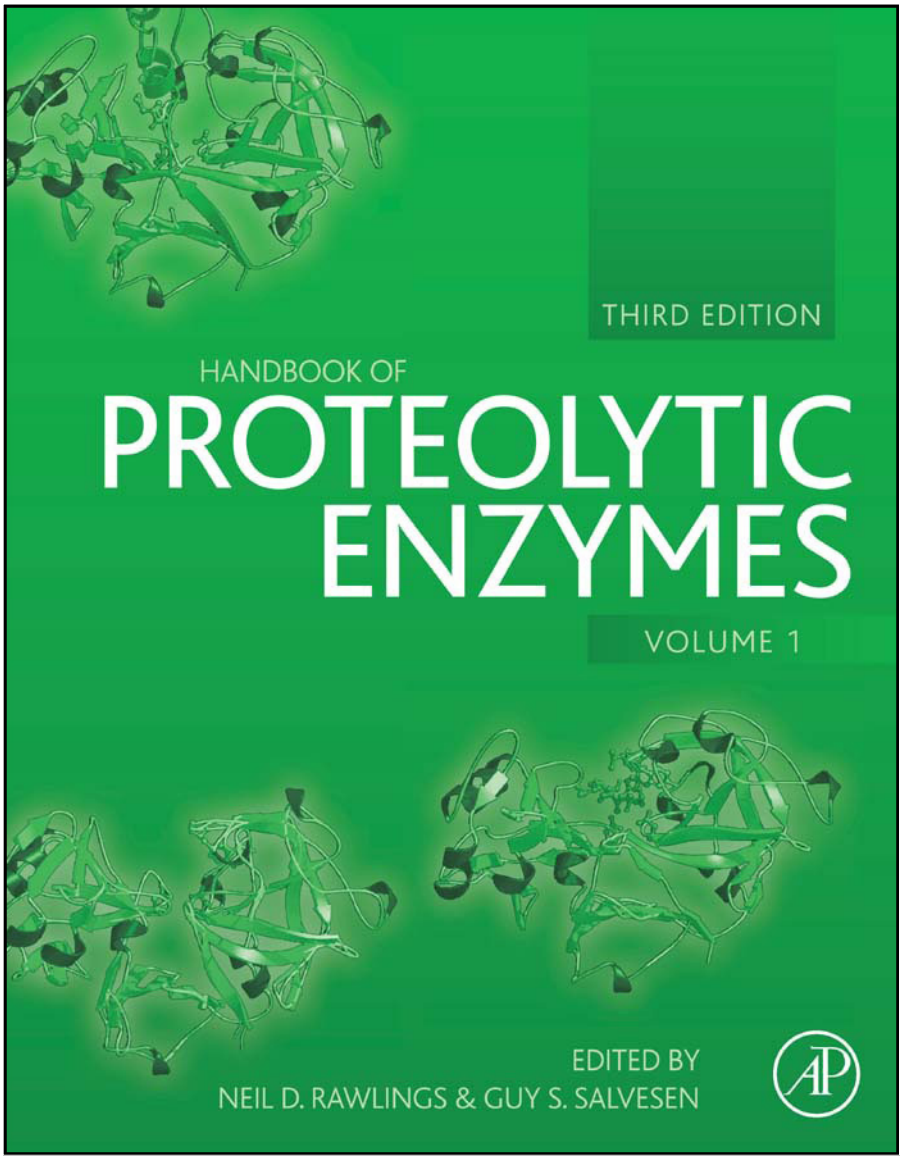

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Chapter 95

The Cold-Active M1 Aminopeptidase from the Arctic Bacterium *Colwellia psychrerythraea*

Databanks

MEROPS name: cold-active aminopeptidase (*Colwellia psychrerythraea*)-type peptidase
MEROPS classification: clan MA, subclan MA(E), family M1, peptidase M01.031
Tertiary structure: Available
Species distribution: superkingdoms Eukaryota, Bacteria
Reference sequence from: *Colwellia psychrerythraea*

