Hydroxynitrile lyases: At the interface of biology and chemistry

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Received 2 February 2005; accepted 25 April 2005

Abstract

Hydroxynitrile lyases are the versatile group of enzymes, which play a significant defensive role in plant system against microbial attack. In chemical industries, hydroxynitrile lyase is used as an important industrial biocatalyst for the synthesis of chiral cyanohydrins by exploiting the reversible enzymatic reaction. Cyanohydrins are biologically active compounds used in synthesis of β-amino alcohols, α-hydroxy ketones and α-hydroxy acids, which have importance as fine chemicals, pharmaceuticals and agrochemicals. NMR and inhibition studies revealed the involvement of different amino acids at the active site and proved that the hydroxynitrile lyases generally utilize acid/base catalysis mechanism. Protein engineering and site directed mutagenesis have been used to change the active site and alter the substrate specificity of various hydroxynitrile lyases. Many recombinant hydroxynitrile lyases have been expressed in Escherichia coli, Saccharomyces cerevisiae and Pichia pastoris.

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Keywords: Hydroxynitrile lyases; Cyanohydrins; Recombinant hydroxynitrile lyases

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1. Introduction

Hydroxynitrile lyases or oxynitrilases (HNLs E.C.4.2.1.10, E.C.4.2.1.11, E.C.4.2.1.37 and E.C.4.2.1.39) are the enzymes, which catalyze enantioselective cleavage and synthesis of cyanohydrins. Cyanohydrins are alcohol containing a cyano group i.e. having a cyano and a hydroxyl group attached to the same carbon atom. In 1837 Friedrich Wöhler detected the hydroxynitrile lyase activity for the first time in almond, which cleaves the cyanohydrins into aldehydes and HCN [1]. In recent years hydroxynitrile lyases have emerged as potential biocatalysts for the synthesis of a range of chiral...
<table>
<thead>
<tr>
<th>S. no.</th>
<th>Products</th>
<th>Chemical structure</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(R)-Salbutamol</td>
<td><img src="image1" alt="Structure" /></td>
<td>Bronchodilator [60]</td>
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<tr>
<td>2</td>
<td>(R)-Terbutaline</td>
<td><img src="image2" alt="Structure" /></td>
<td>Bronchodilator [60]</td>
</tr>
<tr>
<td>3</td>
<td>(S)-13-Hydroxyoctadeca (9Z, 11E)-dienoic acid</td>
<td><img src="image3" alt="Structure" /></td>
<td>Biological activity against rice blast disease and a chiral synthon [60]</td>
</tr>
<tr>
<td>4</td>
<td>(S)-Amphetamines</td>
<td><img src="image4" alt="Structure" /></td>
<td>Designer drug used medically in psychological treatments and central nervous system stimulant [60]</td>
</tr>
<tr>
<td>5</td>
<td>L-Ephedrine</td>
<td><img src="image5" alt="Structure" /></td>
<td>Stimulant and bronchodilator [86]</td>
</tr>
<tr>
<td>6</td>
<td>(R)-Pantolactone</td>
<td><img src="image6" alt="Structure" /></td>
<td>Chiral building block and chiral auxiliaries, constituents of coenzyme A, bactericide, A growth factor [70]</td>
</tr>
<tr>
<td>7</td>
<td>Diltiazem</td>
<td><img src="image7" alt="Structure" /></td>
<td>Cardiac drug [76]</td>
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<tr>
<td>8</td>
<td>Terpenoids [55]</td>
<td><img src="image8" alt="Structure" /></td>
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</tr>
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<td>9</td>
<td>Pheromones [55]</td>
<td><img src="image9" alt="Structure" /></td>
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Table 1 (Continued)

