

Nitrile hydratase

In enzymology, **nitrile hydratases** (NHases; EC 4.2.1.84 (<https://enzyme.expasy.org/EC/4.2.1.84>)) are mononuclear iron or non-corrinoid cobalt enzymes that catalyse the hydration of diverse nitriles to their corresponding amides

R-C≡N + H₂O → R-C(O)NH₂

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Metal cofactor

In biochemistry, cobalt is in general found in a corrin ring, such as in vitamin B₁₂. Nitrile hydratase is one of the rare enzyme types that use cobalt in a non-corrinoid manner. The mechanism by which the cobalt is transported to NHase without causing toxicity is unclear, although a cobalt permease has been identified, which transports cobalt across the cell membrane. The identity of the metal in the active site of a nitrile hydratase can be predicted by analysis of the sequence data of the alpha subunit in the region where the metal is bound. The presence of the amino acid sequence VCTLC indicates a Co-centred NHase and the presence of VCSLC indicates Fe-centred NHase.

Metabolic pathway

Nitrile hydratase and amidase are two hydrating and hydrolytic enzymes responsible for the sequential metabolism of nitriles in bacteria that are capable of utilising nitriles as their sole source of nitrogen and carbon, and in concert act as an alternative to nitrilase activity, which performs nitrile hydrolysis without formation of an intermediate primary amide. A sequence in genome of the choanoflagellate *Monosiga brevicollis* was suggested to encode for a nitrile hydratase.^[1] The *M. brevicollis* gene consisted of both the alpha and beta subunits fused into a single gene. Similar nitrile hydratase genes consisting of a fusion of the beta and alpha subunits have since been identified in several eukaryotic supergroups, suggesting that such nitrile hydratases were present in the last common ancestor of all eukaryotes.^[2]

Industrial applications

NHases have been efficiently used for the industrial production of acrylamide from acrylonitrile^[3] on a scale of 600 000 tons per annum,^[4] and for removal of nitriles from wastewater. Photosensitive NHases intrinsically possess nitric oxide (NO) bound to the iron centre, and its photodissociation activates the enzyme. Nicotinamide is produced industrially^[3] by the hydrolysis of 3-cyanopyridine catalysed by the nitrile hydratase from *Rhodococcus rhodochrous* J1,^{[5][6]} producing 3500 tons per annum of nicotinamide for use in animal feed.^[4]

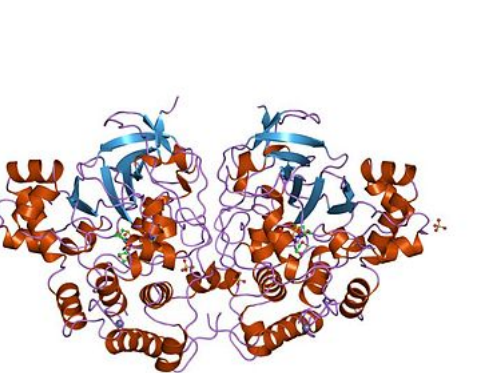
Structure

NHases are composed of two types of subunits, α and β, which are not related in amino acid sequence. NHases exist as αβ dimers or α₂β₂ tetramers and bind one metal atom per αβ unit. The 3-D structures of a number of NHases have been determined. The α subunit consists of a long extended N-terminal "arm", containing two α-helices, and a C-terminal domain with an unusual four-layered structure (α-β-β-α). The β subunit consists of a long N-terminal loop that wraps around the α subunit, a helical domain that packs with N-terminal domain of the α subunit, and a C-terminal domain consisting of a β-roll and one short helix.

