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review

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Hydroxynitrile Lyases: Biological Sources and Application as Biocatalysts

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Summary

We review the state of the art regarding the application of hydroxynitrile lyases to obtain, enantioselectively, (*R*)- and (*S*)-cyanohydrins of aldehydes and ketones. Special emphasis is given to recent preparative applications and to research for extending the number of plants serving as sources for the enzyme. Depending on the plant family, the mechanism of the enzyme-catalysed reaction can be different. A novel area of research is the consideration of evolutionary aspects on the basis of structure comparisons.

Key words: hydroxynitrile lyases, oxynitrilase, biocatalytic cyanohydrin synthesis

Introduction

This review covers the advances that have been achieved in the area of enzyme catalysed cyanohydrin synthesis since our last book chapter addressing this field of biocatalysis (*1*). Hydroxynitrile lyases (HNLs) or oxynitrilases have been isolated from a wide variety of plant sources. These plants can release HCN to protect themselves against herbivores or microbial enemies. This cyanogenesis is mostly a two-step process; first a sugar moiety is cleaved by one or more glycosidases, subsequently the α-hydroxynitrile (cyanohydrin) is degraded either spontaneously or by action of an HNL in order to create the carbonyl compound and prussic acid (*2*). Besides this defence function, cyanogenic glycosides occur in some plants as an alternative nitrogen source for amino acid synthesis by young seedling tissues (*3*).

Hydroxynitrile lyases catalyse the reversible formation of cyanohydrins (see Fig. 1), using HCN and aldehydes or ketones. Four distinctly different types of proteins with oxynitrilase activity have been identified and investigated. These HNLs almost certainly emerge from various protein families (*4*). The first class, the flavine adenine dinucleotide (FAD) dependent HNLs, are to a certain extent highly glycosylated single chain proteins with (*R*)-mandelonitrile as their natural substrate (*5*). FAD-independent HNLs are more heterogeneous and differ in molecular mass, primary structure and subunit polymerisation structure (*6*). The second HNL class, isolated from *Hevea brasiliensis* and *Manihot esculenta*, is closely related to the class of α,β-hydrolases. The oxynitrilase isolated from *Sorghum bicolor*, which has 60 % amino acid sequence homology with a carboxypeptidase, represents the third class of HNLs (*7*). The zinc-dependent HNL from flax (*Linum usitatissimum*) represents a fourth class with homologies to alcohol dehydrogenases (*8*).

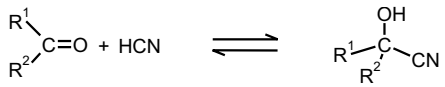

Fig. 1. Cyanohydrin formation: R^1 = alkyl, cycloalkyl, aryl, heteroaryl; R^2 = H, alkyl

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In the last decades these biocatalysts have been extensively investigated and reviewed by several research groups in particular in Stuttgart (*4,9–13*), Leiden (*14–16*) and Graz (*1,5,6,17–22*). In addition, general surveys on the synthesis of enantiopure cyanohydrins, for example, by metal-catalysed addition of cyanide to carbonyl compounds, have been published (*23–26*).

(*R*)-selective HNLs

Some hundred years ago the synthesis of mandelonitrile from benzaldehyde and prussic acid using a crude enzyme preparation extracted from almonds (termed »emulsin«) established the first biocatalytic application of an HNL (*27*). Emulsin was investigated both for the kinetic resolution of racemic cyanohydrins (*28*) as well as for the synthesis of optically active cyanohydrins from different aldehydes (*29–31*). Already at that time it was found that other enzyme preparations from a great variety of plants had oxynitrilase activity (*32*). In the 1960s, purified (*R*)-HNLs from almonds (*Prunus amygdalus*), referred to as PaHNLs (E.C. 4.1.2.10), were used as catalysts in a preparative manner (*33–35*) and thereafter for the first HNL-catalysed continuous synthesis of D(+)-mandelonitrile with 97 % optical purity (*36*). Then in 1987, the synthetic application of PaHNL was rediscovered for the preparation of cyanohydrins with high enantiomeric purity (*37*).

