Note

Hydroxynitrile Lyases from Prunus Seeds in the Preparation of Cyanohydrins

Aida S Oli’s,1,1 Myrna Solis-Oba,2 Herminia Inés Pérez,1 Norberto Manjarrez,1 and Julia Cassani1

1Metropolitan Autonomous University, Campus Xochimilco, Calz. del Hueso 1100, Col. Villa Quietud, CP 04960, D F, Mexico
2Center for Research in Applied Biotechnology, National Polytechnic Institute, Ex-Hacienda San Juan Molino, Carretera Estatal Tecuxcomac-Tepetitla Km 1.5, C.P. 90700, Tlaxcala, México

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The hydroxynitrile lyase (HNL) activity of nine defatted Prunus seeds was compared for catalyzing the addition of HCN to aromatic, heteroaromatic and α,β-unsaturated aldehydes. Although the conversion and enantiomeric excess (ee) of the corresponding cyanohydrins were both influenced by the HNL source and the chemical structure of the aldehyde, Prunus HNLs were all suitable for the enantioselective preparation of cyanohydrins.

Key words: Prunus; biocatalysis; hydroxynitrile lyase; cyanohydrin

Hydroxynitrile lyases (HNLs) have been isolated from a wide variety of plant sources,1–4 including the Prunus genus,5–9 and catalyze the stereoselective addition of HCN to carbonyls to produce optically active cyanohydrins. The importance of cyanohydrins, which has been highlighted in several reviews,5,6 is that they can be readily converted into compounds with diverse biological activities.5,6,9–11 This present study compares the HNL activity of Prunus seeds for catalyzing the addition of HCN to aldehydes (Fig. 1). The seeds were obtained from different types of fresh fruit purchased locally. The fleshy cover was removed to obtain the seeds, and the outer layer of the seeds was cracked to give the soft kernels inside. These kernels were ground three times with acetone, and the resulting powder was air dried and stored at 4°C.2 The defatted seeds were used as the HNL source, and their enzymatic activity was determined by measuring the formation of mandelonitrile (2a) from benzaldehyde (1a, Fig. 1) and HCN. A mixture containing a 0.1 M citrate buffer (pH 5, 0.91 mL), 1 M KCN/citric acid buffer (pH 5, 0.05 mL), the HNL source (4 mg) and 1a at 1 M in DMSO (0.04 mL) was stirred at room temperature (approximately 22°C). Samples (0.05 mL) were extracted every 20 s with hexane/isopropanol:TFA (9:1:0.01, 0.45 mL) and analyzed by high-pressure liquid chromatography (HPLC, Agilent 1100), using a Chiracel OD column (Chiral Technologies) with hexane/isopropanol as the mobile phase (9:1) at a flow rate of 1.0 mL/min. The respective retention times of (S)- and (R)-mandelonitrile were 7.6 and 8.0 min. A blank reaction was performed without the enzyme, and the amount of 2a formed during the blank reaction was subtracted from the amount of 2a formed in the biocatalyzed reaction.8

The enzymatic activity is expressed as the amount of HNL source (mg of defatted seed) that catalyzed the formation of 1 μmol of mandelonitrile from benzaldehyde (1a) per min (Table 1).

The results in Table 1 show that defatted capulin and cherry seeds were the most active HNL sources, the HNL activities of the other defatted seeds being similar to each other. In addition to the HNL activity, it was important to determine the influence of the aldehyde structure (Fig. 1, compounds 1a–1h) on the addition of cyanide catalyzed by the nine types of defatted seeds and their enantioselectivity. HCN from a 1 M KCN/citric acid buffer (pH 5, 1.5 mL) was extracted twice with disopropyl ether (2 mL each), and to this solution were added a 0.1 M citrate buffer solution (pH 5, 0.1% v/v), the HNL source (200 mg), and 0.1 mmol of an aldehyde (1a–1h). The mixture was stirred at 4°C (3 h for 1a and 48 h for 1b–1h), and the enantiomeric excess (ee) and conversion ratio were respectively determined by HPLC under the conditions already described and 1H-NMR.

