Journal of Inorganic Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

The adsorption of allergoids and 3-O-desacyl-4′-monophosphoryl lipid A (MPL®) to microcrystalline tyrosine (MCT) in formulations for use in allergy immunotherapy

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 29 July 2015
Accepted 5 August 2015
Available online xxxx

Keywords:
Adjuvant adsorption
Allergoid
Monophosphoryl lipid A
Microcrystalline tyrosine
Modified allergen tyrosine adsorbed
C–H···π interactions

ABSTRACT

Infectious disease vaccine potency is affected by antigen adjuvant adsorption. WHO and EMA guidelines recommend limits and experimental monitoring of adsorption in vaccines and allergy immunotherapies. Adsorbed allergoids and MPL® in MATA-MPL allergy immunotherapy formulations effectively treat IgE mitigated allergy. Understanding vaccine antigen adjuvant adsorption allows optimisation of potency and should be seen as good practice; however current understanding is seldom applied to allergy immunotherapies.

The allergoid and MPL® adsorption to MCT in MATA-MPL allergy immunotherapy formulations was experimental determination using specific allergen IgE allerginicity and MPL® content methods. Binding forces between MPL® and MCT were investigated by competition binding experiments.

MATA-MPL samples with different allergoids gave results within 100–104% of the theoretical 50 μg/mL MPL® content. Unmodified drug substance samples showed significant desirable IgE antigenicity, 1040–170 QAU/mL. MATA-MPL supernatant samples with different allergoids gave results of ≤2 μg/mL MPL® and ≤0.1–1.4 QAU/mL IgE antigenicity, demonstrating approximately ≥96 & 99% adsorption respectively. Allergoid and MPL® adsorption in different MATA-MPL allergy immunotherapy formulations is consistent and meets guideline recommendations. MCT formulations treated to disrupt electrostatic, hydrophobic and ligand exchange interactions, gave an MPL® content of ≤2 μg/mL in supernatant samples. MCT formulations treated to disrupt aromatic interactions, gave an MPL® content of 73–92 μg/mL in supernatant samples. MPL® adsorption to L-tyrosine in MCT formulations is based on interactions between the 2-deoxy-2-aminoglucose backbone on MPL® and aromatic ring of L-tyrosine in MCT, such as C–H···π interaction. MCT could be an alternative adjuvant depot for some infectious disease antigens.

1. Introduction

Infectious disease vaccines and allergy immunotherapy formulations often include an adjuvant to enhance efficacy, with aluminium salts being the most commonly used adjuvant. The strength and type of adsorption in some infectious disease vaccines, such as anthrax, diphtheria and hepatitis B can affect antigen stability and potency, and therefore should be optimised [1–8]. The World Health Organisation (WHO) recommends adsorption of at least 80% of tetanus and diphtheria toxoid antigen to aluminium adjuvants in vaccines [9].

The WHO and European Medicines Agency (EMA) recommend the degree of antigen to aluminium adjuvant adsorption be characterised on manufacture and over the shelf life of the vaccine [10,11], the presence of human immunoglobulin E (IgE) binding components in the supernatant of adsorbed allergen products should be determined [12] and the content and degree of adsorption of MPL® in Hepatitis B vaccines should be determined using a suitable method e.g., gas chromatography (GC) [13]. The interactions of antigen and adjuvant in infectious disease vaccines have been investigated and documented therefore should be considered good practice in allergy immunotherapy where such understanding is too often not applied [14–16].

The strongest binding force between antigens and aluminium adjuvants is ligand exchange, between the available hydroxyl surface groups on the aluminium adjuvant and terminal phosphates groups on the antigen [17,18].

Formulation conditions such as pH, ionic strength, buffer type and other excipients should be optimised in relation to antigen charge states

Abbreviations: MPL®, monophosphoryl lipid A; MCT, microcrystalline tyrosine; WHO, World Health Organisation; EMA, European Medicines Agency; MATA, modified allergen tyrosine adsorbed; C–H···π, carbon hydrogen delocalised aromatic electron interaction; IgE, immunoglobulin E; GC, gas chromatography; Th2, subset of innate allergen specific immune response; TLR4, toll-like receptor four; IgG, immunoglobulin G; MD2:TLR4, myeloid differential protein 2:toll-like receptor four complex; C–H, carbon hydrogen; GMP, good manufacturing practice; WFI, water for injections; UK, United Kingdom; Ltd, limited; Ph. Eur, European Pharmacopeia; RPM, revolutions per minute; RCF, relative centrifugal force; FID, flame ionisation detector; PSI, pounds per square inch; ELISA, enzyme-linked immunosorbent assay; DPBS, Dulbecco's phosphate buffered saline; BSA, bovine serum albumin; QAU, quality assurance units; MPL-AF, monophosphoryl lipid A aqueous formulation; SD, standard deviation; %RSD, percentage relative standard deviation; π, delocalised aromatic electrons; Plc, public limited company.

