure

Sepharose CL-4B (Pharmacia-LKB, Uppsala, Sweden) was activated by the CNBr method [22]. CB.Hep-1 mAb was coupled following recommendations of the manufacturers [23]. The amount of coupled antibody was determined measuring the total protein before and after the coupling reaction (about 5 mg/mL of gel for each immunogel). Final products were washed with 150 mM phosphate buffered saline solution (PBS) pH 7.4 and stored at 4 °C. Packed gel volumes were determined by low-speed centrifugation (250 g for 1 min). Gels were packed into analytical columns (5 cm × 0.7 cm I.D., Pierce, Rockford, USA) and equilibrated with 20 mM Tris–HCl/1 M NaCl, pH 7.8. The flow-rates used were 20 and 35 cm/h for adsorption and elution, respectively. Columns were loaded with an excess of a partially purified r-HBsAg preparation according to previously standardized conditions (2 mg r-HBsAg/5 mg mAb) [15] in the equilibrium buffer. After washing, the bound r-HBsAg was eluted with 20 mM Tris/3 M KSCN/3 mM EDTA, pH 7.0.

2.7. Size exclusion high performance liquid chromatography (HPLC-SE)

An analytical column TSK gel-5000 PW (600 mm × 7.5 mm I.D., Toso Haas, Japan) with 17 μm particle size was used for characterizing r-HBsAg eluted from immunoaffinity columns. The isocratic chromatographic mobile phase was phosphate buffer adjusted to pH 7.0. The r-HBsAg samples dissolved in 150 mM PBS, pH 7.4 were directly applied into the system. The flow-rate was 0.2 mL/min and chromatograms were recorded and analyzed by a Biocrom interface board (CIGB, Havana, Cuba).

2.8. SDS-agarose electrophoresis

Agarose (Type VI: high gelling temperature, Sigma, St. Louis, MO) was dispersed in the following buffer (1.5 M Tris, 1 mM EDTA, 10% SDS, adjusted to pH 8.8 with HCL) to make a 2% (w/w) solution. The agarose was dissolved by heating in a boiling water bath for 10 min. To prevent evaporation, the flask was covered with aluminum foil. Agarose solution was poured onto a 8 cm × 5 cm × 2.5 cm chamber. Samples were prepared in a standard sample buffer (10% glycerol, 125 mM Tris–HCL, pH 6.8, 2% SDS, 1% 2-mercaptoethanol, 0.0005% bromophenol blue) and were separated by electrophoresis in Tris–glycine buffer (0.1% Tris, 200 mM Glicina, 0.1% SDS pH 8.4) at 15 V/cm constant voltage. Protein bans were stained with 0.125% Coomassie Brillant Blue R-250 in 40% methanol, 10% acetic acid for 1 h at room temperature. Destaining was performed in 5% methanol, 7.5% acetic acid for 3 h with gentle agitation.

2.9. Sandwich monoclonal-based ELISA (m-ELISA)

Polystyrene 96-well microtiter ELISA plates (Nunc-Immunoplate Maxisorp, Nunc, Denmark) were coated 20 min at 50 °C with 100 μL/well of the CB.Hep-1 mAb (10 μg/mL) in carbonate/bicarbonate buffer, pH 9.6. Plates were washed in 150 mM PBS containing 0.05% T20. One hundred microliters of standard, control and samples were added in duplicate to the appropriate wells diluted in working buffer (150 mM PBS containing 0.05% T20 and 0.2% bovine serum albumin pH 7.4), and plates were incubated for 1 h at 37 °C. Subsequently, wells were washed five times and incubated with the same mAb (CB.Hep-1-HRP) conjugate in 1:20,000 dilutions for 1 h at 37 °C. Finally, after another washing, 100 μL of enzyme substrate solution (O-phenylnediamine, 0.015% H_2O_2 in citrate buffer, pH 5.0) was added to each well and the plates were incubated for 10 min in the dark at 23 °C. The reaction was stopped by the addition of 50 μL of 2 M H_2SO_4 and immediately read at 492 using an ELISA reader (Labsystem, Helsinki, Finland).

2.10. Sandwich polyclonal-based ELISA (p-ELISA)

The HBsAg concentration was measured by an enzyme-linked immunosorbent assay (ELISA) system using sheep anti-HBsAg polyclonal antibodies for plate coating, followed by incubation with

Table 1
Intra- and inter-assay coefficient of variation. Recovery values (%) from accuracy study.

