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Screening for hydroxynitrile lyase activity in crude preparations of some edible plants

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Abstract

Enzymatic crude preparations obtained from a variety of food plants were screened for hydroxynitrile lyase activity. The results show that crude material from the seeds of the fruits quince, melon, anona and cherimoya as well as the material from the leaves of plum, peach, cherry and mamey biocatalyze the addition of hydrogen cyanide (HCN) to benzaldehyde with good to excellent enantioselectivities.

Keywords: Cyanohydrins; Cyanogenesis; Hydroxynitrile lyase

1. Introduction

Some living organisms produce hydrogen cyanide to protect themselves against predators [1]. To date, about 3000 plant species are known to release hydrogen cyanide spontaneously from their tissues, activity commonly known as cyanogenesis [2]. When the subcellular structures of the plants are injured or destroyed, several enzymes gain access to the cyanogenic glycosides, which originally are located in separate cell compartments [2,3]. The release of HCN during cyanogenesis is a two-step process; in the first step, the sugar moiety is cleaved by one or more β-glycosidases. In the second step the resulting cyanohydrin, which is relatively unstable, undergoes further degradation either spontaneously or by the action of an α-hydroxynitrile lyase to produce HCN and the corresponding carbonyl compound [4,5]. As many other catalysts, enzymes are able to catalyze chemical reactions in both directions and therefore, α-hydroxynitrile lyases can be used for the preparation of cyanohydrins from aldehydes and a suitable source of HCN.

In 1908, Rosenthaler reported the use of an enzymatic preparation obtained from almonds as the source of hydroxynitrile lyase (HNL) to catalyze the enantioselective synthesis of (R)-mandelonitrile [6]. Later Pfeil et al. [7] informed the preparation of some cyanohydrins using the same source of HNL and mixtures of EtOH–H_2O as the reaction solvent. However, only some substrates afforded cyanohydrins with good optical purities under these reaction conditions. Only recently, after Effenberger et al. [8] reported that the uncatalyzed addition of HCN, observed during the aforementioned biotransformation, could be suppressed by the use of commonly employed organic solvents, and the seminal work by Griengl et al. [9] on the use of (S)-hydroxynitrile lyases from *Hevea brasilensis* for the preparation of cyanohydrins on large scales, this approach has become a practical synthetic methodology which can provide this type of compounds with high optical purity. Nowadays, pure (R)- or (S)-hydroxynitrile lyases (HNLs) or their corresponding crude form, have been used for the synthesis of several optically active cyanohydrins from aldehydes [10], ketones [11,12] as well as fluorinated mandelonitriles [13] and cyanohydrins derived from silicon containing ketones [14].

Cyanohydrins have been used as starting materials during the synthesis of pharmaceuticals and agrochemicals and several methods for the preparation of the enantiopure version of this class of compounds have been reported in the literature [15]. Between them, the enzymatic approaches have become popular because a wide array of substrates can be used and the transformations usually show high

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enantio-, chemo- and regioselectivities [16]. Excellent reviews in this area have been published in recent years [6,16–19].

As part of our ongoing research in this field, namely the search for new sources of this kind of enzymes, we account in this paper the results of the screening for α-hydroxynitrile lyase activity in several plants using the following selection criteria: (i) plants where the presence of cyanogenic glycosides is well established, (ii) plants taxonomically related to other known sources of HNL activity and (iii) readily available plants.

2. Materials and methods

The reagents and solvents were purchased from Baker or Aldrich and were used without further purification. ^{1}H NMR spectra were recorded on a 400 MHz Varian instrument in $CDCl_3$ using tetramethylsilane (TMS) as the internal reference. The optical purity was measured by the analysis of underivatized mandelonitrile using a CHIRACEL OD column (eluent: *n*-hexane/isopropanol 95:5) in a Hewlett Packard 1050 instrument.

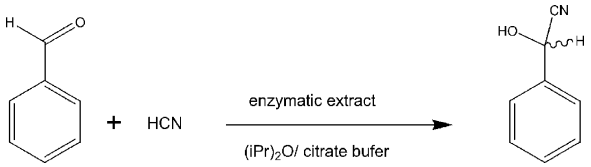
2.1. Preparation of the crude enzymatic materials

The selected seeds were obtained from the commercially available fresh fruits and the leaves were collected from the corresponding garden tree.

In order to remove water, greasy material and some pigments that could interfere with the determinations, the plant material (seeds or leaves) was blended sequentially three times with enough acetone to completely cover the material. After discarding the solvent each time, the resulting solid was air-dried in the fumehood and stored in tight closed jars at 5 °C.

2.2. General procedure for the biotransformation of benzaldehyde into mandelonitrile

In a typical experiment, 1.5 mL of a 1.0 M solution of KCN in a citric acid buffer (pH 4.0) was extracted twice with diisopropyl ether (3.0 mL each time). The organic extracts were combined and added to a vessel containing the enzyme preparation (150 mg) [20], 0.1 M citrate buffer (1%, v/v, pH 4.0) followed by the addition of benzaldehyde (100 mg, 0.94 mmol). The resulting mixture was magnetically stirred at room temperature for 24 h, dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure and the enantiomeric excess of the crude product was determined by HPLC; the conversion percentage was measured by ^{1}H NMR (comparing the area of the signal at 9.95 ppm corresponding to the aldehyde proton with the area of the singlet at 5.54 ppm corresponding to HCCNOH). Gerrits et al. [21] have demonstrated the feasibility of determining both the conversion and the enantiomeric excess during the preparation of several chiral cyanohydrins without O-protection of the corresponding product. The racemic mandelonitrile used as the reference during the measurings was prepared from benzaldehyde according to a reported procedure [21].