Although the protein from almonds had already been crystallized in 1994 (*38*), the complete three dimensional crystal structure was not solved before the year 2000 (*39*). As anticipated from sequence homology of PaHNL to oxidoreductases, two domains were found, an FAD binding and a substrate binding domain (which also represents the active site). This active site is located close to the isoalloxazine ring of the cofactor and was identified by soaking the protein crystals with methylmercury acetate (an inhibitor) and by docking simulations with the natural substrate (*R*)-mandelonitrile (*40*). The proposed mechanism needs His497 as a general base for deprotonating the hydroxyl function of the substrate and also a positive electrostatic potential, which can be cooperatively created by charged residues (*e.g.* Arg300, Lys361). The flavin cofactor is not involved in this mechanism at all, however, only the enzyme with the FAD in the oxidized form has oxynitrilase activity (*41,42*). Moreover, no oxidoreductase activity has been found in flavin-dependent HNLs and the role of the neutral oxidized cofactor seems to be to stabilize the required electrostatic potential in the active site (*5*). Nowadays PaHNL is available not only from natural sources like almonds but also from a fermentation process, which involves the gene for the PaHNL isoenzyme 5 cloned into and overexpressed in the methylotrophic yeast *Pichia pastoris* (*43,44*).

Over the last decade an immense endeavour was made to elucidate the five isoenzymes purified from black cherry (*Prunus serotina*). Comparison of the amino acid sequences of HNLs encoded by the black cherry PsHNL1- to PsHNL5- and PaHnL-cDNAs showed greatest sequence identity between PsHNL5 and PaHNL (93.7 % identity) (*45,46*). This degree of similarity suggests that they represent members of a multigene family rather than multiple alleles (*47*).

Besides the FAD-dependent HNLs, another (*R*)-selective oxynitrilase (LuHNL) has been recognized and isolated from flax (*Linum usitatissimum*) (*32,48,49*). This LuHNL (E.C. 4.1.2.37) has a completely different substrate specificity from that of the *Prunus* enzyme; this acetone cyanohydrin lyase catalyses the addition of HCN to various aliphatic ketones and aldehydes, while aromatic ketones are not converted (*50*). Using this enzyme, it is possible to synthesize (*R*)-butan-2-one cyanohydrin with an enantiomeric excess (e.e.) up to 88 %; this being noteworthy due to the relatively small steric difference between the methyl and ethyl groups in the neighbourhood of the carbonyl functional group of the substrate, 2-butanone. The LuHNL amino acid sequence (1266 base pairs encode for a protein of 46 kDa) shows no overall homologies to the known HNLs but has significant similarities to the members of alcohol dehydrogenase (ADH) family of enzymes. In particular, the cysteine and histidine residues responsible for coordination of an active site Zn^{2+} and a second structurally important Zn^{2+} in alcohol dehydrogenases are conserved. Nevertheless, neither alcohol dehydrogenase activity in LuHNL nor HNL activity in ADH have been detected. Moreover, well known inhibitors of ADHs, which interfere with the coordination of the active site Zn^{2+}, fail to affect the HNL activity of LuHNL, suggesting different mechanisms of cyanohydrin cleavage and alcohol oxidation. Interestingly, LuHNL, like ADH and PsHNL, possesses an ADP-binding βαβ motif, suggesting that the flavoprotein PsHNL and the non-flavoprotein LuHNL have evolved independently from a common ancestor with an ADP-binding βαβ unit (*8*). Initially, cloning of LuHNL was hampered by low expression levels of the recombinant enzyme in *Escherichia coli*. In order to overcome this problem, in Stuttgart the LuHNL-cDNA was cloned into *Pichia pastoris* for overexpression (*51*). In Jülich, an active enzyme was expressed in *Escherichia coli* as an N-terminal hexa-histidine fusion protein, allowing the purification of homogeneous protein in one step. The formation of inclusion bodies was reduced by using a thioreductase deficient *E. coli* strain as the host. Under these conditions, recombinant LuHNL was obtained with a specific activity of 76 U/mg (*52*).