<table>
<thead>
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<td>Antibiotics [55]</td>
<td><img src="image" alt="Tetronic acid structure" /></td>
<td>Synthesis of semi synthetic cephalosporins antibiotics</td>
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<td>11</td>
<td>Amino acids</td>
<td><img src="image" alt="Amino acids structure" /></td>
<td>Component of biologically active compounds [35,85]</td>
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<tr>
<td></td>
<td></td>
<td>N-benzyl-α-hydroxy-β-amino acids</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Thalidomide</td>
<td><img src="image" alt="Thalidomide structure" /></td>
<td>Curb morning sickness in pregnant women [94]</td>
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<tr>
<td>13</td>
<td>Denopamine</td>
<td><img src="image" alt="Denopamine structure" /></td>
<td>Cardiac drug [108]</td>
</tr>
<tr>
<td>14</td>
<td>Tembamide</td>
<td><img src="image" alt="Tembamide structure" /></td>
<td>Hypoglycaemic activity [108,109]</td>
</tr>
<tr>
<td>15</td>
<td>Aegline</td>
<td><img src="image" alt="Aegline structure" /></td>
<td>Adrenaline like and insecticide activity [108,109]</td>
</tr>
<tr>
<td>16</td>
<td>Lipitor</td>
<td><img src="image" alt="Lipitor structure" /></td>
<td>Hypercholesterolaemia treatment [110]</td>
</tr>
<tr>
<td>17</td>
<td>Pyrethroids</td>
<td><img src="image" alt="Pyrethroids structure" /></td>
<td>Insecticide [111]</td>
</tr>
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</table>
compounds, which find applications in pharmaceutical, agrochemical and cosmetic formulations. These enzymes catalyze the carbon-carbon bond formation in organic synthesis [2,3]. Cyanohydrins are biologically active compounds and are excellent synthons as these can be readily converted into a variety of valuable starting materials for synthesis of α-hydroxy ketones, β-amino alcohols and α-hydroxy carboxylic acids [4–6]. Cyanohydrin derivatives are essential constituents of commercial products e.g. pyrethroid insecticides cypermethrin and fluvalinate [6]. In the present article some biochemical characteristics, mechanism of action and possible applications of hydroxynitrile lyases will be reviewed (see Table 1).

### 2. Classification of hydroxynitrile lyases

The hydroxynitrile lyases characterized so far fall into two major groups: HNL I and HNL II based on the presence or absence of flavin adenine dinucleotide (FAD) [7–9] (Table 2). This cofactor is not involved in net redox reaction but their removal causes inactivation of the enzyme [10,11] i.e. it provides structural stability to enzyme structure or it is present as an evolutionary remnant [10,12,13]. The flavin adenine dinucleotide generally binds covalently to a hydrophobic region near the catalytic site of enzyme. The FAD containing (R)-(+)-mandelonitrile lyases (MDL-E.C. 4.2.1.10) have been isolated from plants belonging to Prunoideae and Maloideae subfamilies of the Rosaceae as major seed storage proteins which play an active role in cyanogenesis [14]. FAD lacking hydroxynitrile lyase found in *Sorghum bicolor* [15,16], *Manihot esculenta* [17], *Phleboodium aureum* [18] and *Linum usitatissimum* [19], is less prevalent protein and has diverse physicochemical properties like substrate specificity, mass, glycosylation, isoelectric point, structure and amino acid sequence [7,20].

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<td>Mandelamine</td>
<td><img src="image" alt="Mandelamine structure" /></td>
<td>Urinary antiseptics [112]</td>
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<tr>
<td>19</td>
<td>Mandelonitrile</td>
<td><img src="image" alt="Mandelonitrile structure" /></td>
<td>Germicide [113]</td>
</tr>
<tr>
<td>20</td>
<td>Mandelic marine complexes</td>
<td><img src="image" alt="Mandelic acid structure" /></td>
<td>Cosmetics [113]</td>
</tr>
<tr>
<td>21</td>
<td>SAMMA</td>
<td>Mandelic acid condensation product</td>
<td>Blocks HIV, HSV-1 and HSV-2 [114]</td>
</tr>
<tr>
<td>22</td>
<td>2(R)-Hydroxy-4-phenyl butyric acid</td>
<td><img src="image" alt="2(R)-Hydroxy-4-phenyl butyric acid structure" /></td>
<td>Angiotension converting enzyme inhibitor (Ciba Geigy) and hyperglycemia [115]</td>
</tr>
<tr>
<td>23</td>
<td>Norstatine</td>
<td><img src="image" alt="Norstatine structure" /></td>
<td>Intermediate for antitumor agent, rennin inhibitors and HIV virus protease-inhibitor [116] Asahi chemicals</td>
</tr>
<tr>
<td>24</td>
<td>Sphingosine</td>
<td><img src="image" alt="Sphingosine structure" /></td>
<td>Marker for early detection of cancer and have the HIV binding biological properties [117,118]</td>
</tr>
</tbody>
</table>
Table 2
Characteristics of hydroxynitrile lyases I and II