Sample	Intra-assay precision (CV%)	Inter-assay precision (CV%)	Analytical recovery (%)
SCD	0.77	1.95	100.31
SAP	0.80	6.57	98.18
SSD	1.72	5.61	99.95
NIED	3.43	6.76	100.83
IAF	2.25	3.65	98.68
API	3.12	8.89	100.10

anti-sheep IgG antibodies-horse radish perxidase conjugate as explained elsewhere [24].

2.11. Experiments using several detergents and preparation of production process samples for analysis by ELISA

For sample analysis, these were diluted 1:2 in working buffer containing NaDoc at 0.1% to allow a better recognition of r-HBsAg by CB.Hep-1 mAb. Subsequent 2-fold dilutions were performed in the ELISA working buffer. Similarly, other detergents (TX-100, T20 and SDS) used in the experiments at 0.1% were diluted and applied to samples containing the r-HBsAg to be analyzed in the ELISA assay.

2.12. Linearity, quantification and detection limit

The linearity of the method was established by analyzing standard concentration in a wide range from 0.2 to 800 ng/mL. The least-squared method was applied to obatin a function describing a linear model. Regression coefficient (r^2), *y*-intercept and slope were analyzed. Working range was established between the highest and lowest concentration values with satisfactory accuracy and precision CV < 10%.

The quantification limit (QL) was defined as the smallest concentration of r-HBsAg with an intra- and interday imprecision <20% [25]. We accepted the lowest value. The detection limit of the assay was calculated as follows:

$$\mathrm{DL} = \frac{3.3\,\mathrm{SD}\ [\text{mean of zero standard}]}{\text{slope}}$$

2.13. Precision and accuracy

Intra- and inter-assay precision was determined by repetitive analysis of the production samples ($n = 6$ for intra-plate and $n = 9$ for interplate analysis). For both precision assays the acceptance criteria were coefficient of variation [CV (%) = SD/mean × 100%] lower than 10 and 20%, respectively.

To study the accuracy of the assay, each sample of the purification method was spiked with 95.8, 47.9 and 23.9 ng/mL of the standard curve. Recovery was expressed as the bias of the percentage of error between the observed and the true values. This procedure yields three types of samples, which represent low, medium and high quantification ranges (95.8, 47.9, 23.9 ng/mL).

2.14. Specificity

The assay specificity was investigated as reported by Leyva et al. [26]. A statistical comparison between the curve and other previously analyzed curves was performed in each buffer and protein impurities for detecting possible interferences of the components present in each sample.

2.15. Statistical analysis

All statistical analyses were performed using Microsoft® Office Excel (2007) and the STATGRAPHICS Centurion XV.1 programs (1994–2000).

3. Results

3.1. Standardization and validation of a m-ELISA to quantifying r-HBsAg

CB.Hep-1 mAb has shown a high affinity by the hydrophilic sequence CKTCTT present in the HBsAg immunodominant "a" determinant region [27]. This mAb is currently employed as immunoligand in the purification of the r-HBsAg obtained from yeast for the formulation of a commercial anti-hepatitis B virus vaccine (HeberBiovac, Heber Biotec S.A, Cuba).

In this work, CB.Hep-1 mAb was used both for capturing the r-HBsAg oligomeric particle in the plate and for subsequent revealed with HRP as conjugated. The linearity of the method was evaluated using five r-HBsAg reference standard curves in a concentration ranged 11.9–191.7 ng/mL. The determination coefficient (r^2) value obtained in all cases was higher than 0.99 and the *F*-test shown *p*-value less than 0.01 confirming that this assay is linear in the studied range. The immunoassay detection limit was 1.5 ng/mL of the r-HBsAg. The assay specificity was performed by serial dilutions of r-HBsAg secondary reference material in different buffers employed in the manufacturing process. The comparison of these spiked curves did not show significant differences ($p_{\text{intercepts}} = 0.2733$; $p_{\text{slope}} = 0.6238$, $\alpha = 0.05$) indicating the lack of interference in the working range. The CV of the intra-assay precision parameter ranged 0.77–3.43% while the inter-assay variation ranged 1.95–8.89%. Recovery values ranged 98.2–100.8%, which confirms the reliability of the assay (Table 1). These results allow using this validated m-ELISA for the r-HBsAg quantification with high precision and accuracy throughout the whole manufacturing process.