Other FAD-dependent HNLs have been isolated from *Rosaceae*, especially *Prunus* species and other rosaceous stone fruits, and characterised (see Table 1). Apple, apricot, cherry and plum meals were prepared from the seeds or kernels of mature garden fruits. These preparations and almond meal were used as the source of (*R*)-HNL for the synthesis of aliphatic and aromatic cyanohydrins. Apple seed meal, the most favourable of the crude enzyme preparations, accepts sterically hindered aldehydes (*e.g.* pivaladehyde) as substrates, leading to (*R*)-cyanohydrins with high enantiopurity (e.e.'s better than 90 %) (*53*). Subsequently, the oxynitrilase in apple meal was found to accept methyl ketones as substrates and, when a direct comparison with almond meal was carried out, the apple enzyme gave a slightly higher e.e. (*54*). In Shanghai, the (*R*)-HNL activity of peach and loquat preparations were compared with that of almond meal. The enzyme extracted from loquat had a rather

Table 1. Isolated and characterized (*R*)-selective hydroxynitrile lyases

Species	E.C. No.	Enzyme source	Natural substrate	Substrate acceptance	
				Arom.	Aliphat.
Prunus amygdalus	4.1.2.10	almond bran, overexpression in *Pichia pastoris*	*(R)*-mandelonitrile	+	+
Prunus serotina	4.1.2.10	black cherry kernels	*(R)*-mandelonitrile	+	+
Prunus domestica	4.1.2.10	plum kernel and leaves	*(R)*-mandelonitrile	+	+
Prunus avium	4.1.2.10	cherry kernel and leaves	*(R)*-mandelonitrile	+	+
Prunus persica	4.1.2.10	peach – kernel and leaves	*(R)*-mandelonitrile	+	+
Malus pumila	4.1.2.10	apple seed meal	*(R)*-mandelonitrile	+	+
Eriobotrya japonica		loquat seed meal		+	+
Linum usitatissimum	4.1.2.37	young flax plants overexpression in *Escherichia coli* and *Pichia pastoris*	*(R)*-2-butanone-cyanhydrin	–	+
Phlebodium aureum		leaves of goldfoot fern	*(R)*-mandelonitrile	+	weak
Pouteria sapota		mamey kernels		+	
Cydonia oblonga		quince kernels		+	
Cucumis melo		melon seeds		weak	

narrow substrate range, being restricted to aromatic and heteroaromatic aldehydes, and gave lower e.e.´s than those obtained with almond oxynitrilase. In contrast, peach meal had a substrate range similar to that of almond meal and in some cases gave products with superior e.e.´s. Thus, cinnamaldehyde was converted into its (*R*)-cyanohydrin with 69 % e.e. by peach meal, whilst under the same conditions almond meal gave a product with only 51 % e.e. (*55*).

Recently, oxynitrilase activity has been found in crude enzyme preparations from the leaves of mamey (*Pouteria sapota*), cherry (*Prunus avium*), plum (*Prunus domestica*), peach (*Prunus persica*), capulin (*Prunus serotina*) and the seeds of quince (*Cydonia oblonga*) where e.e.'s of synthesised (*R*)-mandelonitrile were higher than 90 %. For melon seeds (*Cucumis melo*) the e.e. was only 48 %. In the case of catalysis with leaf or seed extracts of guaje, sweet acacia, bonete, tejocote, chicozapote, pomegranate and canistel, the product of the addition of HCN to benzaldehyde was racemic (*56*). High enantiomeric purities (up to 98 % e.e.) were achieved with the defatted meal from capulin and mamey seeds, used as catalysts for the synthesis of cyanohydrins of aromatic aldehydes. There were differences in the reactivities and enantioselectivities of both meals, probably due to different properties of the (*R*)-oxynitrilases. The enzyme from mamey showed higher enantioselectivities (*57*,*58*). The HNL from mamey catalysed the addition of cyanide to imines, prepared from substituted aromatic aldehydes and aniline, producing α-amino-nitriles with a moderate enantioselectivity (23 % e.e.) (*59*). In a related manner, optically active α-amino nitriles were attained by the addition of acetone cyanohydrin to imines. The reaction was catalysed by the (*R*)-oxynitrilase in almond meal (*60*).

