A Brief Report on Immunoassay for 3-chloro-1,2-propanediol and its Esters Derivatives in Rapeseed Oil

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Abstract

(Background) The spontaneous formation of the carcinogen 3-chloropropane-1,2-diol (3-MCPD) in a wide range of cooked foods has recently been discovered. The formation of bound 3-chloro-1,2-propanediol (3-MCPD-ester) was also found in rapeseed oils during refining.

(Objectives) Analysis of 3-MCPD and its esters derivatives has been difficult because of the diminutive size and isomers in the family and the relatively low application level. Immunoassays have been developed for monitoring of these compounds with the merits of rapidity, sensitivity and relatively low cost. The aim of this study is to provide a highly efficient immunoassay approach for the detection of 3-MCPD or its esters derivatives in rapeseed oil.

(Methods) A polyclonal antibody capable of binding the carcinogen was produced by the synthesis of an immunogen comprising 3-MCPD derivatization of 3-chloropropane-1,2-diol as carboxyphenylboronate, and its conjugation to the carrier protein bovine thyroglobulin. Extracted 3-MCPD from the rapeseed oil was then subjected to a rapid 40min derivatisation with phenylboronic acid, pre-analysis. Antiserum from the immunized rabbit was harvested and fully characterised.

(Results and Conclusion) In our experiment, it displayed no affinity for 3-MCPD or bovine thyroglobulin, but for the derivative of 3-MPCD and phenylboronic acid with high affinity. Further biological evaluation and mechanistic studies on the new analytic techniques for the detection of 3-MCPD and its esters derivatives in rapeseed oil are currently being studied and will be reported.

Keywords: rapeseed oil, 3-chloro-1,2-propanediol (3-MCPD), immunoassay, antibody

1. Introduction

3-Chloropropane-1,2-diol (3-MCPD) is a food borne contaminant that was identified in acid-hydrolysed vegetable protein (acid-HVP) in 1980 (Velíšek et al., 1980)and recently showed to be a substance which exhibits genotoxic effects in in-vitro tests but not in in-vivo studies. JECFA recommended a maximum tolerable daily intake of 2 mg/kg BW (Opinion, 2001). So far, soy products especially hydrolyzed soy proteins in sauces were found with higher amounts of free 3-MCPD and for these products a provisional maximum level of 0.02 mg/kg on a liquid basis was fixed by the European Commission (Commission regulation 466/2001). A provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bodyweight per day was recommended for 3-MCPD by JEFCA and SCF (Opinion, 2001).

3-MCPD was detected in many other types of food, like bakery products, meat and fish products, and soups (Hamlet et al., 2002). A part of 3-MCPD is ester-bound with fatty acids (Svejkovska et al., 2004). Recently, the occurrence of high levels of 3-MCPD-esters (monoesters and diesters with higher fatty acids) in some edible oils has been reported(Zelinková et al, 2006). Analysis of unrefined, de-gummed, bleached, and deodorized rapeseed oil showed that the level of bound MCPD changed during the refining process(Franke et al., 2009).

So far, many methods have been developed for the quantitative determination of 3-MCPD and its esters derivatives. Most of them demand sophisticated instruments such as gas chromatography (GC) flame ionization detection (Plantinga et al., 1991) and electron capture detection (Matthew et al., 2000), GC combined with mass spectrometric detection (Wenzl et al., 2007). The conventional pretreatment method for 3-MCPD in soy sauce or other matrices consists of purification with a column loaded with diatomaceous earth or an Extrelut column, followed by concentration and derivatization with, e.g., butaneboronic acid, heptafluorobutyric acid anhydride. The pretreatment procedures are not only tedious and troublesome but also require large volumes of organic solvents with influence on the

detection limit of trace analysis. However, the high demand for skillful operators and the narrow dynamic detection limits its wide application.

Owing to the complexity of existing methods, the development of a low-cost, simpler immunoassay is strongly needed(Li et al., 2009). A major reason for this is the lack of strong epitope groups in the 3-MCPD molecule. Besides, direct conjugation of 3-MCPD to a carrier protein may result in low efficiency and even cause further loss of the limited antigenic epitopes. To address such an issue, we propose a unique strategy to synthesize a complete antigen for 3-MCPD by using 4-carboxyphenylboronic acid as the hapten instead of 3-MCPD itself. By using the above approaches, we successfully obtained antibodies with satisfactory affinity and specificity towards the derivative of 3-MCPD and phenylboronic acid.