Nitrile hydratase, alpha chain	
Identifiers	
Symbol	NHase_alpha
Pfam	PF02979 (http://pfam.xfam.org/family?acc=PF02979)
InterPro	IPR004232 (https://www.ebi.ac.uk/interpro/entry/IPR004232)
SCOP2	2ahj (http://scop2.mrc-lmb.cam.ac.uk/search?t=txt;q=2ahj) / SCOPe (https://scop.berkeley.edu/pdb/code=2ahj) / SUPFAM (http://supfam.org/SUPERFAMILY/cgi-bin/search.cgi?search_field=2ahj)
Available protein structures:	
Pfam	structures (http://pfam.xfam.org/family/PF02979?tab=pdbBlock) / ECOD (http://prodata.swmmed.edu/ecod/complete/search?kw=PF02979)
PDB	RCSB PDB (https://www.rcsb.org/search?q=rcsb_polymer_entity_annotation.annotation_id:PF02979%20AND%20rcsb_polymer_entity_annotation.type:Pfam); PDBe (https://www.ebi.ac.uk/pdbe/entry/search/index?pfam_accession:PF02979); PDBj (https://pdbj.org/searchFor?query=PF02979)
PDBsum	structure summary (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPfamStr.pl?pfam_id=PF02979)
PDB	1ahj (https://www.rcsb.org/structure/1ahj), 1ire (https://www.rcsb.org/structure/1ire), 1ugp (https://www.rcsb.org/structure/1ugp), 1ugq (https://www.rcsb.org/structure/1ugq), 1ugr (https://www.rcsb.org/structure/1ugr), 1ugs (https://www.rcsb.org/structure/1ugs), 1v29 (https://www.rcsb.org/structure/1v29), 2ahj (https://www.rcsb.org/structure/2ahj), 2cyz (https://www.rcsb.org/structure/2cyz), 2cz0 (https://www.rcsb.org/structure/2cz0), 2cz1 (https://www.rcsb.org/structure/2cz1)

Nitrile hydratase beta subunit	
Identifiers	
Symbol	NHase_beta
Pfam	PF02211 (http://pfam.xfam.org/family?acc=PF02211)
InterPro	IPR003168 (https://www.ebi.ac.uk/interpro/entry/IPR003168)
SCOP2	2ahj (http://scop2.mrc-lmb.cam.ac.uk/search?t=txt;q=2ahj) / SCOPe (https://scop.berkeley.edu/pdb/code=2ahj) / SUPFAM (http://supfam.org/SUPERFAMILY/cgi-bin/search.cgi?search_field=2ahj)
Available protein structures:	
Pfam	structures (http://pfam.xfam.org/family/PF02211?tab=pdbBlock) / ECOD (http://prodata.swmmed.edu/ecod/complete/search?kw=PF02211)
PDB	RCSB PDB (https://www.rcsb.org/search?q=rcsb_polymer_entity_annotation.annotation_id:PF02211%20AND%20rcsb_polymer_entity_annotation.type:Pfam); PDBe (https://www.ebi.ac.uk/pdbe/entry/search/index?pfam_accession:PF02211); PDBj (https://pdbj.org/searchFor?query=PF02211)
PDBsum	structure summary (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPfamStr.pl?pfam_id=PF02211)
PDB	1ahj (https://www.rcsb.org/structure/1ahj), 1ire (https://www.rcsb.org/structure/1ire), 1ugp (https://www.rcsb.org/structure/1ugp), 1ugq (https://www.rcsb.org/structure/1ugq), 1ugr (https://www.rcsb.org/structure/1ugr), 1ugs (https://www.rcsb.org/structure/1ugs), 2ahj (https://www.rcsb.org/structure/2ahj), 2cyz (https://www.rcsb.org/structure/2cyz), 2cz0 (https://www.rcsb.org/structure/2cz0), 2cz1 (https://www.rcsb.org/structure/2cz1), 2cz6 (https://www.rcsb.org/structure/2cz6)