In 1995, Wajant described the purification of a novel (*R*)-HNL, which contains no FAD, from the fern *Phlebodium aureum*. This PhaHNL has no properties in common with the flavoprotein lyases from *Rosaceae*, except that it has the same natural substrate, (*R*)-mandelonitrile, which is released from prunasin in *Prunus* species and from vicianin in *Phlebodium aureum*. PhaHNL is a multimer of 20 kDa subunits and is suitable for synthesis of (*R*)-cyanohydrins in organic media (*61*).

(*S*)-selective HNLs

Rosenthaler reported the first (*S*)-selective oxynitrilase: »The leaves of *Taraktogenos blumei* contain an enzyme capable of yielding not D-benzaldehyde cyanohydrin like enzymes of the *Prunacae* but the L-form or L-oxynitriles. The same enzyme is possibly also present in the flowers of *Achillea millefolium*« (*32*).

In the 1960s, an oxynitrilase that catalyses the cleavage of *p*-hydroxymandelonitrile was purified from etiolated seedlings of *Sorghum vulgare* (*62*). Six years later it was found that *Sorghum* oxynitrilase is stereoselective and decomposes the L-isomer of *p*-hydroxymandelonitrile (*63*). The native molecular weight for the enzyme purified from *Sorghum bicolor* (SbHNL, E.C. 4.1.2.11) was determined by SDS-PAGE as 95 kDa (*64*). The enzyme is a 510-amino acid polypeptide chain, composed of two different subunits α and β. The active enzyme form is an $\alpha_2\beta_2$ heterotetramer assembled as a dimer of αβ units (*65*,*66*). The mature enzyme is N-glycosylated and exists in three different isoforms. The SbHNL amino acid sequence is not similar to that of any other known HNL, but the sequence of SbHNL is 60 % identical and 73 % similar to that of wheat serine carboxypeptidase (*7*). Recently, SbHNL has been crystallized in the presence of the inhibitor benzoic acid and the three-dimensional structure has been determined to a 2.3 Å resolution (*67*). The suggested mechanism for the cyanohydrin cleavage predicts the substrate to form a hydrogen bond between the cyanohydrin hydroxyl group and Ser158, while the nitrile group is directed towards Leu190 and Gly159. The phenyl ring is nicely sandwiched between the side chain of His160 and the nonpolar face of Pro64, and the additional hydroxyl group at C4 is within 2.7 Å of Asp126

and thereby assists in binding the substrate in the active site. A docking simulation further revealed that the carboxylate moiety of the C-terminal residue Trp270 undergoes a movement to form a good hydrogen bond contact between the Trp270 carboxylate and the cyanohydrin hydroxyl group, while an active site water adjusts to maintain a hydrogen bond contact between the Trp270 carboxylate and the substrate nitrile group. Cyclohexane carbaldehyde differs from benzaldehyde in that it has sp^3-hybridized C atoms with additional axial protons, rather than exclusively equatorial protons as in the phenyl ring of benzaldehyde, and is not accepted as aliphatic substrate. Additionally, carbonyl substrates with small aliphatic side chains such as butyraldehyde and isobutyraldehyde are not accepted as substrates (*68*). Models of complexes of SbHNL with cyclohexane carbaldehyde reveal several closer contacts than van der Waals of the axial protons, in particular at C2-C4 of cyclohexane carbaldehyde with Pro64 and His160. Similar disfavoured interactions are observed for C4 of butyraldehyde (*67*).