Name and History

Colwellia psychrerythraea 34H is a Gram-negative marine psychrophilic (literally cold-loving) bacterium that has been isolated from Greenland sediments [1]. The observed temperature growth range of this microorganism is −6°C to 19°C, and the optimal temperature for growth is 8 to 9°C [2]. The genome of this Arctic strain has been sequenced and has revealed many adaptive features linked to microbial lifestyle at low temperatures [3]. *C. psychrerythraea* 34H secretes a family M1 aminopeptidase designated ***ColAP***, which displays at least two remarkable characteristics. Firstly, this is a cold-active protease that has contributed to understand the structure-function relationships in cold-adapted enzymes, and secondly it is a close homolog of human leukotriene A4 hydrolase (LTA4H; Chapter 96) involved in inflammation processes. The latter is a bifunctional zinc metalloenzyme which integrates aminopeptidase activity with a sophisticated epoxide hydrolase activity in a common active center [4]. This chapter summarizes the essential functional and structural properties of the M1 aminopeptidase ColAP from *C. psychrerythraea*.

Activity and Specificity

Aminopeptidase Activity

Purification procedures of ColAP have been described for both the wild-type and the recombinant enzyme expressed in *E. coli* [2,5]. ColAP displays the highest levels of activity with methylcoumarin substrates containing alanine and arginine residues and intermediate levels of activity with leucine. Lower levels of activity were observed with serine, while no detectable activity was observed with proline and aspartic acid. Activity was also observed with AAPL p-nitroanilide and the macromolecular substrate azocasein. ColAP exhibits a narrow pH range for activity, and the maximal activity was observed at neutral pH. At pH 6.0 and 8.5, only 10 and 5% of the maximal activity is retained, respectively. PMSF does not affect enzyme activity significantly, while 10 mM dithiothreitol or 1 mM EDTA completely inhibits ColAP activity. Activity also appeared to be strongly dependent upon chloride salts as no activity has been recorded in buffer lacking NaCl [2]. ColAP does not exhibit epoxide hydrolase activity as has been previously observed with other M1 aminopeptidases.

Cold Adapted Activity

Figure 95.1 and Table 95.1 both illustrate the thermodependence of activity using L-leucine 7-amido-4-methylcoumarin (MCA-L) as substrate. The highest k_{cat} value was observed at ∼19°C and 12% of the maximal activity was still recorded at −1°C. Furthermore, the K_m value was found to be optimum at this low temperature (Table 95.1). Interestingly, at all temperatures measured, the aminopeptidase activity of ColAP is significantly higher than for the human homolog LTA4H by at least a factor of 7. The latter property is considered as the main physiological adaptation to cold at the enzyme level as it compensates for the reduction of chemical reaction rates at low temperatures [6–8]. In the case of ColAP, it has been shown that a significant contribution to this high activity at low temperatures is provided by the low activation enthalpy ΔH that primarily reflects a weaker temperature dependence of the activity [5]. Accordingly,

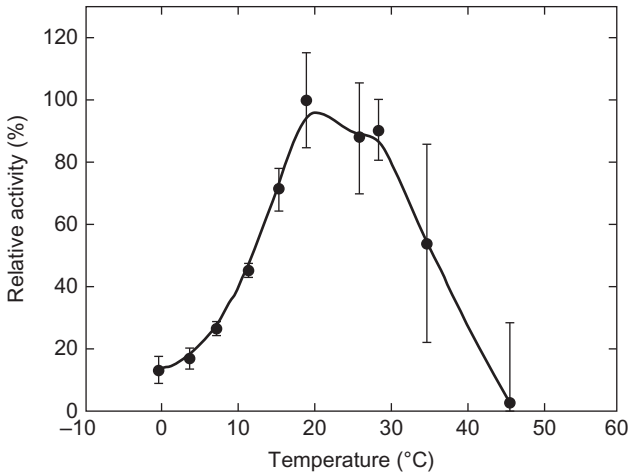

FIGURE 95.1 Activity of ColAP using 200 μM MCA-L as the substrate. Reprinted from Huston *et al.* [2] with permission from American Society for Microbiology, © 2004.

