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Micellar electrokinetic capillary chromatography—Synchronous monitoring of substrate and products in the myrosinase catalysed hydrolysis of glucosinolates

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Abstract

A micellar electrokinetic capillary chromatography (MECC) method has been developed for monitoring the myrosinase catalysed hydrolysis of 2-hydroxy substituted glucosinolates and the simultaneous formation of the corresponding degradation products (oxazolidine-2-thiones (OZTs) and nitriles). Glucosibarin ((2*R*)-2-hydroxy-2-phenylethylglucosinolate) was chosen as the model glucosinolate owing to the difficulties in determining hydrolysis rates of this type of substrates in traditional UV-assays. The method was afterwards validated with glucobarbarin ((2*S*)-2-hydroxy-2-phenylethylglucosinolate) and progoitrin ((2*R*)-2-hydroxybut-3-enylglucosinolate). Aromatic glucosinolates without a 2-hydroxy group in their side chains, such as glucotropaeolin (benzylglucosinolate) and gluconasturtiin (phenethylglucosinolate) were also tested. Formation of the glucosinolate hydrolysis products was monitored simultaneously at 206 nm and 230 nm. This allowed estimation of the extinction coefficient of the OZT derived from glucosibarin, which was found to be 18,000 M^{-1} cm^{-1} and 12,000 M^{-1} cm^{-1} at 206 nm and 230 nm, respectively. The developed method has limit of detection of 0.04 mM and 0.06 mM and limit of quantification of 0.2 mM and 0.3 mM for the glucosibarin derived OZT and nitrile, respectively. Linearity of the glucosinolate concentration was examined at six concentration levels from 2.5 mM to 100 mM and at 206 nm a straight line ($R^2 = 0.9996$) was obtained. The number of theoretical plates (N) at the optimal system conditions was 245,000 for the intact glucosibarin, 264,000 for the OZT and 252,000 for the nitrile.

Keywords: Glucosinolates; Myrosinase; Nitriles; OZTs; MECC; Synchronous method

1. Introduction

Glucosinolates are alkyl-*N*-hydroximine esters with a β-D-thioglucopyranoside group attached to the hydroximine carbon in *Z* configuration to the sulphate group [1–3]. These allelochemicals are present in all plants of the order Capparales [4] and have as well been reported in some few other species [1,5]. Myrosinase isoenzymes (EC 3.2.1.147) co-exist with glucosinolates and catalyze the hydrolysis of the β-D-thioglucopyranoside bond releasing an aglucone that further rearranges to a variety of products depending on the parent glucosinolate and the environmental conditions [6–10]. At neutral pH, aliphatic glucosinolates generally yield isothiocyanates, while at acidic pH or in the presence of ferrous ions the formation of nitriles is favoured (Fig. 1) [11–16].

Glucosinolate-containing plants have traditionally attracted much attention due to the physiological effects of the various glucosinolate degradation compounds. Oxazolidine-2-thiones (OZTs), especially goitrin from progoitrin (Fig. 2), have appreciable antinutritional or negative effects on the quality of food and feed used for monogastric animals, whereas other OZTs do not have so strong physiological effects [17,18]. Other glucosinolate and isothiocyanate derived products, especially sulphoraphane from glucoraphanin and its dithiocarbamates, and the degradation compounds of the indol-3-ylmethylglucosinolates have been reported to have antioxidant and anticarcinogenic properties [6,19–21]. Isothiocyanates have also been found to be fungicidal [22,23], nematocidal [24] and bactericidal [25]. The great variety of physiological effects of the different glucosinolate derived compounds calls for further attention, especially with respect to the factors controlling glucosinolate hydrolysis and the conditions under which the different transformation products are produced. The development of analytical methods able to

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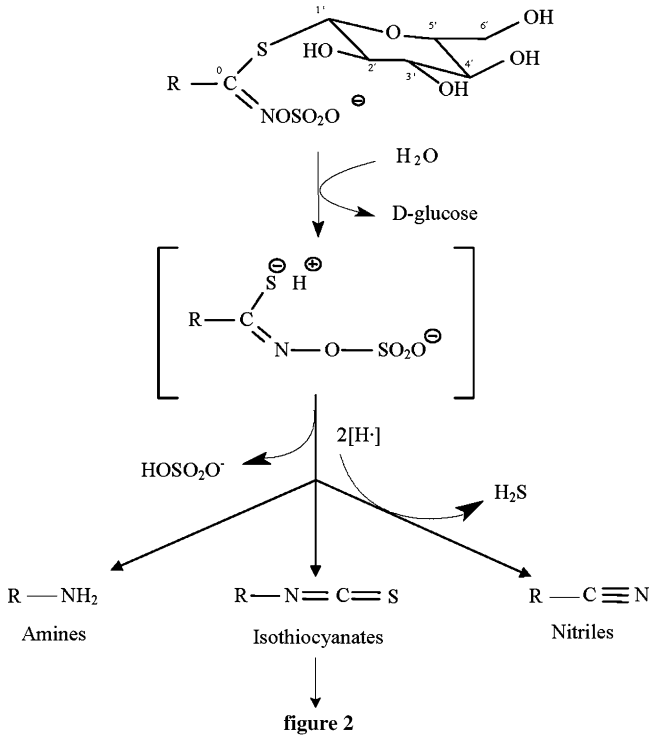

Fig. 1. Glucosinolate degradation by myrosinase and products obtained from aliphatic glucosinolates [6].

determine the compounds produced under different conditions and capable of studying the role that the type of myrosinase, cofactors and additional proteins play in the hydrolysis of glucosinolates is therefore of great importance in this respect.