The cyanoglucoside linamarin occurs in varying proportions in seedlings of some higher plants as well as in *Manihot* and *Hevea brasiliensis* (*69*). The appending HNL from the tropical rubber tree (*Hevea brasiliensis*, HbHNL) was first isolated and studied in terms of cyanogenesis by Selmar (*70*), then characterized in terms of substrate selectivity by Klempier (*71*). The oxynitrilase from cassava (*Manihot esculenta*, MeHNL) was first purified, characterised and cloned by Hughes (*72–74*). Both enzymes (E.C. 4.1.2.39) are highly homologous (77 % sequence identity), have no cofactor, are nonglycosylated and belong to the α/β-hydrolase superfamily (*75,76*). The crystal structure of the HbHNL, resolved to 1.9 Å, shows an active site that is buried deep within the protein and connected with the outside by a narrow tunnel (*77*). Subsequently, structural parameters were reported for the same enzyme, refined against crystallographic data collected to 1.1 Å resolution (*78*). Crystallographic data were also measured and solved for HbHNL complexed with the natural substrate acetone as well as with a variety of inhibitors, including trichloroacetaldehyde, hexafluoroacetone, and rhodanide (*79*). The crystal structures of MeHNL complexed with acetone and the product analogue chloroacetone were determined and refined at 2.2 Å resolution. The substrates are positioned in the active site by hydrogen-bond interactions of the carbonyl O atom with Thr11, Ser80 and, to a lesser extent, Cys81 (*80*). In order to elucidate the mechanism, the three-dimensional structure of the MeHNL-Ser80Ala-acetone cyanohydrin complex was solved at the same resolution (*81*). Surprisingly, the structure of the crystal complex of mutant Trp128Ala with 4-hydroxybenzaldehyde, at 2.1 Å resolution, showed the presence of two molecules in a sandwich type arrangement in the active site with an additional hydrogen bridge to the reacting centre (*82*). In most cases of synthetic reactions, the MeHNL-Trp128Ala mutant proved to be superior as the wild-type (*83*). HbHNL-mutant Lys236Leu is inactive, although its three-dimensional structure is similar to the wild-type enzyme. However, the crystal structure of the Lys236-Leu-acetone cyanohydrin complex shows the substrate in a different orientation as compared to the wild-type complex (*75*).

The current view on the molecular reaction mechanism of the HbHNL-catalysed cyanohydrin cleavage, deduced from crystallographic experiments (*77–79*), NMR (*84*) and molecular modelling (*85*), involves the following four key steps: (*i*) the substrate cyanohydrin is attached to the active site by hydrophobic interactions and by hydrogen bonding between its hydroxy group and the OH groups of Thr11 and Ser80; (*ii*) after the substrate binds, the OH-Ser80 is deprotonated by His235, which induces the simultaneous deprotonation of the substrate hydroxyl by Ser80; (*iii*) the subsequent cleavage of the cyanohydrin is assisted by stabilization of the charge of the nascent cyanide through interaction with the positive charge of Lys236; (*iv*) the cyanide ion formed is eventually protonated by His235 (*84*). All these conclusions are confirmed by enzyme-kinetic data (*86*).

For the homologous MeHNL, structural analyses have resulted in similar but not completely identical mechanistic ideas. They differ with respect to the role of the lysine residue in the active site (Lys236 in the *Hevea* enzyme or Lys237 in the *Manihot* enzyme), which is not believed to contribute to the catalysis of MeHNL (*80,81*).