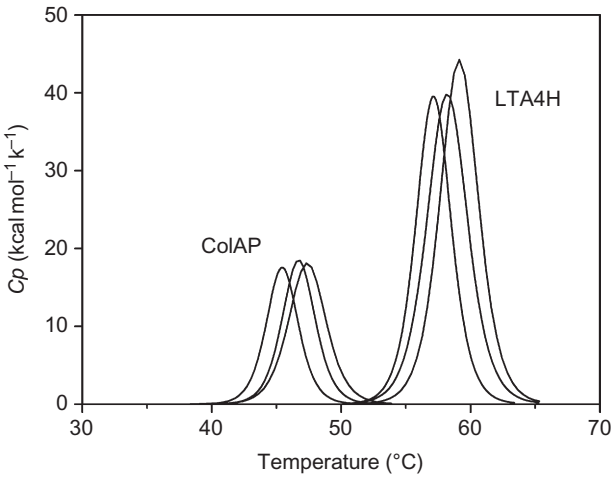

FIGURE 95.2 Thermal unfolding as monitored by DSC at scan rates of 0.5, 1.0, and 1.5 K min^{-1} for ColAP and human LTA4H. Adapted from Huston *et al.* [5] with permission from Elsevier, © 2008.

TABLE 95.1 Kinetic parameters for purified ColAP

Temperature °C	k_{cat} s^{-1}	K_m μM	k_{cat}/K_m s^{-1} mM^{-1}
−1	0.043 ± 0.006	43 ± 22	1.0
9	0.13 ± 0.02	58 ± 31	2.2
19	0.36 ± 0.06	72 ± 34	5.0

when the reaction temperature is reduced, the activity of ColAP is less reduced than that of human LTA4H.

Structural Chemistry

Gene Sequence and Homology

The ColAP gene sequence (GenBank accession number AY302752) encodes for a family M1 aminopeptidase with a predicted molecular mass of 71 273 Da [2]. Following removal of the signal sequence (determined experimentally), native ColAP has a predicted molecular weight of 68 593 Da and a pI = 5.25. ColAP exhibits the highest overall levels of amino acid identity (45 to 55%) with putative M1 aminopeptidases from mesophilic members of γ-proteobacteria and the next highest levels of identity (35 to 36%) with LTA4 hydrolases from various sources. The results of a multiple-sequence alignment indicated that there was perfect conservation of the putative substrate binding site (GGMEN), the zinc binding motif (HEXXH-X18-E), and catalytic Glu and Tyr residues involved in aminopeptidase activity which are thought to act as a general base and a proton donor, respectively.

Conformational Stability

Thermal unfolding of ColAP and LTA4H has been monitored by differential scanning calorimetry (DSC). As seen in Figure 95.2, conformational unfolding (as determined by T_m and ΔH_{cal} values) was scan rate-dependent, indicating that the thermal denaturation of these proteins is under kinetic control. Unfolding was also found to be irreversible. The stability of ColAP is significantly weaker, with average apparent T_m values of 46.5 and 58.2°C, and average ΔH_{cal} values of 265 and 670 kJ mol^{-1} for ColAP and LTA4H, respectively. The decreased microcalorimetric enthalpy values, ΔH_{cal}, indicate that fewer enthalpy-driven interactions are disrupted during thermal unfolding. These results suggest that enthalpic destabilization contributes to the weak structural stability of the cold-active enzyme relative to its human homolog. In addition, the structure of the psychrophilic ColAP has an improved propensity to be penetrated by a small quencher molecule when compared to human LTA4H [5]. This reveals a less compact conformation undergoing frequent micro-unfolding events of the native state and larger native state fluctuations for the cold-adapted protein.

