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Influence of pH and type of myrosinase complex on the products obtained in the myrosinase catalysed hydrolysis of glucosinolates – a MECC study

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Environmental conditions, e.g. pH and the presence of Fe^{2+} are well known factors that influence the product profile of the myrosinase catalysed hydrolysis of glucosinolates. Depending on the plant genera, the species and tissue of origin myrosinase isoenzymes (thioglucohydrolase EC 3.2.1.147) have different characteristics in terms of MW, subunit composition and pI. However, the influence of these parameters on the outcome of glucosinolate hydrolysis has not been traditionally studied, which hinders the full exploitation of the catalytic potential of these enzymes. In the present experiments the effect of myrosinase type on the products obtained in the hydrolysis of glucosibarin was studied by MECC using two *B. carinata* myrosinase preparations differing on their affinity to the Con A material, Con A 1 (first eluting fractions) and Con A 2 (last eluting fractions). At pH 3 Con A 1 isoenzymes were more active than Con A 2 isoenzymes. At pH 5 and 6.5 Con A 1 isoenzymes produced oxazolidine-2-thione to a higher extent than Con A 2 isoenzymes. The production of nitriles by Con A 1 isoenzymes was not influenced by pH and at pH 5 and 6.5 the amount of nitrile produced by Con A 1 isoenzymes was lower than that produced by Con A 2 isoenzymes. Formation of nitriles requires the presence of two redox equivalents which leads to the release of the sulphur atom from the aglucone. Isothiocyanates and nitriles differ in their bioactivity towards different targets; therefore the possibility for directing the glucosinolate hydrolysis towards the desired compound in a particular situation is of great relevance.

Introduction

Glucosinolates are amino acid derived allelochemicals present in all plants of the order Capparales. They co-exist with myrosinase isoenzymes (EC 3.2.1.147), which are oligomeric glycoproteins that catalyze the hydrolysis of the β-D-thioglucopyranoside bond. The aglucone released in the hydrolysis of the glucosinolate further rearranges to a variety of products depending on the parent glucosinolate and the environmental conditions (Sørensen, 1990; Bjergegaard et al., 1994). The great variety of physiological effects of the different glucosinolate derived compounds makes it very relevant to study the factors controlling glucosinolate hydrolysis and the conditions under which the different transformation products are produced. It is well established that pH and the presence of certain cofactors have an influence on the compounds formed, e. g. at neutral pH aliphatic glucosinolates generally yield isothiocyanates, while at acidic pH or in the presence of Fe^{2+} the formation of nitriles is favoured (Sørensen, 1990; Bjergegaard et al., 1994). Myrosinase isoenzymes have different molecular weight, pI and subunit composition (Bellostas et al., 2003), however, little is known about whether these parameters influence the type and quantity of compounds formed upon glucosinolate hydrolysis. In a previous study we have developed a method for the on-line monitoring of the myrosinase hydrolysis of glucosinolates which allows for the simultaneous detection of the degradation compounds (Bellostas et al., 2006). With the use of this method in the present

experiments we have studied the influence of the type of myrosinase isoenzymes on the outcome of the hydrolysis of glucosibarin at three pH values (3, 5 and 6.5).

Materials and methods

Glucosinolates and myrosinases

The intact glucosinolates were from the laboratory collection (Sørensen, 1990, Sørensen, 2001). Myrosinase isoenzymes were obtained from *B. carinata* cv. BRK-147-A by the method used in our laboratories (Bellostas et al., 2003). After affinity chromatography by Con A, two pools were made (Figure 1): Con A 1 (first six fractions from the Con A column) and Con A 2 (seven last fractions from the Con A column).