The kinetic behaviour of this class of enzymes has been the subject of a number of recent investigations. The inhibition pattern observed for benzaldehyde and HCN corresponds well to an ordered Uni Bi mechanism including the formation of a dead-end complex of enzyme, (*S*)-mandelonitrile and HCN. The latter is the first product released from the enzyme followed by benzaldehyde, while in the synthesis reaction benzaldehyde is the first substrate bound to the enzyme followed by HCN (*86*). The analyses indicate that the reaction does not follow the three-step, reversible-ordered Uni–Bi reaction scheme proposed by Jorns for the cleavage of (*R*)-mandelonitrile to HCN and benzaldehyde catalysed by PaHNL (*42,87*). Under similar experimental conditions, the HbHNL enzymatic reversible conversion of (*S*)-mandelonitrile to HCN and benzaldehyde was adequately described by a three-step, reversible-ordered Uni–Bi reaction scheme and the non-enzymatic conversion rate was just about small enough to be neglected (*88*). In the often-used two-phase systems, the mass transfer of benzaldehyde from organic to aqueous phase is enhanced by the biocatalytic reaction of the (*S*)-HNL from *Hevea brasiliensis* (*89*).

Another HNL that catalyses the dissociation of (*S*)-mandelonitrile to benzaldehyde and HCN, purified from the leaves of *Ximenia americana* (*Olacaceae*) (*90*), has been investigated only once with respect to structural organization and substrate specificity (*16*).

In addition, completely new sources for (*S*)-selective oxynitrilase have recently been reported (see Table 2). Anona (*Annona squamosa*) and cherimoya (*Annona cherimolia*) seeds produce very interesting results, where the negative value for the optical rotation of the produced mandelonitrile strongly suggests the 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 of the reaction conditions could improve the outcome of this transformation (*56*). It is noteworthy that the leaves of anona and cherimoya do not catalyse the same reaction. These findings are consistent with recent

Table 2. Isolated and characterized (*S*)-selective hydroxynitrile lyases

Species	E.C. No.	Enzyme source	Natural substrate	Substrate acceptance	
				Arom.	Aliphat.
Manihot esculenta	4.1.2.38	manioc leaves over-expression in *Escherichia coli*	acetone-cyanhydrin	+	+
Hevea brasiliensis	4.1.2.38	rubber tree leaves overexpression in *Pichia pastoris*	acetone-cyanhydrin	+	+
Sorghum bicolor	4.1.2.11	millet seedlings	*(S)*-4-hydroxy-mandelonitrile	+	–
Ximenia americana		–	*(S)*-mandelonitrile	+	–
Annonia muricata		guanabana seed meal		+	
Annonia cherimolia		cherimoya seed meal		weak	
Annonia squamosa		anona seed meal		weak	

results reported by the same group: the defatted meal of guanabana (*Annona muricata)* seeds catalyses the (*S*)-selective addition of HCN to heteroaromatic, aromatic and α,β-unsaturated aldehydes, but does not work with aliphatic aldehydes. For example, the cyanohydrin of 2-furfuraldehyde prepared by this biocatalyst showed 87 % e.e. after 95 % conversion. The reaction with cinnamaldehyde proceeded with a high e.e. (82 %) but low conversion (*91*).

HNLs in Organic Synthesis

In 1987, it was recognized that for obtaining good e.e.'s the non enzymatic cyanohydrin reaction has to be suppressed by working in organic solvents (*37*). Later, for working in aqueous medium it was acknowledged that the reaction has to be performed at low pH (*92*). The Stuttgart group reported the transformation of a set of aromatic and aliphatic aldehydes into their corresponding cyanohydrins. Ethyl acetate was used as solvent. Under these conditions the (*R*)-oxynitrilase from almonds bound to cellulose gave appropriate yields and e.e.'s between 96 and 99 % (*37*). Recently, besides organic solvents with microaqueous conditions (*55,93,94*), highly emulsified two phase systems (buffer/organic solvent) have been used (*95*) and, for the first time, ionic liquids have successfully been employed for Pa- and HbHNL-catalysed cyanohydrin formation (*96*). Besides these searches for new solvents for oxynitrilase-catalysed reactions, innovative HCN sources have also been explored. The commonly used method of transhydrocyanation, namely cyanide transfer from acetone cyanohydrin or from a more complex ketone cyanohydrin to aldehydes (*97–100)*, was modified by the application of ethyl cyanoformate as cyanide donor. In a chemoenzymic one-pot reaction of ethyl cyanoformate with benzaldehyde, catalysed by PaHNL, ethoxycarbonylated (*R*)-mandelonitrile was formed. Investigations revealed a two-step procedure consisting of an enzyme-catalysed addition of HCN, which was generated by hydrolysis of ethyl cyanoformate to the aldehyde, followed by the protection of the free cyanohydrin in the second step (*95*).