Scheme 1.

3. Results and discussion

We selected the synthesis of mandelonitrile from benzaldehyde (Scheme 1) for the evaluation of the hydroxynitrile lyase activity of each crude preparation, and the results are shown in Table 1.

The seeds of capulin (*Prunus serotina* var *capuli*) and almond (*Prunus amygdalus*), were used as standard and according to the results shown in Table 1, only melon, quince, cherimoya and anona seeds, as well as the leaves of peach, cherry, plum, capulin and mamey biocatalyzed the conversion of benzaldehyde into mandelonitrile. The lack of enantioselectivity and the low conversions using the seeds of guaje, bonete, huisache, tejocote, canistel and chico zapote were presumably due to unwanted non-biocatalyzed chemical reactions. Some evidence to support this hypothesis was obtained by performing the reaction in the absence of the source of biocatalyst observing only conversions between 6 and 13%.[1] Unexpectedly hydroxynitrile lyase activity was observed for melon seeds, although only modest values for enantiomeric excess and percentage of conversion were obtained (48 and 40%, respectively). A possible explanation for this numbers may be that the reaction conditions employed are not optimal for the enzyme involved in this biotransformation. Indeed, this is a remarkable result, particularly because no HNL activity neither evidence of cyanogenesis or the presence of cyanogenetic glycosides has been previously reported for the Cucurbitaceae family. The results from the enzymatic preparation of quince (92% ee, R configuration and 70% conversion) and the wide distribution of the seeds of this fruit, lead us to consider the potential use of this material as an alternative biocatalyst readily available for the multi-gram scale preparation of (R)-cyanohydrins.

Anona and cherimoya seeds produced very interesting results, notoriously the negative value for the optical rotation of the produced mandelonitrile, which strongly suggest the

[1] In the case of bonete conversion of 17% was observed. However we believe that this result is not significantly different to the result for the non-biocatalyzed reaction, particularly because crude material was used for the experiment.

Table 1
Screening for hydroxynitrile lyase activity in enzyme preparations[a]

Plant	Common name	Part of the plant	ee (%)[b]	Conversion (%)[c]	Configuration	Chemical conversion
Acacia glauca	Guaje	Seeds	0	6	–	9
Acacia farnesiana	Sweet acacia (huisache)	Seeds	0	23	–	13
Annona	Cherimoya	Seeds	16	30	S	13
Cherimolia		Leaves	0	10	–	9
Annona squamosa	Sugar-apple (anona)	Seeds	18	54	S	13
		Leaves	0	3	–	6
Carica mexicana	Bonete	Seeds	0	17	–	9
Crataegus mexicana	Tejocote	Seeds	0	4	–	6
		Leaves	0	10	–	9
Cucumis melo	Melon	Seeds	48	40	R	13
Cydonia oblonga	Quince	Seeds	92	77	R	13
Manilkara zapota	Chicozapote	Seeds	0	5	–	6
		Leaves	0	4	–	6
Passiflora ligularis	Pomergranate	Seeds	0	14	–	9
Pouteria campechiana	Canistel	Seeds	0	18	–	9
		Leaves	0	8	–	9
Pouteria sapota	Mamey	Leaves	92	58	R	6
Prunus avium	Cherry	Leaves	96	56	R	6
Prunus domestica	Plum	Leaves	98	73	R	6
Prunus persica	Peach	Leaves	96	83	R	6
Prunus serotina var *capuli*	Capulin	Leaves	98	98	R	6
		Seeds[d]	86	60	R	6
Prunus amygdalus	Almond	Seeds[d]	94	56	R	6
Trifolum repens	Clover	Leaves	0	17	–	13

[a] Reaction time of 24 h and pH 4.0 buffer were used for all the experiments.
[b] Determined by HPLC on a CHIRACEL OD column.
[c] Determined by ^{1}H NMR.
[d] Known sources of HNLs.

presence of an (S)-HNL in this plant. Although the enantiomeric excess is low (18 and 16%, respectively) and the percentage of conversion is modest (54 and 30%, respectively), modifications to the employed reaction conditions could improve the outcome of this transformation. It is noteworthy to mention that the leaves of anona and cherimoya do not biocatalyze the same reaction. This finding is consistent with recent results reported by our group [22].

The presence of HNLs activity for the seeds of capulin [23], cherry, peach, plum [24] and mamey [23], has been previously described, but to the best of our knowledge, HNL activity has never been reported for leaves of these plants. Our findings provide strong evidence that the activity of crude preparations of cherry, peach, plums and mamey leaves is similar to that displayed for the corresponding seeds, at least for the conversion of benzaldehyde into mandelonitrile. The enzymatic activity observed for the aforementioned leaves seems to be significantly important mainly because they can provide larger amounts of biological material to be used as biocatalysts and secondly because the leaves are readily available since they are not seasonal.

In conclusion, HNL activity was observed for the seeds of melon, quince anona and cherimoya, as well as for the leaves of cherry, plum peach and mamey. Crude preparations from these sources biocatalyzed the transformation of benzaldehyde into mandelonitrile with enantioselectivities ranging form modest to excellent. This methodology represents an interesting and attractive alternative for the preparation of quiral non-racemic cyanohydrins.

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