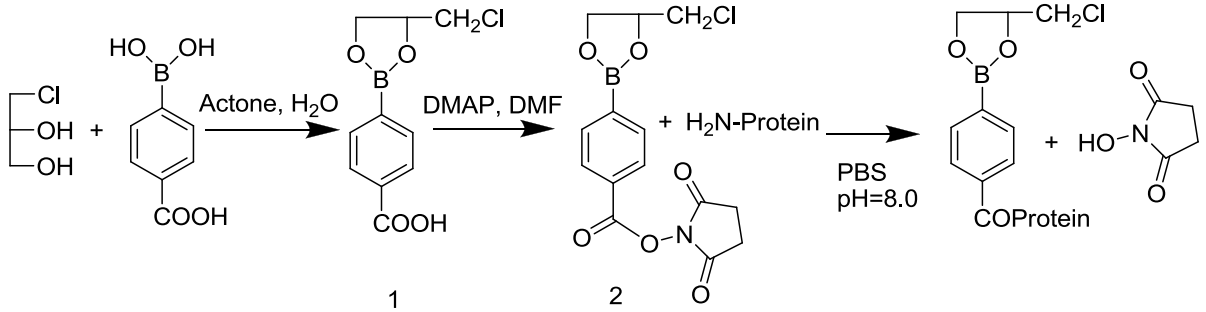
2. Materials and methods

2.1. Chemicals and instruments

The 3-MCPD standard, 4-carboxyphenylboronic acid and chemicals for hapten synthesis were obtained from Aladdin Chemical Co., Ltd. (Shanghai, China) or Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). Biochemicals of Goat anti-mouse IgG-HRP, BSA, culture elements were purchased from Sigma-Aldrich (USA). The ELISA was carried out in 96-well polystyrene microplates (Costar, USA) and red by a SpectraMax M2e microplate reader (Molecular Devices, USA).

2.2. Preparation of the complete antigen

Synthesis of hapten was carried out and illustrated in Fig. 1. 4-carboxyphenylboronic acid (112 mg, 0.68 mmol) and 3-MCPD (75 mg, 0.68 mmol) were dissolved in 4mL actone/H_2O (19:1). The reaction mixture was stirred at 90°C for 30 min. The reactant was evaporated to dryness under reduced pressure to obtain the product as light yellow solid in nearly quantitative yield. The NHS (14 mg, 0.06 mmol) was added to the solution of hapten (9.6 mg, 0.04 mmol) in 0.8mL of dry DMF, then the mixture reacted for 1 h. The DCC (20 mg, 0.08 mmol) in 0.2mL of
DMF was added dropwise to the aforementioned hapten/NHS solution. After the reaction mixture was stirred overnight at room temperature, the precipitate was removed by centrifugation. The 20 mg of BSA and OVA were dissolved in 10mL of PBS (pH 8), respectively. Aliquots of the activated hapten solution were separately added dropwise to the two protein solutions. The mixture was stirred for 30 min at room temperature, then overnight at 4°C, then dialyzed against PBS over 72 h at 4°C and stored in aliquots at -20°C.



1 2

Figure 1 Synthetic schemes for the compound 1, 2 and immunogen of 3-MPCD

2.3. Production of the antibodies

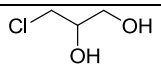
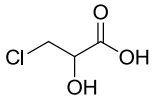
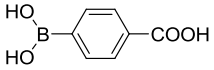
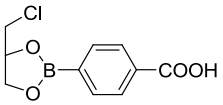
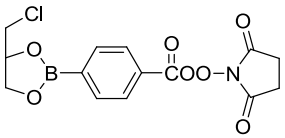
The mice were immunized with the above hapten–BSA conjugate. One week after the last injection, the mice were eye-bled, the antihapten antibody titers of the sera were tested by indirect ELISA and the analyte recognition properties were examined by competitive indirect ELISA according to the description of Zhang's(Zhang et al., 2007).

3. Results

Characterization of polyclonal antibodies

Specificity of mAb were tested and both of them shown little or no cross-reactivity (0.0-27.1%) with other tested compounds and their derivatives.

Table 1 Cross-reactivity of the antibody with acrylamide and structural analogues

Analyte	Structure	Cross-reactivity (%)
3-MPCD		0.0
β-Chlorolactic acid,		<0.01
4-carboxyphenylboronic acid		7.1
Compound 1		100
Compound 2		55.4

4. Conclusion

In our experiment, it displayed no affinity for 3-MCPD or bovine thyroglobulin, but for the derivative of 3-MPCD and phenylboronic acid with high affinity. Further biological evaluation and mechanistic studies on the new analytic techniques for the detection of 3-MCPD and its esters derivatives in rapeseed oil are currently being studied and will be reported.

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