Numerous methods have been developed for the measurement of the activity of myrosinase. Indirect methods involve the measurement of the released products, such as glucose [26] isothiocyanates and nitriles [1,2,14]. GC, LC and HPLC techniques for determination of glucosinolates and glucosinolate degradation products require in most cases more or less time consuming sample preparation steps prior to the chromatographic separation [3,5,14,27–29]. Micellar electrokinetic capillary chromatography (MECC) has also been developed as a method of analysis for determination of glucosinolates, desulphoglucosinolates [30,31] and some of the glucosinolate degradation products, such as OZTs and nitriles [32–34]. There is however, no GC, HPLC or HPCE method described up to date for the simultaneous determination of glucosinolates and their degradation products. Direct methods have been used both for determination of myrosinase activity and kinetic parameters, such as the determination of liberated acid, which is titrated with alkali using pH-stat apparatus (pHSA) [35], direct spectrophotometric assay (DSA) of glucosinolate degradation [12,36], the spectrophotometric coupled enzyme assay (SCEA) of liberated glucose [37,38] and the polarographic coupled assay (PCA) [39]. The pHSA, the DSA and the SCEA are used in routine analysis of myrosinase activity of *Brassica* extracts due to their being simple, fast and inexpensive. However, despite their accuracy in the quantitative determination of the amount of glucosinolate degraded in assays based on only one glucosinolate, they are neither able to determine differences in the speed of degradation of glucosinolate mixtures nor the lack of degradation of thioglucoside substituted glucosinolates [3,5]. Furthermore, these methods do not allow determining the types of products formed and in which proportions they are produced.

In recent years, capillary electrophoresis has been increasingly used for monitoring of enzymatic reactions [40–42], both for determination of the total amount of reaction products [43] as well as for the determination of the enzyme kinetic parameters and the efficiency of the reaction [44,45]. In the present study, we have developed a method for the simultaneous monitoring of the hydrolysis of glucosinolates by myrosinase and the formation of the degradation compounds. The method is based on the separation of glucosinolates in crude extracts by MECC [34]. Glucosibarin ((2*R*)-2-hydroxy-2-phenylethylglucosinolate) was chosen as the model glucosinolate and the method was afterwards validated with other glucosinolates, such as glucobarbarin ((2*S*)-2-hydroxy-2-phenylethylglucosinolate), progoitrin ((2*R*)-2-hydroxybut-3-enylglucosinolate), glucotropaeolin (benzylglucosinolate) and gluconasturtiin (phenethylglucosinolate). Formation of the corresponding degradation products was mon-

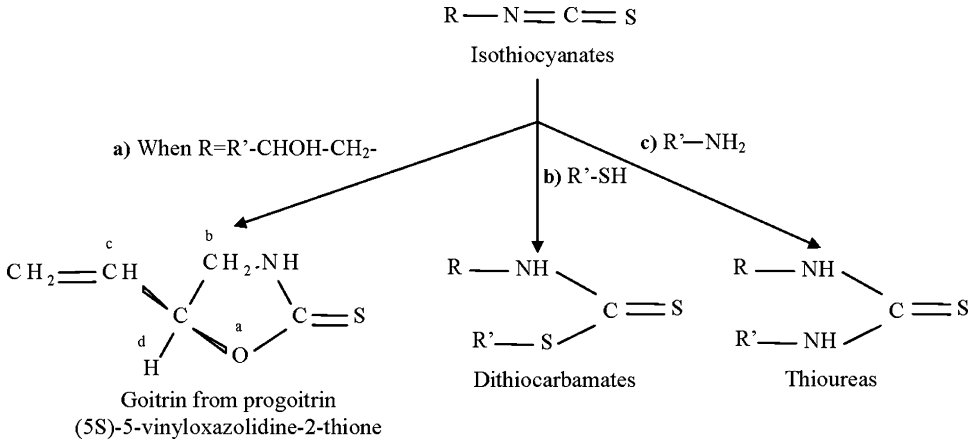

Fig. 2. Products formed from isothiocyanates: (a) the isothiocyanates derived from glucosinolates with a 2-OH group cyclise spontaneously to form the corresponding OZT; (b) dithiocarbamates are formed by the reaction of isothiocyanates with thiol groups; (c) thioureas are formed by the reaction of isothiocyanates with free amino groups.