Crystal Structure

The crystal structure of the cold-active aminopeptidase [9] has been solved at 2.7 Å resolution (PDB code 3CIA). As expected from the high sequence similarity, the overall fold and the main secondary structures of ColAP are very similar to those of LTA4H (Figure 95.3). The backbone structures of both enzymes can be superimposed with a root mean square deviation of 1.21 Å for 509 Cα. These monomeric enzymes are folded into three distinct domains

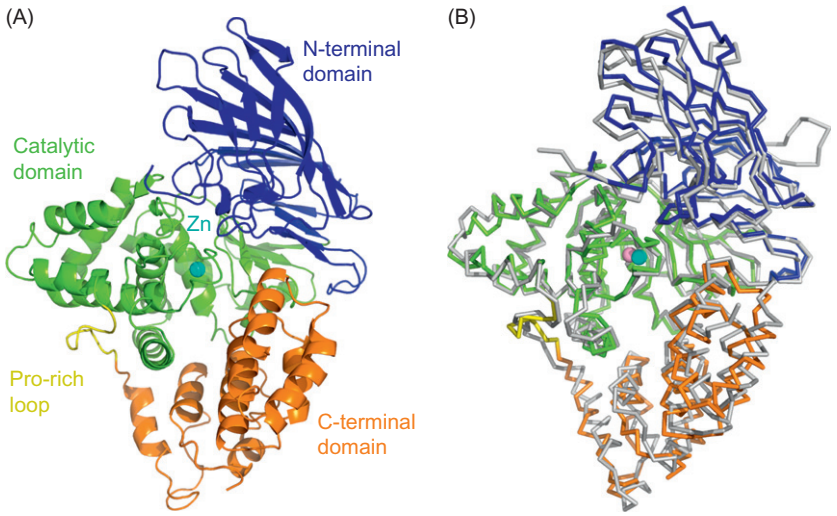

FIGURE 95.3 Overall structure of ColAP. The structural domains in ColAP are colored as follows: N-terminal domain in blue (residues 38–236), catalytic domain in green (residues 237–477), Pro-rich loops in yellow (residues 478–487), and C-terminal domain in orange (residues 488–629). (A) backbone schematic diagram of the crystal structure of ColAP; (B) superimposition of the Cα trace of ColAP and LTA4H (gray). Adapted from Bauvois *et al.* [9] with permission from the American Society for Biochemistry and Molecular Biology, © 2008.

in which the topologies are largely conserved. The N-terminal domain comprises three β-sheets, the major one consisting of seven mixed β-strands and two minor ones consisting of three and four antiparallel β-strands, respectively. On the whole, N-terminal domain β-sheets resemble a kind of saddle, presenting its large concave surface to the solvent. As already observed for LTA4H, the architecture of the ColAP catalytic domain is similar to that of thermolysin (Chapter 111). It comprises two lobes: one is composed of α-helices only, whereas the other is a mix of α-helices and β-strands. Between the lobes, a depression contains the zinc binding site. The C-terminal domain of ColAP is α-helical. In this domain, eight successive helices are arranged in a right-handed flat spiral.

Structural Determinants of Cold Adaptation

It has been argued that the high activity of psychrophilic enzymes arises from an improved flexibility and dynamics of the structure, allowing appropriate molecular motions at low temperature, which in turn render the enzyme heat-labile and unstable [6–8]. The structure of ColAP displays several features that can be related to this improved flexibility [9]. For instance, the Ala content is higher in ColAP and seems to be correlated to a reduction of more aliphatic residues, suggesting a global decrease in packing. A lower aliphatic index also suggests a lower hydrophobic effect stabilizing the core of ColAP. The Pro content is significantly lower in ColAP and the replacement of these Pro by residues possessing larger dihedral angles probably contributes to the increase in backbone flexibility. The analysis of ColAP secondary structures revealed a striking increase in the loop content as compared with LTA4H, which corresponds mainly to a shortening of the regular secondary structures. These observations suggest that ColAP, by having a higher loop content exposed to the solvent, has improved the breathing (or micro-unfolding) of its external shell in comparison with its human counterpart. ColAP exposes a higher proportion of aromatic residues to the solvent, which would result in entropic destabilization by weakening the hydrophobic effect. The number of complex ion pairs and of interdomain ion pairs is higher in the human LTA4H enzyme whereas no interdomain ion pair was found between the different ColAP domains. This suggests that the localization and complexity of salt bridges are determining factors for ColAP thermal adaptation and that cohesion between the different domains is lower in the psychrophilic enzyme. ColAP is also characterized by a lower number of hydrogen bonds that can contribute to a less stable native structure.