From the beginning of this millennium many novel substrates have been investigated in terms of initial reaction rate, substrate conversion and product e.e. For example, α-alkoxy and α,β-di-alkoxy substituted aldehydes were submitted to the catalytic action of the oxynitrilases from almond (PaHNL) and from *Hevea brasiliensis* (HbHNL) to give the corresponding cyanohydrins **1** (see Fig. 2) (*101*). (*R*)-(cyanohydroxymethyl)ferrocene (**2**) and (*R,R*)-1,1′-bis(cyanohydroxymethyl)ferrocene (**3**) were obtained with 99 and 96 % optical purity, respectively, by the catalysis of HbHNL in a standard biphasic reaction protocol (*102*). In a similar manner, the bifunctional cyanohydrin of methyl 4-oxobutanoate (**4**) was achieved in 99 % e.e. (*103*). Recent reports include the first enantioselective synthesis of fluorinated (*R*)-mandelonitriles (**5**)

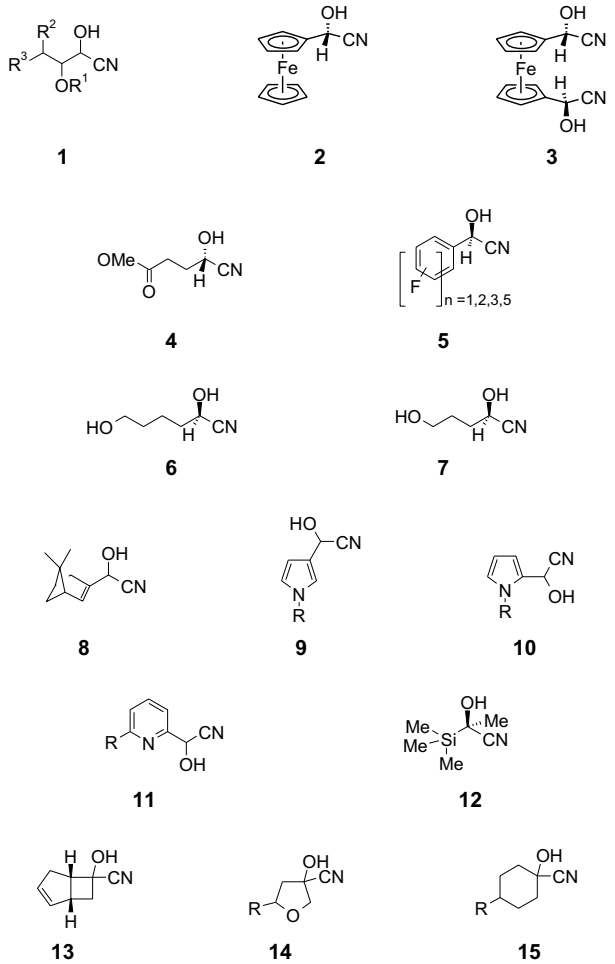

Fig. 2. Selected cyanohydrins recently synthesized using HNLs as catalyst

by hydrocyanation under microaqueous conditions with almond meal as catalyst (*104*) and almond-(*R*)-oxynitrilase-catalysed synthesis of optically enriched aliphatic ω-hydroxycyanohydrins from 4-hydroxybutanal (**6**) and 5-hydroxypentanal (**7**), which are mainly found as hemiacetals in the reaction media (*105*).