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HNL I</th>
<th>HNL II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cofactor</td>
<td>FAD containing</td>
<td>FAD lacking</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>N-glycosylated</td>
<td>–</td>
</tr>
<tr>
<td>Chiral aglycon</td>
<td>Mandelonitrile</td>
<td>Acetone cyanohydrin, (S)-mandelonitrile, (S)-4-hydroxy mandelonitrile, (R)-2-butanone cyanohydrin</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>(S)-prunasin and (R)-amylidin</td>
<td>(S)-Dhurrin, (S)-sambunigrin, linamarin, lotaustraline</td>
</tr>
<tr>
<td>Carbohydrate content</td>
<td>30%</td>
<td>9%</td>
</tr>
<tr>
<td>Isoforms</td>
<td>Presence of Isoenzymes</td>
<td>–</td>
</tr>
<tr>
<td>Homology</td>
<td>Oxidoreductases (30%)</td>
<td>–</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>50–80 kDa</td>
<td>28–42 kDa</td>
</tr>
<tr>
<td>Isoelectric pH</td>
<td>4.2–4.8</td>
<td>3.9–4.6</td>
</tr>
</tbody>
</table>

3. Distribution of hydroxynitrile lyases and their function in nature

The phenomenon of cyanogenesis (i.e. hydroxynitrile lyase mediated release of cyanide group in the form of HCN on microbial attack) is widely observed in higher plants. It has been reported in more than the 3000 species of vascular plant taxa comprising 105 families of flowering plants, pteridophytes (ferns) and gymnosperms. Prominent among these are the plants belonging to families like Linaceae, Euphorbiaceae, Clusiaceae, Olacaceae, Rosaceae, Gramineae (monocotyledons) and Filitaceae (ferns) [21]. In addition to plants, the cyanogenesis is also reported in taxonomically diverse group of organisms like bacteria (Chromobacterium violaceum, few species of Pseudomonas) [22–24], fungi, lichen, millipedes (Apheloria corrugata), arthropods and insects (Zygaena trifolii, a moth). Based on amino acid sequence homology and other characteristics of the HNLs, these are considered to be evolved by convergent evolution (Fig. 1).

Endogenous cyanide containing compounds occur mostly as cyanoglycosides (cyanohydrin stabilized by a glycosidically linked sugar moiety) and cyanolipids in plants [7]. Major function of these compounds in plant system seems to be defense against herbivoral, fungal attack or mechanical injury [25]. Besides their role in defense mechanism, cyanogenic glycosides are also utilized as nitrogen source in amino acid anabolism where HCN is refixed by β-cyanolalanine synthetase and catabolized to β-cyanolalanine with L-cysteine. This β-cyanolalanine is further hydrolyzed to L-asparagine by β-cyanolalanine hydrodase [26–28] (Fig. 2).

Sixty different types of cyanogenic glycosides has been reported which are derived from amino acid precursors like valine, isoleucine, leucine, phenylalanine and tyrosine (Fig. 3).

4. Hydroxynitrile lyase reactions

Hydrocyanation of aldehydes and ketones to produce chiral cyanohydrin was first carried out by Rosenthaler in 1909...
using hydroxynitrile lyases from almond [29]. It catalyzes the asymmetric condensation of HCN with aldehydes or ketones to yield cyanohydrins [30–32] (Fig. 4). Almond is most common source of (R)-hydroxynitrile lyase. Defatted almond meal is used as an inexpensive catalyst and does not require immobilization as enzyme is naturally immobilized in the meal matrix that also provide stability to it [33–35]. (S)-HNL is not easily available and thus can be cloned and over expressed in microbial expression system [32,36–41].