3.2. Application of m-ELISA for the r-HBsAg manufacturing process control. Comparative quantification of the r-HBsAg with an established p-ELISA

In order to assess the m-ELISA efficiency to control the r-HBsAg manufacturing process, all of its samples were also comparatively quantified by using an established and validated ELISA with polyclonal antibodies (p-ELISA) [24]. Significant differences (*p*-value < 0.0001), among quantification values of these samples before IAC by both immunoassays were observed (Fig. 1). On the contrary, there were not significant differences ($p = 0.380$) in the quantification of r-HBsAg in the IAC elution fractions analyzed by both ELISA systems. The difference factor (DF) between both immunoassays ranged 2.0–2.67 in samples previous to the IAC and up to 3.58 in the non-bound fractions. However, in the eluted fractions of the IAC this DF was 1 (Fig. 1). These results suggest the presence of some inhibitory elements (IE) that could prevent the molecular binding of r-HBsAg to CB.Hep-1 mab.

Taking into account that this mAb is used as ligand, some decrease in the r-HBsAg recovery can be expected in the IAC step as well. Thus, it is important to remove the IE for increasing the recovery of the r-HBsAg in the IAC. In order to eliminate this IE, the non-bound samples from (with the highest DF) were treated with several detergents and quantified with the m-ELISA. As shown in

Table 2
Effect of detergent NaDoc at 0.1% on the recovery of immunoaffinity chromatography purification.

Series number	Total r-HBsAg by ELISA (mg)	HBsAg recovery (%)	Purification fold
No.1			1.41
Starting material	3.46		
Treated	2.56	74.06	
Untreated	1.81	52.36	
No.2			
Starting material	3.74		1.55
Treated	1.84	49.14	
Untreated	1.19	31.74	
No.3			
Starting material	3.34		1.35
Treated	1.47	43.98	
Untreated	1.09	32.58	

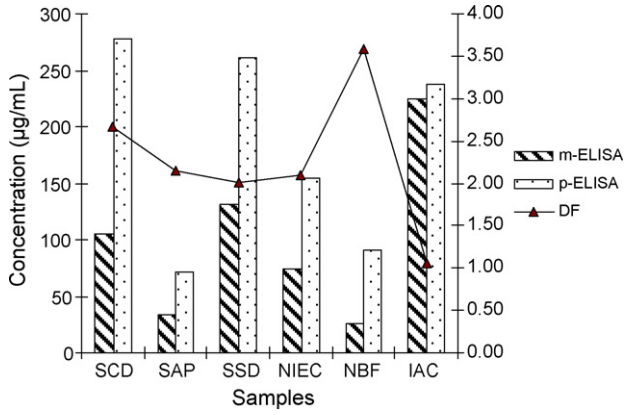

Fig. 1. Comparison of quantification efficiency between both m-ELISA and p-ELISA.

Fig. 2, the application of 0.1% NaDoc increased up to 2.4-fold the r-HBsAg quantification values compared with untreated samples, suggesting that the specific IE binding was eliminated.

3.3. Effect of NaDoc on the r-HBsAg recovery in the immunoaffinity chromatography

In order to probe the hypothesis formulated from the application of the NaDoc in the ELISA studies, three independent analytical IAC processes were performed by using this detergent. Data obtained are shown in Table 2. Statistical differences ($p = 0.001$) between the r-HBsAg quantification values in the elution fractions from both, NaDoc treated and untreated samples, were

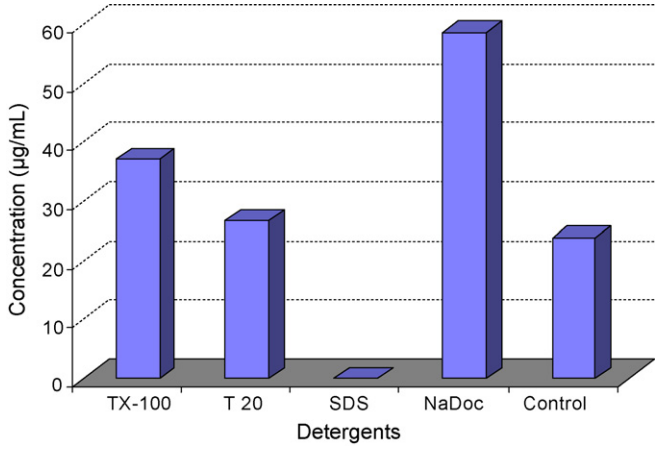

Fig. 2. Detergent effect in elimination of the inhibition present in r-HBsAg. Detergent concentration used in the experiment was 0.1%.

