Journal of Immunological Methods 388 (2013) 60–67

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

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

Research paper

Hapten modification approach for switching immunoassay specificity from selective to generic

Maksim A. Burkin *, Inna A. Galvidis

Department of Hybridomas, Mechnikov Research Institute for Vaccines and Sera, Russian Academy of Medical Sciences, Moscow 105064, Russia

ARTICLE INFO

Article history:
Received 1 October 2012
Received in revised form 29 October 2012
Accepted 4 December 2012
Available online 9 December 2012

Keywords:
ELISA specificity
Low molecular weight analyte
Cross-reactivity profile
Hapten conjugate design
Macrolides
Glycopeptide antibiotics

ABSTRACT

The cross-reactivity profile of polyclonal antibodies against a low molecular weight analyte is strongly influenced by design of the coating or enzyme-linked hapten. The hapten modification effect on immunoassay specificity was studied. Heterology in hapten type and linking method were applied. The influence of these factors on analyses of two groups of antibiotics, 16-membered macrolides and glycopeptides was studied. This approach was used to convert the selective ELISAs to tylosin and eremomycin for group determination of tylosin\tilmicosin, tylosin\spiramycin and eremomycin\vancomycin. It was shown that the analytical spectrum of the developed polyclonal antibody-based immunoassays could be expanded and depended mainly on the type of coating hapten but not on the linking method. Modification of the hapten type in coating conjugates applied in present study served as a mechanism for switching specificity of the ELISA between selective and group.

1. Introduction

Numerous low molecular weight compounds of natural and anthropogenic origin are physiologically active. Therefore they are not indifferent for human, animals, plants and ecosphere as a whole. The following groups of compounds: anti-infective agents, hormones and endocrine disruptors, growth promoters, abused drugs, other pharmaceuticals, pesticides, pollutants and toxins are the target analytes in areas of food control, environmental and drug monitoring, forensic medical examination, veterinary and sanitary inspection, research activities and others. Thus the development of analytical methods should be a match to the growing number of analytes.

Although technologies are being intensively developed based on antibody-like properties of artificial biomimetic receptors, such as molecular-imprinted polymers (Baggiani et al., 2006) and aptamers (Jayasena, 1999; Giovannoli et al., 2008; Fodey et al., 2011), which are not time-consuming and require no animal involvement the traditional immunoassay remains popular and widely used. The immunoassay is well-known to be specific, sensitive and inexpensive tool capable for high throughput screening in the mentioned areas of activities (Rebe Raz and Haasnoot, 2011; Kumar et al., 2004; Galve et al., 2002; Schwenzer et al., 1983; Tsai and Lin, 2005).

Here we demonstrate an approach permitting versatile usage of immune sera or already developed polyclonal antibodies-based immunoassays. The principle of this approach is to change assay specificity converting cross-reactivity profile of related analogs, metabolites or derivatives. Owing to structurally modified hapten conjugates used as coating antigens in competitive ELISA only a part of an antibody population of antiserum binds whereas the remaining part appears to be nonreactive. A part of antibodies express some different epitope specificity from initial one. The modified (heterologous) antigen may differ from the homologous antigen and immunogen in hapten type, its spatial orientation on the carrier, in presence/absence and length of the spacer arm, in method of conjugation that determines the chemical structure of the linkage.

In present paper the attempts to extend analytical spectrum for existing methods using coating antigen modification are described. The effect of modifications in hapten type and linking method on analysis was studied. The object under study was the ELISA developed for determination of veterinary

* Corresponding author. Tel./fax: +7 495 9170393.
E-mail address: burma68@yandex.ru (M.A. Burkin).

0022-1759/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2012.12.002

macrolide antibiotics tylosin and tilmicosin. Spiramycin (SPIR) is another 16-membered macrolide used to combat bacterial and mycoplasmal infections in cattle, pigs and poultry. The residues of SPIR are therefore also to be estimated in tissues of edible animals (Council Regulation (EU), 2010; Codex Alimentarius Commission, 2011). To convert the assay for SPIR identification the same antibodies against tylosin were used.

One more model was selective ELISA for determination of glycopeptide eremomycin, a human antibiotic candidate. The possibility of recognition in the other members of glycopeptide family, vancomycin, teicoplanin and ristomycin A using this assay was also assessed.

2. Methods

2.1. Chemicals

Tylosin base (TYL) and desmycosin (DMN) were the gift from Prof. G.A. Korshunova (Belozersky Research Institute of Physicochemical Biology, Moscow State University). Eremomycin (ERM) and ristomycin A were the gift from Prof. G.S. Katrukha (Gause Institute of New Antibiotics). Tilmicosin (TMN), spiramycin (SPIR), vancomycin (VCM), teicoplanin, glucose oxidase (GO), bovine serum albumin (BSA), gelatine (Gel), N-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (edc), Freund's complete adjuvant, *o*-phenylenediamine, and glutaraldehyde (ga) were purchased from Chimmed (Moscow, Russia). Antisera against BSA–TYL and GO–ERM(ga) were developed in recent works (Burkin and Galvidis, 2012; Burkin and Burkin, 2009).