Enantiomeric or specific synthesis of cyanohydrin is influenced by reaction medium, cyanide source, water content, buffer pH and temperature during the hydroxynitrile lyase catalyzed reaction. Increase in enantiomeric excess of the product can be obtained by carrying out reaction in aqueous medium and at low pH, however, at low pH the stability of enzyme is affected significantly and using excess enzyme in the reaction can circumvent this problem [42,43]. Alternatively, the use of HNL in biphasic system provides many advantages like increased substrate concentration (1–1.5 M), high product yield, easy down stream processing as enzyme remain partitioned to one of the phases [43–45].

Most solvent used in biphasic reactions are ethyl acetate, diisopropyl and other ethers [46–55]. Enzymes in diisopropyl ether (DIPE)/water interface is more active than in ethyl acetate. Studies have shown that while using biphasic system enzyme activity was at interface rather than in one phase of solvent system [56–58]. After complete reaction in biphasic

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**Fig. 1.** Phylogenetic tree of hydroxynitrile lyases. Sources of HNLs: LuHNL—Linum usitatissimum, HbHNL—Hevea brasiliensis, MaHNL—Mammea americana, MeHNL—Manihot esculenta, XaHNL—Ximenia americana, SvHNL—Sorghum vulgare, SnHNL—Sambucus nigra, PhaHNL—Phlebodium aureum, PaHNL—Prunus amygdalus, PcHNL—Prunus capuli, PsHNL—Prunus serotina.

**Fig. 2.** HCN released due to catabolism of cyanogenic glycoside can be used as nitrogen source for amino acid synthesis.
system the aqueous phase having enzyme can be removed by simple phase separation step and can be reused, however, biphasic systems have few disadvantages like enzyme activity is highly affected by the solvent hydrophobicity [42]. In the absence of moisture or water, catalytic activity of enzyme reduces to zero, while in excess of water significant chemical background reaction occurs [59].

Hydrogen cyanide is the most preferred cyanide source in cyanohydrin synthesis [35,46,48,50,60–62]. Besides HCN, several different cyanide sources like potassium cyanide are being used in biotransformation. It acts as an effective source of cyanide in the low pH range of aqueous buffer [63–65]. The spontaneous addition of HCN will result in reduction of enantiomeric purity of the product because at high concentration HCN becomes insoluble leading to its instantaneous release from reaction mixture. Alternatively, the addition of hydrogen cyanide in the reaction can be replaced by its indirect generation by addition of the acid to the aqueous solution of alkali cyanide in transhydrocyanation process. This slow diffusion of HCN gives advantage over spontaneous addition and results in high enantiomeric purity and yield [53,54,66].

Temperature has profound effect on the course of reaction. Enzyme stability as well as enantiomeric product yield is enhanced at low temperature —5 to 4 °C [57]. However, further decrease in temperature (i.e. below —5 °C) freezes the water present in reaction mixture, which leads to complete loss of enzyme activity. The enantiomeric excess of product decreases significantly in aqueous/alcoholic buffer system because of the background chemical reactions. Engineering of reaction conditions of hydroxynitrile lyase mediated organic synthesis can minimize the background non-enzymatic reaction and will further improve yield and enantiomeric purity of the product.
The enantiomeric purity of the cyanohydrin or derivatives produced through HNL mediated reactions is routinely determined by using chiral HPLC [67,68], chiral GC [48,49,63,73–75] and NMR [33,34,73,75,76] techniques. Thin layer chromatography involving KMnO$_4$ dip is most convenient technique for the detection of cyanohydrins in the reaction mixture [77]. NMR is also used to confirm the desired product formed by hydroxynitrile lyase.

Many workers have also carried out immobilization of hydroxynitrile lyases on different matrices like ECTEOLA cellulose (ion exchanger) [74,78,79], DEAE cellulose, sepharose 4B, pore glass beads [74], silica gel [80], microcrystalline cellulose (Avicel) [49,74], supergel C, nitrocellulose [50], celite, liquid crystals and PV AL hydrogels [81]. Recently CLEA (cross linked enzyme aggregate) technology has emerged for immobilization of hydroxynitrile lyases [82].