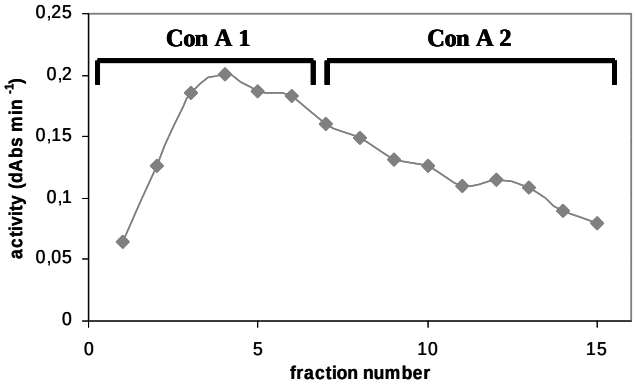

Figure 1. Activity (dAbs min^{-1} at 227 nm) of the fifteen different fractions eluted from the Con A affinity column.

CE instrumentation, buffer and procedure for the in-vial reaction

The CE instrumentation, buffer composition and procedure used for the in-vial reaction have been described elsewhere (Bellostas et al., 2006).

Results and discussion

Profile of degradation products depending on pH and myrosinase type

As previously described (Sørensen, 1990) pH has a great influence on myrosinase activity and therefore the rate of degradation of glucosibarin by both groups of isoenzymes decreased when pH was decreased from 6.5 to 3. Differences between the two groups of isoenzymes could still be observed, and whereas no activity of Con A 2 isoenzymes was detected at pH 3, Con A 1 isoenzymes still degraded glucosibarin at this low pH. At pH 6.5 and 5 Con A 1 isoenzymes produced OZT almost in a linear fashion with time, whereas Con A 2 isoenzymes seemed to produce OZT only in the first minutes of the reaction. At pH 3 only low amounts of OZT were produced by both isoenzyme groups (Figure 2 above). At pH 5 and 6.5 Con A 2 isoenzymes produced the nitrile in a higher concentration than Con A 1 isoenzymes, the highest amount of nitrile being produced by Con A 2 isoenzymes at pH 5. At pH 3 Con A 2 isoenzymes produced the nitrile only up to a plateau, probably due to a low activity of the isoenzymes at this pH. Although the final amount of nitrile produced at the

different pHs by Con A 1 isoenzymes was very similar, this compound was produced at different moments in time: the lower the pH the earlier the nitrile was produced (Figure 2 below).