Zinc Binding Site

The amino acid sequence of ColAP is characterized by the conserved motif ^{324}HEXXH^{328} in which the histidine residues are the first two ligands of the metallic atom. The third ligand corresponds to a glutamate residue (Glu347) and is found toward the C-terminus. Superimposition of both the LTA4H and ColAP metal binding motifs clearly indicates that the three ligand residues are located at equivalent positions and that secondary structure, including those residues, fits very well for the most part. In ColAP and LTA4H, the environments of canonical zinc binding residues are nearly identical. This part of the active site is thus particularly well conserved in these two enzymes. However, significant differences are observed between ColAP and LTA4H metal coordination geometry: in ColAP, the zinc cation is more distant from the chelating residues. Furthermore, zinc cation occupancies are partial, between 30 and 40% depending on the ColAP monomer considered. These data suggest that zinc affinity is probably weaker in ColAP than in its

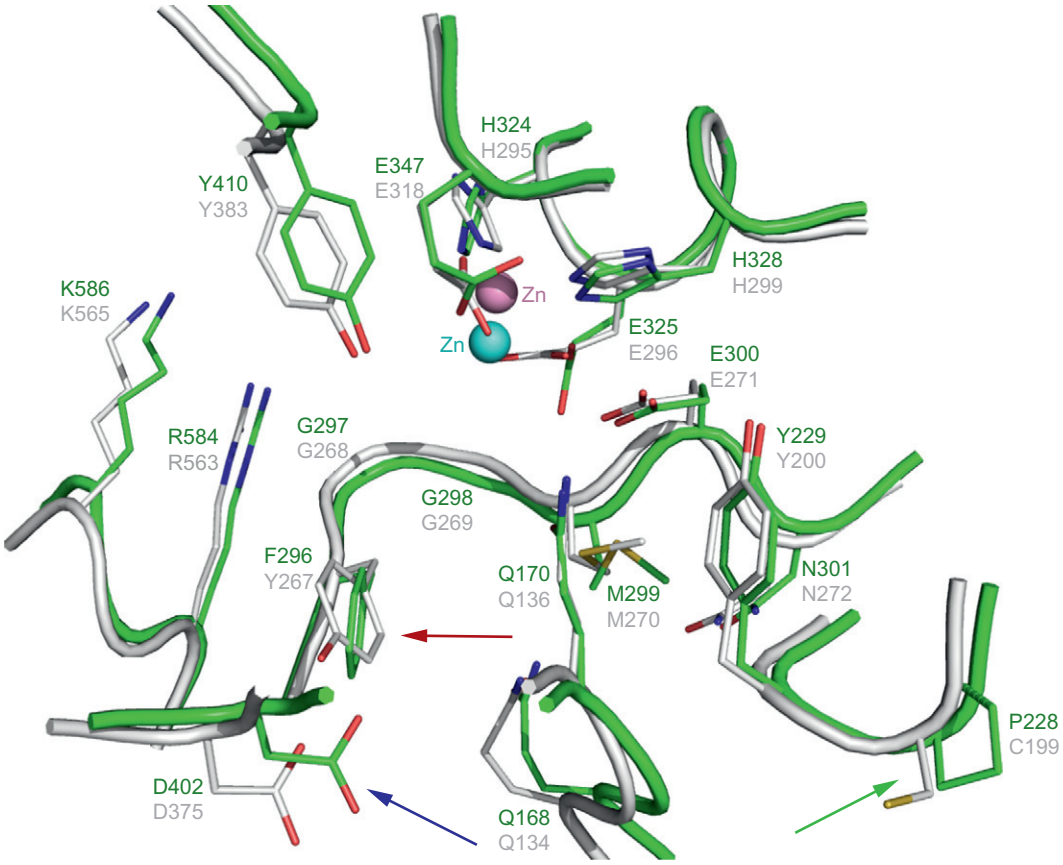

FIGURE 95.4 ColAP and LTA4H aminopeptidase active sites. Superimposition of ColAP (green) and LTA4H (gray) aminopeptidase active sites. The Cα trace and catalytic residue side chains are shown as smooth ribbons and sticks, respectively. The metallic cations are represented by a pink and a cyan sphere for ColAP and LTA4H, respectively. Differences are indicated by arrows. Adapted from Bauvois *et al.* [9] with permission from the American Society for Biochemistry and Molecular Biology, © 2008.

mesophilic counterpart. It should be noted that all psychrophilic proteins investigated thus far display low binding affinities for metal ions as a result of their flexible conformation [7].

Aminopeptidase Active Site

By analogy with LTA4H, ColAP aminopeptidase activity is also expected to follow a zinc-assisted general base mechanism. Among the catalytic residues (Figure 95.4), some are required for the scissile bond rupture, such as Glu325 and Tyr410, presumably as general base and proton donor, respectively [10,11]. Several residues included in the ^{297}GGMEN ColAP motif would be implicated in both binding and alignment of substrate. Glu300, probably assisted by Asn301/Gln170, would interact with the free N-terminal substrate conferring its exopeptidase specificity to ColAP. Further interactions could be made