5. Substrate specificity of hydroxynitrile lyases

Hydroxynitrile lyase differs in their stereospecificity and substrate affinity. PaHNL has broad substrate specificity and applicability [83]. Kyler explored the active site dimension of this enzyme with different substrates [84]. Brussee [33–35,62,85] and Kanerva [52,86] studied the substrate affinity using crude almond extract and almond bran, which are cheaper sources of enzyme and do not require immobilization. (S)-hydroxynitrile lyase enzyme of Hevea brasiliensis and Manihot esculenta have been altered through protein engineering or site directed mutagenesis by substituting bulky amino acids (e.g. tryptophan) which constrict the entrance of active sites with less bulky amino acids like alanine and this made the enzyme to accept large size of substrates [87]. LuHNL is a less available $R$-selective enzyme and has a narrow substrate range [41,83] while HbHNL and MeHNL catalyse the synthesis of cyanohydrins from a wide range of substrate like aliphatic, aromatic, heteroaromatic carbonyls and ketones. XaHNL has limited occurrence in nature and it has fairly good affinity for aromatic aldehydes [88]. New sources of hydroxynitrile lyases like PhaHNL catalyse aromatic and heterocyclic carbonyls rather than aliphatic substrates and find use in selected applications [17].

6. Biochemical and molecular properties of hydroxynitrile lyases

Hydroxynitrile lyases from about 15 sources have been reported so far and few of them are fully characterized while search for novel hydroxynitrile lyases is going on. These HNLs have different conformations, molecular weights, pH optima, $p_I$ values, FAD requirements and kinetic properties (Table 4).

Structures of catalytic site of hydroxynitrile lyases have been explored and studied by chemical mutagenesis and X-ray crystallography. HbHNL was the first enzyme whose 3D crystal structure was elucidated followed by other HNLs [36,89]. The X-ray diffraction studies have been carried out with crystal of PaHNL and MeHNL [88]. Enzymes are of widely different phylogenetic origin like subtilisin, cysteine proteases, eucaryotic serine proteases and $\alpha/\beta$ hydrolases and share the common structural motif of eight $\beta$ sheets connected by a helices beside similar protein folds [90,91]. HbHNL belongs to $\alpha/\beta$ hydrolase super family having deeply buried active site within protein and linked to outer environment by a narrow channel flanked by apolar residues.

The amino acid sequence of FAD containing hydroxynitrile lyases showed that they are related to members of Glucose–methanol–choline (GMC) oxidoreductase family (30% sequence identity) [59]. Hydroxynitrile lyases are the proteins of size ranging from 250 to 664 amino acid residues, which share a number of highly conserved regions (>89%). One of these regions, located in the N-terminal section, corresponds to the FAD ADP-binding domain, while other one is located in the center of the enzyme protein as a signature pattern [92].

<table>
<thead>
<tr>
<th>HNLs</th>
<th>Mol. wt. (kDa)</th>
<th>$K_M$ (mM)</th>
<th>$V_{max}$ (nM/min)</th>
<th>pH</th>
<th>Isoelectric points</th>
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<tr>
<td>MeHNL</td>
<td>30</td>
<td>1.10 mM</td>
<td>1.10 mM</td>
<td>3.5–5.4</td>
<td>4.4, 4.1, 4.6</td>
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<tr>
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<td>0.058 mM/min</td>
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<td>–</td>
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<tr>
<td>PhaHNL</td>
<td>20</td>
<td>0.85 mM</td>
<td>–</td>
<td>6.5</td>
<td>–</td>
</tr>
<tr>
<td>SbHNL</td>
<td>38, 18</td>
<td>0.80 &amp;D</td>
<td>–</td>
<td>5.5</td>
<td>–</td>
</tr>
<tr>
<td>SbHNL</td>
<td>38, 18</td>
<td>–</td>
<td>–</td>
<td>5.0–5.5</td>
<td>–</td>
</tr>
<tr>
<td>XaHNL</td>
<td>36.5</td>
<td>0.53 mM</td>
<td>1031 IU</td>
<td>5.5</td>
<td>–</td>
</tr>
<tr>
<td>PbhNL</td>
<td>59</td>
<td>93 $\mu$M</td>
<td>450 $\times$ 10$^3$</td>
<td>5.5</td>
<td>4.75</td>
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<tr>
<td>PaHNL</td>
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<td>0.59 mM</td>
<td>3780 $\mu$M/nM</td>
<td>5.5–6.0</td>
<td>–</td>
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<tr>
<td>PbhNL</td>
<td>60</td>
<td>0.17 $\mu$M</td>
<td>–</td>
<td>6.0–7.0</td>
<td>4.58–4.63</td>
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<tr>
<td>PhbNL</td>
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<td>–</td>
<td>–</td>
<td>5.5–6.0</td>
<td>4.2–4.4</td>
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<tr>
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<td>75-84</td>
<td>–</td>
<td>–</td>
<td>5.5</td>
<td>–</td>
</tr>
<tr>
<td>LuHNL</td>
<td>42</td>
<td>2.5, 1.9 $\mu$</td>
<td>–</td>
<td>5.5</td>
<td>4.70–4.85</td>
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R—recombinant enzyme.