The results in Table 1 show that substituents on the aromatic ring had an important effect on ee and the extent of the biocatalyzed addition of cyanide to aromatic compounds 1a–1d. The reaction was enantioselective with all the seeds (>99% ee) and semi-quantitative if the ring had no substituents (1a). Ee and conversion were strongly dependent on the position of the substituent (–Cl) for the three chlorobenzalde-
Table 1. HNL Activity of the Defatted *Prunus* Seeds and Influence of the Aldehyde (1) Structure on the Biocatalyzed Addition of HCN for Preparing Cyanohydrin (2)

<table>
<thead>
<tr>
<th>Seed</th>
<th>HNL activity* (U/mg of defatted seed)</th>
<th>2a†</th>
<th>2b†</th>
<th>2c†</th>
<th>2d†</th>
<th>2e†</th>
<th>2f†</th>
<th>2g†</th>
<th>2h†</th>
</tr>
</thead>
<tbody>
<tr>
<td>almond (P. amygdalus)</td>
<td>0.37 ± 0.04</td>
<td>99 ± 1</td>
<td>100</td>
<td>14 ± 2</td>
<td>17 ± 3</td>
<td>85 ± 3</td>
<td>48 ± 2</td>
<td>99 ± 1</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>capulin (P. serotina, Var. capuli)</td>
<td>0.77 ± 0.06</td>
<td>99 ± 1</td>
<td>100</td>
<td>6 ± 1</td>
<td>21 ± 1</td>
<td>83 ± 3</td>
<td>53 ± 1</td>
<td>99 ± 1</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>cherry (P. avium)</td>
<td>0.53 ± 0.06</td>
<td>99 ± 2</td>
<td>100</td>
<td>16 ± 1</td>
<td>19 ± 1</td>
<td>97 ± 3</td>
<td>53 ± 3</td>
<td>92 ± 3</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>red plum (P. domestica, cv moscato)</td>
<td>0.34 ± 0.03</td>
<td>99 ± 2</td>
<td>100</td>
<td>12 ± 2</td>
<td>35 ± 2</td>
<td>80 ± 5</td>
<td>49 ± 4</td>
<td>88 ± 4</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>plum (P. domestica)</td>
<td>0.34 ± 0.03</td>
<td>99 ± 1</td>
<td>92</td>
<td>12 ± 1</td>
<td>26 ± 2</td>
<td>97 ± 3</td>
<td>70 ± 2</td>
<td>86 ± 2</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>apricot (P. armeniaca)</td>
<td>0.24 ± 0.02</td>
<td>99 ± 1</td>
<td>74</td>
<td>9 ± 1</td>
<td>21 ± 3</td>
<td>59 ± 1</td>
<td>27 ± 4</td>
<td>99 ± 1</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>yellow peach (P. persica, cv diamante)</td>
<td>0.26 ± 0.02</td>
<td>99 ± 2</td>
<td>100</td>
<td>10 ± 1</td>
<td>16 ± 3</td>
<td>69 ± 4</td>
<td>35 ± 4</td>
<td>91 ± 3</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>peach (P. persica)</td>
<td>0.38 ± 0.04</td>
<td>99 ± 1</td>
<td>94</td>
<td>6 ± 1</td>
<td>20 ± 1</td>
<td>60 ± 5</td>
<td>39 ± 3</td>
<td>81 ± 4</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>nectarine (P. persica, L. Batsch)</td>
<td>0.43 ± 0.04</td>
<td>99 ± 1</td>
<td>93</td>
<td>10 ± 1</td>
<td>16 ± 3</td>
<td>69 ± 5</td>
<td>43 ± 4</td>
<td>87 ± 2</td>
<td>60 ± 5</td>
</tr>
</tbody>
</table>

*The HNL activity is expressed as the amount of the HNL source (mg of defatted seed) that catalyzes the formation of 1 μmol of mandelonitrile from benzaldehyde per min.
†The configuration of the main enantiomer was “R.”
‡The configuration of the main enantiomer was “S,” assigned according to Cahn-Ingold-Prelog. %ee, % enantiomeric excess; %con, % conversion; mean ± SD (n = 3).

Hydrenes (1b–1d). Ee and the conversion were higher if the substituent was in the para position (1d), but the reaction was less enantioselective and slower when –Cl was closer to the aldehyde. The conversion to cyanohydrin was much lower and not enantioselective if the substituent was in the ortho position (1b). The more enantioselective HNL sources for 1e were capulin, cherry, apricot, peach, and nectarine, although the conversion was low, except with red plum and apricot. Nearly optically pure 2f was obtained only with plum for heteroaromatic 1f. Interestingly, the HNL activity of all the seeds towards the addition of cyanide to α,β-unsaturated aldehydes 1g and 1h displayed ee of greater than 92% for 2h. However, the reaction was more dependent on the HNL source than on the substrate for 1g. The conversion was variable in both cases.

Defatted seeds from *Prunus* are very cheap and readily accessible crude sources of HNL. They generally showed good enantioselectivity towards the addition of HCN to various aldehydes. An advantage of these multiple crude sources is that they can be complementary for preparing enantiomerically pure cyanohydrins. With the exception of almond, *Prunus* seeds can also be considered as waste material.

**References**