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http://dx.doi.org/10.1016/j.jinorgbio.2015.08.007

or isoelectric points, to provide appropriate vaccine adsorption, stability and potency [19–22]. Commonly aluminium adjuvants are formulated in excess with the antigen or allergen, to ensure sufficient adsorption and increased efficacy.

However the inflammatory response produced by aluminium adjuvants may not be aligned with the Th2 hypothesis in allergy treatment [23–26].

Safety concerns regarding the accumulation of aluminium following multiple sub-cutaneous allergy immunotherapy injections [27–29] and the need for more appropriate adjuvants in allergy immunotherapy formulations, has led to the use of toll-like receptor (TLR4) agonist monophosphoryl lipid A (MPL®) [30–33]. Glutaraldehyde modified allergenic extracts (allergoids) from grass, tree and ragweed pollens have reduced IgE reactivity, but maintain IgG reactivity [34] and are readily adsorbed to the microcrystalline tyrosine (MCT) in modified allergen tyrosine adsorbed (MATA) formulations. Clinical studies have shown that the addition of MPL® to MATA allergy immunotherapy formulations provides a more effective treatment for IgE mediated allergy [35–40]. The contribution of electrostatic, hydrophobic and ligand exchange binding forces between antigens and aluminium adjuvants has been investigated by the addition of ethylene glycol and ionic species [41,42]. The nature of allergoid and MPL® association in commercially available MATA-MPL allergy immunotherapy formulations has not previously been investigated and compared to guideline recommendations.

The structure of MPL® consists of a B′1 -> 6-linked disaccharide backbone of 2-deoxy-2-aminoglucose phosphorylated at the 4′ position, which differs in the length of fatty acid substitutions at the 2, 2′ and 3′ positions [43], see Fig. 2. The distinct hydrophilic 2-deoxy-2-aminoglucose phosphorylated and hydrophobic fatty acid chain regions of MPL® lead lipid A motifs to form aggregates in aqueous solutions [44,45]. The formation and shape of these aggregates is essential to MD2:TLR4 complex dimerization and species specific pro-inflammatory cytokine pathway activation [46,47]. Carbohydrate protein interactions have been shown to depend significantly on C–H···π attraction between the saccharide structures in carbohydrates and aromatic amino acids in proteins including L-tyrosine [48–50].

Studies have shown that saccharide structures with three or more upward facing C–H groups like the 2-deoxy-2-aminoglucose in the MPL® backbone can interact with aromatic centres through C–H···π attraction and this is the predominant associated seen within carbohydrate protein stacked structures [51]. Commercially available MATA-MPL allergy immunotherapy formulations were tested for adsorption of MPL® and allergoid, for comparison against guideline recommendations. Specific MPL® content and allergen IgE reactivity methods used are presented. The nature of the adsorption of MPL® to MCT is investigated and the possible mechanism of adsorption between MPL® and MCT is described in the context of experimental results seen.

2. Experimental

2.1. Materials

2.1.1. 100 μg/mL MPL® aqueous formulation

Commercially available good manufacturing practice (GMP) grade (Avanti Polar Lipids Inc.) triethylamine salt of MPL® (100 mg) and surfactant dipalmitoyl phosphatidyl choline (11 mg) were dispersed in 1 L of water for injections (WFI) to give concentrations of 100 μg/mL and 11 μg/mL, respectively. Microfluidisation with an Avestin Emulsiflex C/55 at 130 revolutions per minute (RPM) for 30 min was used to give a homogeneous aqueous formulation, before sterile filtration with a 0.22 μm filter (Sterile Millipak®-200). This solution was used as a standard in the Gas Chromatography (GC) MPL® content assay [52].