between the substrate and main chain atoms of Gly297 and Gly298. Arg584 and Lys586 would be involved in recognizing the C-terminus of substrate and could limit the substrate length. In such a binding mode, the oxygen carbonyl of the scissile bond would be located near the zinc cation, and a water molecule polarized by the metallic cofactor and Glu325 would be able to make a nucleophilic attack on the carbonyl. A carbocation would thus be generated in which the charge would be stabilized by interactions with the zinc cation, Glu325, Glu347, and maybe Tyr229. Finally, in the last step of the mechanism, Tyr-410 could act as a proton donor, and the products would then be released.

Epoxide Hydrolase Active Site

In addition to its aminopeptidase activity, human LTA4H retains the particularity of being an epoxide hydrolase with the ability to convert LTA4 into LTB4, a lipidic chemoattractant involved in inflammation. This second highly specific reaction also requires the zinc cation and occurs in an overlapping active site [4]. This is made possible only via a precise positioning of LTA4 in the active site [10,12]. The aminopeptidase active sites of ColAP and LTA4H enzymes are very similar but this is not the case for the part of the active site supposed to be more

specific for LTA4 hydrolysis. For instance, Asp402, corresponding to the essential Asp375 in LTA4H, is displaced from ~1 Å. Furthermore, its hydrophilic environment is only partially conserved. Further differences are observed between the hypothetical olefinic tail binding pockets. The main chain of residues 394–399 adopts a different conformation and occludes the pocket (opened to solvent in the human enzyme). Also, in ColAP, the Ile394 side chain (Val367 in LTA4H) points directly inside the pocket. These differences suggest that ColAP is probably unable to bind LTA4. In conclusion, the crystal structure suggests that the aminopeptidase catalytic mechanism is conserved in these two homologs. By contrast, the olefinic LTA4 binding region differs significantly in both enzymes, suggesting that the aminopeptidase active site has evolved from a common ancestor and that the second activity of LTA4H appeared later by the reshaping of the lipidic binding region.

Further Reading

For reviews on cold-active proteases, see Davail *et al.* [13], Aghajari *et al.* [14] and Smalås *et al.* [15].

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