Table 3
Comparative analysis by both ELISA systems of different fractions containing several aggregation stages of r-HBsAg.

Replicate numbers	Fractions	Determinations (µg/mL)		
		m-ELISA	p-ELISA	DF
F 1-1	F1	261.24	108.05	2.42
F 1-2		172.27	70.19	2.45
F 1-3		268.65	114.21	2.35
F 1-4		240.05	104.09	2.31
F 2-1	F2	1070.33	927.04	1.15
F 2-2		1004.25	1001.56	1.00
F 2-3		998.45	1018.45	0.98
F 2-4		1100.87	1004.83	1.10
F 3-1	F3	123.61	148.58	0.83
F 3-2		112.64	128.51	0.88
F 3-3		322.14	270.08	1.19
F 3-4		433.92	362.32	1.20
Positive control		2162.94[a]	2068.36[a]	1.05

[a]These values correspond to the media of tree independent determinations.

detected. In those analytical runs the r-HBsAg amount obtained from the samples treated with detergent ranged 1.35–1.55-fold over those observed in untreated samples. These results corroborate the presence of a molecular factor (s) blockading the specific binding of the r-HBsAg by the CB.Hep-1 mAb coupled to the IAC matrix. The detergent was able to dissociate this IE from the r-HBsAg and consequently increase antigen recovery. Therefore, the hypothesis formulated from ELISAs experiment was ratified.

3.4. Efficiency of the m-ELISA for quantifying several aggregation stages of r-HBsAg

To explore the quantification efficiency of both ELISAs, the m-ELISA and p-ELISA, we compare them using three molecular populations containing several stages of the r-HBsAg aggregation obtained from HPLC-SE. As shown in Fig. 3, the HPLC-SE profile of fraction 1 corresponds to an over aggregate of particles with more than 100 monomers, fraction 2 with r-HBsAg of 24 nm and fraction 3 with a mixture of that observed in fraction 2 and other molecular species of smaller size (Fig. 3A and B). All these fractions were collected and analyzed by both ELISA systems. The p-ELISA values were underestimated in the over-particulate population. On the contrary, comparable results were obtained by both immunoassays in the fractions 2 and 3 (Table 3).

4. Discussion

The CB.Hep-1 mAb is a mouse IgG-2b, specific for the HBsAg [20], routinely used as immunoligand in the antigen-purification