2.1.2. MATA-MPL immunotherapy formulations

Commercially available MATA-MPL immunotherapy formulations for grass, birch, tree, grass & tree, grass & birch and grass & mugwort were all sourced from Allergy Therapeutics (UK) Ltd and contain 2% w/v tyrosine, 0.5% w/v phenol, 50 μg/mL MPL® and a clinically standardised specific allergoid amount. These MATA-MPL immunotherapy formulations were used to investigate MPL® and allergoid adsorption. MPL® content was tested in both formulation and supernatant, while allergen specific IgE reactivity was tested for in the supernatant.

2.1.3. Unmodified drug substance

Unmodified drug substance solutions were sourced from Allergy Therapeutics (UK) Ltd and comprised of 5% w/v grass, tree, birch and mugwort pollen species aqueous extracts in Evans solution (pH 7). The Evans solution consisted of commercially available (Fisher Scientific Ph. Eur.) anhydrous salts of disodium hydrogen phosphate (0.121% w/v), potassium dihydrogen phosphate (0.03% w/v), sodium chloride (0.417% w/v) and phenol (0.60% w/v) in WFI. Evans solution and aqueous pollen extracts were sterile filtered to 0.22 μm (Sterile Millipak®-200). Manufacture and source materials comply with EMA and European Pharmacopeia (Ph. Eur.) guidelines [12,53]. These unmodified drug substance solutions were used to confirm allergen specific IgE reactivity for comparison to MATA-MPL supernatant results.

2.1.4. MCT test formulation (4% w/v L-tyrosine and 0.5% w/v phenol)

Commercially available (Fisher Scientific Ph.Eur.) sodium hydroxide pellets (152 g) were dissolved volumetrically in 1 L of WFI giving a 3.8 M sodium hydroxide solution. Commercially available (Sigma. Ph. Eur.) 37% (36.46 g/mol) hydrochloric acid (138.55 g) was diluted volumetrically in 1 L of WFI giving a 3.8 M hydrochloric acid solution. Commercially available (Ph. Eur.) L-tyrosine (240 g) was dissolved volumetrically in 1 L of 3.8 M hydrochloric acid, to give a 24% w/v L-tyrosine in 3.8 M hydrochloric acid solution.

Sodium hydroxide and hydrochloric acid solutions were sterile filtered to 0.22 μm (Sterile Millipak®-200). MCT was precipitated by adding together equal volumes of 3.8 M sodium hydroxide and 24% w/v L-tyrosine in 3.8 M hydrochloric acid solution, using a Silverson homogeniser at 6000 RPM and equal flow rates of 50 mL/min using Flexicon peristaltic pumps. The resulting 12% w/v L-tyrosine suspension was then diluted 1:4 with a sterile filtered to 0.22 μm (Sterile Millipak®-200), Evans solution (pH 7). The MCT test formulation without MPL® was used in MPL® competition binding experiments.

2.1.5. Grass MATA test formulation

Grass MATA formulation without MPL® was sourced from Allergy Therapeutics (UK) Ltd and comprises of a 13 grass species drug substance Evans solution (pH 7) aqueous extract, modified with aqueous 0.05% v/v glutaraldehyde (Fisher Scientific Ph. Eur.). Modified drug substance aqueous extracts are diafiltered using Millipore Pelican 2 ultrafiltration cassettes, to remove low molecular weight moieties. The allergoids in the aqueous extract are adsorbed to MCT on precipitation and then diluted with MCT to the required clinical strength. Manufacture and source materials comply with EMA and Ph. Eur. guidelines [12,53]. The Grass MATA test formulation without MPL® was used in MPL® competition binding experiments.

2.2. Methods

All chemicals and reagents used in methods are commercially available analytical grade unless otherwise stated.

2.2.1. MPL content assay

Samples were prepared and tested in duplicate. Test formulations and MATA-MPL samples were mixed for 10 min on a roller mixer at 35 RPM. Following mixing 1 mL aliquots were taken for MPL® content testing of the formulation. Test formulations and MATA-MPL samples

Please cite this article as: A.J. Bell, et al., The adsorption of allergoids and 3-O-desacyl-4′-monophosphoryl lipid A (MPL®) to microcrystalline tyrosine (MCT) in formulation..., J. Inorg. Biochem. (2015), http://dx.doi.org/10.1016/j.jinorgbio.2015.08.007