The enzyme-mediated preparation of enantiomerically or diastereomerically enriched cyanohydrins of polycyclic aldehydes has been investigated. Oxynitrilase-catalysed cyanurations gave excellent results with the bicyclic aldehydes tested. The results obtained with (*R*)-myrtenal proved that this aldehyde is a very good substrate for both PaHNL and HbHNL, giving the corresponding epimeric cyanohydrins **8** with extremely high diastereomeric purity (no formation of the other stereoisomer was observed) (*106*). The asymmetric hydrocyanation of *N*-substituted pyrrole-2- and -3-carboxaldehydes into their corresponding α-hydroxycyanides **9** and **10** catalyzed by HbHNL and PaHNL was reported, with moderate to good enantiomeric purities being obtained. Thus, *N*-benzylpyrrole-3-carboxaldehyde gave 91 % optical purity with both enzymes, Pa- and HbHNL (*107*). (*R*)-oxynitrilase (almond meal) catalysed hydrocyanation of 2-pyridinecarboxaldehyde was reported to give the corresponding cyanohydrin **11** in 42 % yield as a racemate, while the reaction with 6-bromo-2-pyridinecarboxaldehyde gave the corresponding cyanohydrin in 92 % yield and with 65 % e.e. (*108*).

(*S*)-Ketone cyanohydrins were synthesized by enantioselective HCN addition to methylketones by using hydroxynitrile lyase from *Manihot esculenta* as a biocatalyst (*109*). The synthesis of a member of a new class of compounds, optically active silicon-containing aliphatic (*R*)-ketone-cyanohydrin (*R*)-2-trimethylsilyl-2-hydroxylethylcyanide (**12**), was achieved with (*R*)-oxynitrilase from defatted almond meal (*110*) and apple seed meal (*111*) in a water/organic solvent biphasic system. Apple seed meal seems to be more efficient for the reaction than almond meal (*111*). This trend was observed as well for the synthesis of additional silicon-containing aliphatic (*R*)-ketone-cyanohydrins (*112*). The first enantioselective PaHNL-catalysed addition of HCN to a complex ketone, namely bicyclo[3.2.0]hept-2-en-6-one, gives access to a valuable single enantiomer of a bicyclic ketone *via* cyanohydrin **13** and was published by Gregory (*113*). In a comparable manner, heterocyclic saturated five- and six--membered ring ketones occasionally bearing a methyl substituent reacted with HCN under enzyme catalysis using recombinant HbHNL and PaHNL (*114*). These ring ketones were accepted as substrates as well as substituted cyclohexanone derivatives by PaHNL and MeHNL, yielding cyanohydrins **14** and **15** (*115,116*).

Conclusion

Numerous contributions of several research groups have led to the enantioselective cyanohydrin reaction that is catalysed by hydroxynitrile lyases being developed into a valuable tool for obtaining both (*R*)- and (*S*)-configured products in high enantiomeric purity. In principle, the structural requirements of the substrates to be accepted by HNLs have been evaluated. The HNLs are and will be genetically modified for optimisation of the catalytic performance of the enzyme. Presently HNLs from novel plant sources are being studied and efforts are being made for gaining insight into evolutionary aspects.

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Hidroksilnitril-liaze

Prirodna nalazišta i njihova primjena kao biokatalizatora

Sažetak

U ovom je revijalnom prikazu opisana primjena hidroksinitril-liaza pri dobivanju enantioselektivnih (*R*)- i (*S*)-cijanohidrina od aldehida i ketona. Osobito su istaknuti suvremena primjena i istraživanje što većeg broja biljaka kao izvora tih enzima. Mehanizam enzimom katalizirane reakcije može biti različit ovisno o porodici biljke. Usporedba struktura liaza na osnovi evolucijskoga gledišta novi je pristup istraživanju na tom području.