Table 4: Biochemical properties of hydroxynitrile lyases

<table>
<thead>
<tr>
<th>HNLs</th>
<th>Mol. wt. (kDa)</th>
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<tr>
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<td>–</td>
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<tr>
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<td>–</td>
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<td>4.75</td>
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<tr>
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<td>5.5–6.0</td>
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<td>–</td>
<td>5.5</td>
<td>–</td>
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<tr>
<td>LuHNL</td>
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<td>2.5, 1.9 $\mu$</td>
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</table>

R—recombinant enzyme.
Topology of HbHNL differs slightly from α/β hydrolase family. It lacks first two β sheets and has a continued cap region where lies catalytic active site [90]. A study on molecular cloning of ShHNL gene has shown that it lacks introns and has a stretch of 23 A (adenine) bases which suggests that it might have evolved by a reverse transcription event during evolution from serine carboxypeptidase. It has 11 stranded β sheets surrounded by more than a dozen α helices [93].

PaHNL has 4 N-glycosylation sites at Asn118, Asn135, Asn352, Asn392 and Asn-X-Ser/Thr on the consensus sequence [59]. N-glycosylation constituents are Manα,GlcNAc2 (Mannose-N-acetyl glucosamine). PaHNL has two domains one for FAD binding and other for substrate binding. The FAD cofactor is completely isolated from solvent and is deeply buried within the enzyme molecule. The FAD binding domain mainly has an α and β sheets and surrounded by six α helices (H1, H2, H3, H4, H6 and H9) and two 310 type helices (a protein secondary structure containing three amino acid residues per turn and hydrogen bonds in 10 atom rings), β meander (single sheet formed by β hairpin repeats) and βαβ motif in central parallel sheet and the later is responsible for FAD binding. The substrate-binding site is characterized by 6-stranded β sheets (C0–C5), surrounded by helices H4, H7 and H8 and one 310 type helix. PaHNL has 24% homology to glucose oxidase (GOX) of Penicillium amagasakiense and cholestrol oxidase (CHOX) of Streptomyces sp. [59].

LuHNL has an ADP binding βαβ domain and catalytic domain containing two Zn2+, which are not directly involved in catalysis [19]. Some hydroxynitrile lyases require post-translational modifications for activity e.g. (R)-hydroxynitrile lyase and (S)-hydroxynitrile lyase of S. bicolor are highly glycosylated and require post-translational proteolytic processing for their activity [36]. Hydroxynitrile lyase of Prunus serotina is present in isoforms and this multiplicity is either due to allelic differences at the structural loci or due to gene duplication and post-translational modifications ultimately resulting in isoenzyme forms [7]. This heterogeneity in genes provides organisms specificity, regulation and differential expression.

7. Recombinant hydroxynitrile lyases

The hydroxynitrile lyases of some plants have been cloned in microbial systems since the latter can be easily transformed and cultured in a short time for large-scale production. HbHNL gene has been over expressed in Escherichia coli using strong inducible tac promoter and the gene product formed inclusion bodies, which were insoluble and inactive [36,37,43]. The most successful heterologous expression of HbHNL was achieved in Saccharomyces cerevisiae and methanol inducible Pichia pastoris expression.
Fig. 6. Reaction mechanism of hydroxynitrile lyase of Prunus amygdalus [99].