2.2. Synthesis of conjugated antigens based on 16-membered macrolides

2.2.1. Gel–SPIR

The solutions containing 4 mg of Gel (0.025 μmol) in 1 mL of 0.05 M carbonate buffer pH 9.6 (CB) were combined with the solutions of SPIR (10 mg/mL) corresponding 10-, 30- and 100-fold molar excess of antibiotic over carrier (0.21, 0.63, 2.11 mg, respectively) and stirred for 3 h at room temperature. Then each mixture was supplemented with 0.1 mL of sodium borohydride solution (2 mg/mL) and stirred for another 1 h. The resulting conjugates were dialyzed exhaustively against 0.15 M phosphate buffered saline, pH 7.0 (PBS).

2.2.2. Gel–TYL, Gel–DMN and BSA–TYL

The syntheses of coating antigens Gel–TYL×10, Gel–DMN×175 and immunogen, BSA–TYL×100 with corresponding hapten load by synthesis did not differ from the preceding description and detailed in Burkin and Galvidis (2012).

2.3. Synthesis of conjugated antigens based on glycopeptide antibiotics

Several functionally active groups in glycopeptide molecule allowed conjugating of hapten in different sites.

2.3.1. Conjugating using hapten NH_2 group

2.3.1.1. BSA–ERM(ga), BSA–VCM(ga) and GO–ERM(ga). Two solutions containing 4 mg of BSA (0.06 μmol) in 1 mL of distilled water were supplemented with 25-fold molar excess of ERM and VCM (234 μL and 217 μL, 10 mg/mL solutions, respectively) and 30 μL of freshly prepared 2.5% glutaraldehyde. After incubation at room temperature for 2 h under stirring, 100 μL of 2 mg/mL sodium borohydride was added to each reaction mixture and stirred for another 2 h. Thus obtained conjugates BSA–ERM×25(ga) and BSA–VCM×25(ga) were dialyzed for 2 days against PBS (3×5 L). The immunogen GO–ERM×50(ga) was prepared before using the same procedure.

2.3.1.2. Gel(pi)–ERM, Gel(pi)–VCM. Crystal sodium periodate (2.4 mg, 114 nmol) was added to 18 mg of Gel (2×57 nmol) in 2 mL of 0.01 M acetate buffer (pH 5.0) and mixed for 15 min with magnet stirrer. Oxidized glycoprotein was dialyzed against 2×5 L of 0.01 M acetate buffer (pH 5.0) during the night at 4 °C. The resulting volume of dialyzate was divided into two equal portions, which were supplemented with 2 mL-solution of ERM (2.22 mg) and of VCM (2.06 mg) in CB (pH 9.6). These mixtures of protein and hapten taken in molar ratio 1/25 were stirred for 2 h and then for another 2 h after addition of sodium borohydride solution (0.1 mL, 2 mg/mL). The resultant conjugates were dialyzed exhaustively against PBS.

2.3.2. Conjugating using hapten COOH group

2.3.2.1. BSA–ERM(edc), BSA–VCM(edc). Two portions of VCM 1.3 mg and 4.35 mg (0.9 and 3 μmol, respectively) in 0.5 mL of water were supplemented with 15 mg of EDC and stirred for 30 min. The 1 mL water solutions of BSA (4 mg, 0.06 μmol) were added to activated VCM and mixed during the night. The resultant conjugates BSA–VCM×15(edc) and BSA–VCM×50(edc) were purified from the unreacted ingredients using dialysis. BSA–ERM×50(edc) was prepared using the same procedure detailed in Burkin and Burkin (2009).

2.3.2.2. BSA–ERM(ae) and BSA–VCM(ae). Two solutions containing 4.67 mg of ERM (3 μmol) in 1 mL of DMSO and 4.35 mg of VCM (3 μmol) in 1 mL of DMF were supplemented with 52 μL of N-hydroxysuccinimide (4.5 μmol) and 87 μL of EDC (4.5 μmol) each one from 10 mg/mL solutions in DMF and mixed using magnet stirrer for 1.5 h. The solutions of activated glycopeptides 860 μl (2.25 μmol) were added to BSA (3 mg, 45 nmol) in 0.5 mL of CB (pH 9.6). Thus, the mixtures BSA/hapten prepared with molar ratio 1/50 were incubated overnight under stirring and then dialyzed exhaustively.

2.3.3. Conjugating using hapten phenyl and resorcyl groups

2.3.3.1. BSA–ERM(f), BSA–VCM(f). The synthesis of VCM conjugate using formaldehyde condensation method was made according to the procedure described for BSA–ERM(f) (Burkin and Burkin, 2009). The mixture of BSA (4 mg, 0.06 μmol), VCM (2.34 mg, 3 μmol) and formaldehyde (0.3 mL of 37% solution, 3690 μmol) was incubated overnight at room temperature under stirring and then dialyzed.