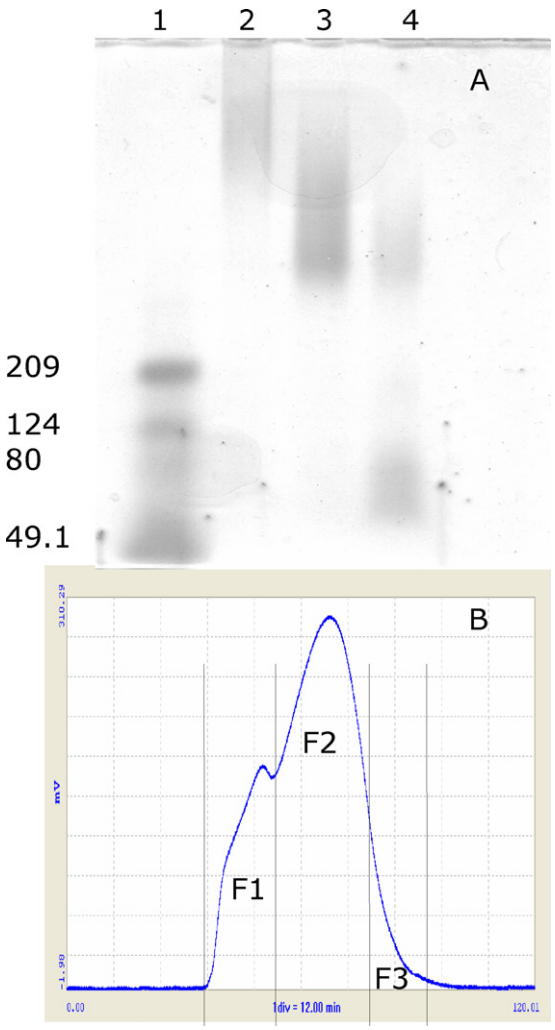

Fig. 3. Analysis of molecular aggregate and non-aggregate forms of r-HBsAg eluted from SEC in SDS-agarose electrophoresis (A). Profile of HPLC-SE corresponding to different aggregation stages of r-HBsAg (B). Lane 1, molecular weight marker (myosin, 209 kDa; β-galactosidase, 124 kDa; bovine serum albumin, 80 kDa; ovalbumin, 49.1 kDa; carbinic anhydrase, 34.8 kDa; soybean trypsin inhibitor, 28.9 kDa; lysozyme, 20.6 kDa; aprotinin, 7.1 kDa). Lane 2, fraction containing r-HBsAg aggregation. Lane 3, fraction aggregate-free r-HBsAg. Lane 4, fraction containing a mixture of aggregate-free and other isoforms of r-HBsAg.

process, which is one of the steps in the manufacturing of the Hepatitis B vaccine for human use [9,13]. In this work, we reported the validation and implementation of a sandwich ELISA using this mAb, as a tool, to quantify up to 11.9 ng/mL of the r-HBsAg produced in methylotrophic Pichia pastoris yeast. The linearity of an analytical method represents its ability to elicit test results that are either directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [28]. The linear range of this m-ELISA showed similar sensitivity compared with other analogue ELISA systems [29,30]. The results of the statistical processing corresponding to accuracy and precision parameters were also comparable with those reported by other researchers [31] and with the acceptance criteria of the ICH guidelines [32], confirming the reliability of this assay. Specificity is the ability of the bioanalytical method to unequivocally measure and differentiate among the analyte the components present in the matrix solution [28]. Specificity study demonstrated that the r-HBsAg can be detected in the established working range without interference.

The comparative quantification by both m-ELISA and p-ELISA of the r-HBsAg samples corresponding to those prior to the IAC step, showed a DF with the highest value in non-bound fractions. However, this difference was not found in the IAC elution fractions. These results suggest the presence of some IE affecting the r-HBsAg recognition by the CB.Hep-1. Therefore, the recovery of this antigen in the IAC step would also be affected.

The r-HBsAg is initially liberated from yeast cells as a non-disulfide-bonded aggregate of monomeric subunits [9,33]. This aggregate can be converted into fully disulfide-bonded particles that resemble the natural HBsAg by treatment with 3 mol L^{-1} thiocyanate, which seems to facilitate the exchange (intra-chain to inter-chain) within oxidized HBsAg polypeptides [33]. During this complex assembly process, contaminant proteins, nucleic acids, carbohydrates and host lipids can be non-covalently linked to the particle. Some of these molecules could be the cause of the suggested IE. In that sense, the addition of a detergent would be able to dissociate those r-HBsAg undesired elements. Surfactant such as SDS [34], Triton X-100 [35] and NaDoc [36] have been shown to provide a structure forming environment in which native order conformation of solubilized protein can be induced [37,38]. Among them, NaDoc is a very mild ionic detergent used to solubilizing membrane-bound proteins without affecting the biological activity of several proteins like r-HBsAg [39–41]. The results obtained with the m-ELISA in presence of NaDoc showed an increase in the recognition of the r-HBsAg by CB.Hep-1 mAb in non-bound IAC samples. Similar results were obtained in three independent analytical IAC batches in which the recovery values were increased with the application of this detergent. All of these results propose a dissociation of the IE from the r-HBsAg. Taking into account the validation experiment for specificity analysis, we suggest that the possible association between IE and the antigen would take effect during the intracellular folding process of the multimeric protein.

The lipid composition of r-HBsAg has previously been determined [42,43]. They represent approximately between 25 and 30% of the particulate protein total mass [44,45]. Its association to r-HBsAg has been reported to occur during self-folding inside the yeast cell [4,45]. This molecular binding could overlap the specific epitopes exposure of that multimeric antigen [4,43]. Therefore, the lipids could be a candidate to represent this detected IE. The use of NaDoc could promote the partial delipidation of r-HBsAg. From the results of r-HBsAg quantification through both ELISA systems, in three fractions eluted from HPLC-SE, an underestimation in the over-particulate population with the p-ELISA was shown. Considering that this fact is not present neither in the second and third fractions containing homogeneous 24 nm or any other molecular species of smallest size r-HBsAg particles, we propose that this underestimation by polyclonal antibodies could be explained by the impossibility to recognize all over-particulated antigen by steric impediment.