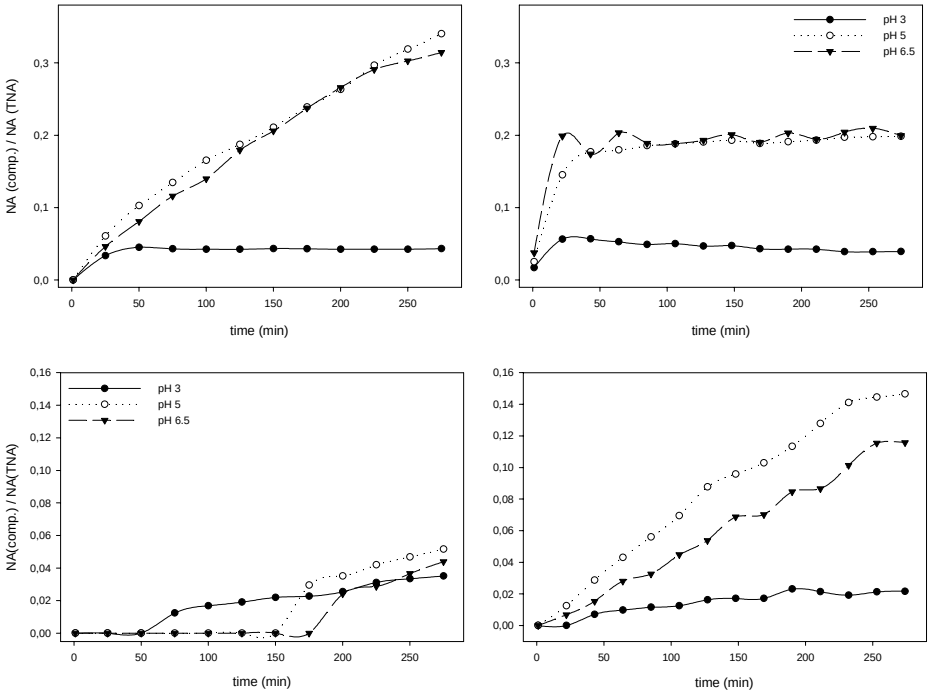

Figure 2. Production of the OZT (above) and the nitrile (below) at three different pH by Con A 1 (left) and Con A 2 (right) isoenzymes. Values are presented as relative normalized area of the compound with respect to TNA.

Physico-chemical properties of myrosinase isoenzymes and their relation to the profile of glucosinolate hydrolysis products

Con A 1 and Con A 2 isoenzymes groups are retained in the affinity chromatography column to different extents, therefore it is likely that differences in the glycosylation level of the protein subunits play a role in the activity of the isoenzymes. Figure 3 shows the SDS-PAGE gels of *B. carinata* Con A 1 and Con A 2 isoenzymes groups before and after G-200 gel filtration (Bellostas et al., 2003). Although showing very similar profiles, a number of subunits present in one group of isoenzymes seem to be absent in the other one and vice versa (see arrows). Whether a different protein profile of the myrosinase complexes may have an influence on the myrosinase catalysed glucosinolate hydrolysis to the extent of changing the product profile has not been described, although the presence of some proteins in the myrosinase complexes leads to the production of special compounds, such as epithionitriles (de Torres Zabala et al., 2006).

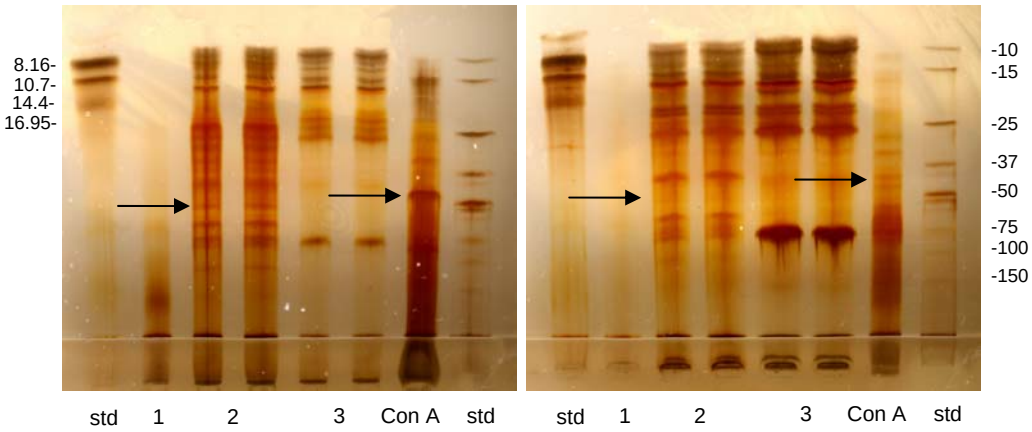

Figure 3. SDS-PAGE of *B. carinata* isoenzymes after G-200 gel filtration. Left: Con A 1 isoenzymes. Right: Con A 2 isoenzymes. Legend: std (standard), 1, 2 and 3 (first, second and third peaks in G-200), Con A (Con A pool before G-200 gel filtration).

Conclusions

The MECC method allowed for the observation of different degradation patterns of the two groups of isoenzymes that would not have been observed with a standard spectrophotometric assay, which confirms the validity of the method developed. The different pools of myrosinase isoenzymes obtained after Con A affinity chromatography produced different proportions of OZT and nitrile at the three pH studied. This opens the door for further research, as the use of different myrosinase isoenzymes may allow directing the hydrolysis of glucosinolates towards the wanted compounds.

Acknowledgement

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