nitrile hydratase	
Identifiers	
EC no.	4.2.1.84 (https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC4/2/1/84.html)
CAS no.	82391-37-5 (http://www.commonchemistry.org/ChemicalDetail.aspx?ref=82391-37-5&title=)
Databases	
IntEnz	IntEnz view (https://www.ebi.ac.uk/intenz/query?cmd=SearchEC&ec=4.2.1.84)
BRENDA	BRENDA entry (http://www.brenda-enzymes.org/enzyme.php?ecno=4.2.1.84)
ExPASy	NiceZyme view (https://enzyme.expasy.org/EC/4.2.1.84)
KEGG	KEGG entry (https://www.genome.jp/dbget-bin/www_bget?enzyme+4.2.1.84)
MetaCyc	metabolic pathway (https://biocyc.org/META/substring-search?type=NIL&object=4.2.1.84)
PRIAM	profile (http://priam.prabi.fr/cgi-bin/PRIAM_profiles/CurrentRelease.pl?EC=4.2.1.84)
PDB structures	RCSB PDB (https://www.rcsb.org/search?q=rcsb_polymer_entity.rcsb_ec_library.id:4.2.1.84) PDBe (https://www.ebi.ac.uk/pdbe/entry/search/index?ec_number:4.2.1.84) PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=4.2.1.84)
Gene Ontology	AmiGO (http://amigo.geneontology.org/amigo/term/GO:0018822) / QuickGO (https://www.ebi.ac.uk/QuickGO/term/GO:0018822)
Search	
PMC	articles (https://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&term=4.2.1.84%5BEC/RN%20Number%5D%20AND%20pubmed%20pmc%20local%5Bsb%5D)
PubMed	articles (https://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&term=4.2.1.84%5BEC/RN%20Number%5D)
NCBI	proteins (https://www.ncbi.nlm.nih.gov/protein?term=4.2.1.84%5BEC/RN%20Number%5D)



Structure of nitrile hydratase.[7]

	ucture/2cz1), 2cz6 (https://www.rcsb.org/structure/2cz6), 2cz7 (https://www.rcsb.org/structure/2cz7), 2d0q (https://www.rcsb.org/structure/2d0q), 2qdy (https://www.rcsb.org/structure/2qdy)
	ucture/2cz6), 2cz7 (https://www.rcsb.org/structure/2cz7), 2d0q (https://www.rcsb.org/structure/2d0q), 2dpp (https://www.rcsb.org/structure/2dpp), 2qdy (https://www.rcsb.org/structure/2qdy), 2zcf (https://www.rcsb.org/structure/2zcf), 2zpb (https://www.rcsb.org/structure/2zpb), 2zpe (https://www.rcsb.org/structure/2zpe), 2zpf (https://www.rcsb.org/structure/2zpf), 2zpg (https://www.rcsb.org/structure/2zpg), 2zph (https://www.rcsb.org/structure/2zph), 2zpi (https://www.rcsb.org/structure/2zpi)

Assembly

An assembly pathway for nitrile hydratase was first proposed when gel filtration experiments found that the complex exists in both αβ and α2β2 forms.^[8] In vitro experiments using mass spectrometry further revealed that the α and β subunits first assemble to form the αβ dimer. The dimers can then subsequently interact to form a tetramer.^[9]

Mechanism

The metal centre is located in the central cavity at the interface between two subunits. All protein ligands to the metal atom are provided by the α subunit. The protein ligands to the iron are the sidechains of the three cysteine (Cys) residues and two mainchain amide nitrogens. The metal ion is octahedrally coordinated, with the protein ligands at the five vertices of an octahedron. The sixth position, accessible to the active site cleft, is occupied either by NO or by a solvent-exchangeable ligand (hydroxide or water). The two Cys residues coordinated to the metal are post-translationally modified to Cys-sulfinic (Cys-SO₂H) and -sulfenic (Cys-SOH) acids.

Quantum chemical studies predicted that the Cys-SOH residue might play a role as either a base (activating a nucleophilic water molecule)^[10] or as a nucleophile.^[11] Subsequently, the functional role of the SOH center as nucleophile has obtained experimental support.^[12]

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