All of these results bring several facts to use the proposed m-ELISA in conjunction with IAC system, with the same mAb, to assess a possible presence of some IE that could affect IAC recovery. In that case, it is possible to explore the right way to avoid it. Besides that, the m-ELISA implemented in this paper can be useful as a quality control technique for determining the purity of r-HBsAg, considering the total protein values.

5. Conclusion

In this paper, we developed and validated a novel sandwich ELISA to quantify HBsAg obtained from yeast cells. This immunoassay uses the same CB.Hep-1 mAb employed in the IAC to purify this multimeric antigen. The combination of both analytical methods allowed to detect an IE and to avoid its negative effect to increase the r-HBsAg immunopurification recovery by using 0.1% of NaDoc.

This procedure combination could be usefully extended to assess the possible presence of some IE with its potential immunopurification recovering affectations. Finally, that can be used for multimeric proteins like Hepatitis B vaccine r-HBsAg.

Acknowledgments

The authors wish to thank the Hepatitis B Department for providing the manufacturing process samples and the Process Control Department for the financial support of this study. We are also grateful to Jose L.Fdez.Sierra for reviewing the English.

References

[1] D.S. Dane, C.H. Cameron, M. Briggs, Lancet (1970) 695–698.
[2] W.S. Robinson, D.A. Clayton, R.L. Greenman, J. Virol. 14 (1974) 384–391.
[3] P. Tiollais, C. Pourcel, A. Dejean, Nature 317 (1985) 489–495.
[4] F. Gavilanes, J.M. Gonzales-Ros, D.L. Peterson, J. Biol. Chem. 257 (1982) 7770–7777.
[5] E. Pentón, V. Muzio, M. Griego-González, Biotecnología Aplicada (Cuba) 11 (1994) 1–11.
[6] A. Miyanohara, A. Toh-e, C. Nozaki, F. Hamada, N. Ohtomo, K. Matsubara, Proc. Natl. Acad. Sci. 80 (1983) 1–5.
[7] M.L. Michel, P. Tiollais, D.R. Milich, et al., Vaccines 86, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1986, pp. 359–363.
[8] G.W. Holzer, J. Mayrhofer, J. Leitner, M. Blum, G. Webersinke, S. Heuritsch, F.G. Falkner, Protein Expr. Purif. 29 (2003) 58–69.
[9] E. Hardy, E. Martínez, D. Diago, R. Díaz, D. González, L. Herrera, J. Biotechnol. 77 (2000) 157–167.
[10] H.W. Chang, E. Bock, Anal. Biochem. 104 (1980) 112–117.
[11] R. Coleman, S. Iqbal, P.P. Godfrey, D. Billington, Biochem. J. 178 (1979) 201–208.
[12] J.B. Robinson, J.M. Stritmann, D.G. Wick, R. Stellwagen, Proc. Natl. Acad. Sci. 77 (1980) 5847–5851.
[13] M.E. Fernández de Cossio, T. Días, A. Galván, R. Valdés, E. González, M. Ayala, J. Días, M. Bestagno, O. Borrune, J. Gavilondo, J. Biotechnol. 56 (1997) 69–80.
[14] R. Valdés, B. Reyes, T. Álvarez, J. García, J.A. Montero, A. Figueroa, L. Gómez, S. Padilla, D. Geada, M.C. Abrahantes, L. Dorta, D. Hernández, O. Mendoza, N. Ramírez, M. Rodríguez, M. Pujol, C. Borroto, J. Brito, Biochem. Biophys. Res. Commun. 310 (2003) 742–747.
[15] R. Hernández, L. Plana, L. Gómez, N. Expósito, J. Valdés, R. Páez, E. Martínez, A. Beldarraín, J. Chromatogr. B 816 (2005) 1–6.
[16] N. Ibarra, A. Caballero, E. González, R. Valdés, J. Chromatogr. B 735 (1999) 271–277.