Fig. 7. Reaction mechanism of hydroxynitrile lyase of Sorghum bicolor [100].
Histidine 496 acts as a base and deprotonates the hydroxyl group of the substrate [6]. Negativity of cyanide ion is neutralized by pronounced positive electrostatic potential at the active site by two positively charged residues arginine 300 and lysine 361. PaHNL binds FAD cofactor in its oxidized form. The reversal of electrostatic potential upon FAD removal or modification makes the complex unstable at active site leading to inactivation of enzyme [99] (Fig. 6).

During catalysis in SbHNL hydrogen bonds are formed between substrate hydroxyl group, serine 158 and oxygen atom of tryptophan 270-carboxyl group. A water molecule is suspended between other carboxylate group of tryptophan-270 and nitrile group of the substrate. Here the carboxylate moiety of tryptophan acts as catalytic base causing abstraction of proton from cyanohydrin hydroxyl group, while water at active site transfers this proton to cyanide resulting in carbon–carbon bond cleavage [100] (Fig. 7).

In MeHNL serine was reported to play a pivotal role in catalytic nucleophile addition reactions i.e. different residues involved in the catalysis are joined by hydrogen bonding e.g. serine-80 [101,102] forms hydrogen bond to imidazole nitrogen of histidine-236, which is further stabilized by hydrogen bonding with aspartate. Serine-80 functions as a strong base resulting in the formation of negatively charged oxyanion hole. This is further stabilized by an oxygen hole formed by the amide nitrogen of serine-80 and glycine-78 backbone, which is present as a consensus motif. Due to this stabilization there is an increase in positive charge on alpha carbon of hydroxynitrile resulting in cyanide group release [16,101,102] (Fig. 8).

In hydroxynitrile lyases of P. serotina, P. lyonii, X. americana, L. usitatissimum and S. vulgare cysteine was found to be involved at the active site [89]. During catalysis the substrate and product access the active site in a sequential manner e.g. PaHNL follows uni-bi mechanism in which aldehydes binds first to active site followed by HCN. Inhibition studies showed that cyanide ion and its analogues act as inhibitors of enzyme aldehydes complex, and this suggests that during cyanogenesis HCN binds to enzyme aldehydes complex rather than free cyanide ion [71]. Generally hydroxynitrile lyases utilize acid/base catalysis mechanism. The amino acid residues at...
Fig. 10. Transformation of hydroxyl group.

Fig. 11. Transformation of O-protected cyanohydrins.
active site of these enzymes differ significantly but share the common motif for cyanogenesis.

9. Chemo enzymatic transformation of cyanohydrins

Cyanohydrins can be synthesized using hydroxynitrile lyases, which offer high enantioselectivity, broad substrate variability and high yield in the synthesis of cyanohydrins or their derivatives. Chiral cyanohydrins are promising versatile building blocks since they can be used to create tailored molecules and have large number of applications in chemical industries. These cyanohydrins either serve as intermediate substrates or precursors that can further be converted into valuable products. Cyanohydins so produced can be transformed into variety of products using chemical reactions like (a) reaction of cyano group [51,55,103,104] (solvolysis, grignard reaction and hydride addition) (Fig. 9) (b) reaction of hydroxyl group [33] (c) reaction of R substituents and (d) conversion of OH group to a good leaving group to allow nucleophilic displacement with inversion of configuration [93]. Cyanohydrins and a range of their structural variants have wide applications in pharmaceutical industry [35,60,68,86]. Alpha-sulfonyloxynitriles undergo S N 2 reactions to give valuable products. Cyanohydrins so produced can be transformed into variety of products using chemical reactions like (a) reaction of cyano group [51,55,103,104] (solvolysis, grignard reaction and hydride addition) (Fig. 9) (b) reaction of hydroxyl group [33] (c) reaction of R substituents and (d) conversion of OH group to a good leaving group to allow nucleophilic displacement with inversion of configuration [93].

Acknowledgements

We thank the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for providing financial assistance in the form of Junior Research Fellowship to Ms. Monica Sharma and Mr. Nitya Nand Sharma.

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