[17] J. Sánchez, J.M. País, Y. Pestana, I. López, Y. Masso, M. Linares, G. Marquez, Biochem. Eng. J. 38 (2008) 1–8.
[18] E. Pentón, L. Herrera, V. Muzio, V. Ramirez, A. Gracía, C. Duarte, European Patent 480525, 1992.
[19] G. Fontirrochi, M. Dueñas, M.E. Fernandez de Cossio, P. Fuentes, M. Pérez, D. Mainet, et al., Biotecnología Aplicada (Cuba) 10 (1993) 24–30.
[20] A. Danielson, A. Ljunglöf, H. Lindblom, J. Immunol. Methods 115 (1988) 79–88.
[21] T. Zor, Z. Selinge, Anal. Biochem. 236 (1996) 302–308.
[22] M. Wilchek, T. Miron, J. Kohn, Methods Enzymol. 104 (1984) 3–55.
[23] Affinity chromatography, principles and methods, Pharmacia [29] LKB Biotechnology, 1993.
[24] A. González, A. Alerm, A. Salgado, V. Ramírez, T. González, Biotecnología Aplicada (Cuba) 10 (1993) 104–105.
[25] V.P. Shah, K.K. Midha, J.W.A. Findlay, et al., Conference Report Pharm. Res. 17 (2000) 1551.
[26] A. Leyva, A. Franco, T. González, J.C. Sánchez, I. López, D. Geada, N. Hernández, M. Montanés, I. Delgado, R. Valdés, Biologicals 35 (2007) 19–25.
[27] M.E. Fernández de Cossío, R. Valdés, A. Agraz, L. Pérez, M. González, G. García, I. Valdivia, J.V. Gavilondo, Minerva, Biotechnol 9 (1997) 76–84.
[28] The United States Pharmacopeia XXXI, Validation of Compendial Methods, 33(1), USP Convention Inc., 1225, Rockville, MD, 2008, p. 96.
[29] M.H. van Roosmalen, J.J. de Jong, W. Haenen, T. Jacobs, F. Couwenberg, G.J.C.M. Ahlers-de Boer, J.A. Hellings, Intervirology 49 (2006) 127–132.
[30] V. Hutse, E. Verhaegen, L. De Cock, S. Quoilin, H. Vandenberghe, Y. Horsmans, P. Michielsen, P. Van Damme, H. Van Vlierberghe, F. Claeys, R. Vranckx, H. Van Oyen, J. Med. Virol. 77 (2005) 53–56.
[31] R. Karakuş, L.A. Aral, B.B. Türk, C. Aybay, Turk. J. Med. Sci. 37 (2007) 87–92.
[32] ICH, International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures, 1996, pp. 1–8.
[33] D.E. Wampler, E.D. Lehman, J. Boger, W.J. McAleer, E.M. Scolnick, Proc. Natl. Acad. Sci. 82 (1985) 6830–6834.
[34] J.C.H. Steele, J.A. Reynolds, J. Biol. Chem. 254 (1979) 1633–1638.
[35] A. Helenius, K. Simons, J. Biol. Chem. 247 (1972) 3656–3661.
[36] C.H. Chen, F. Aladjem, Biochem. Biophys. Res. Commun. 60 (1974) 549–554.
[37] W.L. Mattice, J.M. Riser, D.S. Clark, Biochemistry 15 (1976) 4264–4272.
[38] C.S. Wu, J.T. Yang, Biochem. Biophys. Res. Commun. 82 (1978) 85–91.
[39] I. Desombere, A. Willems, Y. Gijbels, G. Leroux-Roels, J. Virol. 80 (2006) 3506–3514.
[40] C.Y. Kim, D.M. Bissell, J. Infect. Dis. 123 (1971) 470–476.
[41] A. Goudeau, F. Barin, P. Coursaget, P. Sizaret, M. Andre, P. Maupas, in: G.N. Vyas, S.N. Cohen, R. Schmid (Eds.), Effect of Deoxycholate on Ultrastructure and Antigenicity of Hepatitis B Surface Antigen (HBsAg), The Franklin Institute Press, Philadelphia, 1978, p. 707.
[42] J.L. Marcelo, J.V. Hormaza, G. Padrón, Biotecnología Aplicada (Cuba) 18 (2000) 24–32, 7.
[43] O. Satoh, M. Umeda, H. Imai, H. Tunoo, K. Inoue, J. Lipid Res. 31 (1990) 1293–1300.
[44] D.L. Peterson, J. Biol. Chem. 256 (1981) 6975–6983.
[45] N. Sonveaux, D. Thines, J.M. Ruysschaert, Res. Virol. 146 (1995) 43–51.