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Carboxypeptidase Y

Cat No: ME92729

I.U.B.: 3.4.16.5
C.A.S.: 9046-67-7

Enzymatic Reaction

Carboxypeptidase Y (CPDY) is a glycoprotein exopeptidase of the acid and serine class.

History:
CPDY was originally referred to as proteinase C by Hata *et al.* in 1967. In 1970, Hayashi *et al.* found that CPDY released C-terminal amino acids from peptides and determined it is inactivated by DFP at the active site serine residue, thus classifying it as a serine peptidase (Hayashi *et al.* 1970, and Mortensen *et al.* 2004).

Wolf and Fink (1975) first identified the structural gene for CPDY, which was later confirmed by Hemmings *et al.* (1981). In the 1980s, extensive work was done to better understand the signal sequence of CPDY and it was determined that the protein can be translocated without its amino-terminal sequence (Hemmings *et al.* 1981 and Blachly-Dyson and Stevens 1987). Throughout the 1990s, the carbohydrate contents of CPDY were studied (Ballou *et al.* 1990), and in 1994 the crystal structure was solved to a 2.8 Å resolution by Endrizzi *et al.*.

Recent work has focused on transport of the nascent protein (Gharakhanian *et al.* 2011, Mukaiyama *et al.* 2010, and Burston *et al.* 2008) and inhibitory proteins (Gombault *et al.* 2009).

Specificity:
CPDY has broad amino acid specificity. It shows preference for hydrophobic amino acids in the P1' position of the substrate. CPDY is also able to catalyze aminolysis, the reverse reaction of hydrolysis (Remington and Breddam 1994). For details on the random order bi-bi mechanism CPDY employs, see Mortensen *et al.* 1994.

Composition:
Unlike carboxypeptidases A and B, CPDY contains no metal ion. It is glycosylated at four positions, and contains 15% mannose. Some of the carbohydrate chains are phosphorylated (Trimble and Maley 1977, Hasilik and Tanner 1978, Hashimoto *et al.* 1981, Winther *et al.* 1991, and Mortensen *et al.* 2004). Each molecule of CPDY contains 4-5 diesterfied phosphates (Hashimoto *et al.* 1981).
The CPDY structure consists of fourteen a-helices, eleven strands of mixed ß-sheets, five disulfide bridges, and one free cysteine residue (Endrizzi*et al.* 1994).

Molecular Characteristics:
The mature chain of CPDY contains 421 amino acid residues. The gene, *prc1*, has been cloned and sequenced and encodes a prepro form of the enzyme (Stevens *et al.* 1986, Blachly-Dyson and Stevens 1987, Valls *et al.* 1987, and Mortensen *et al.* 2004).

Protein Accession Number: P00729

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CATH Classification (v. 3.2.0):
CPDY contains two domains:

- Class: Alpha Beta; Mainly Alpha
- Architecture: 3-Layer(aba) Sandwich; Orthogonal Bundle
- Topology: Rossmann Fold; Helix Hairpins

Molecular Weight:

- 64 kDa (Hayashi *et al.* 1973, and Mortensen *et al.* 2004)

Optimal pH: 4.5-6.0

Isoelectric Point: 3.6 (Hayashi *et al.* 1973)

Extinction Coefficient:

- 88,940 $cm^{-1}M^{-1}$ (Theoretical)
- $E_{1\%,280}$ = 15.0 (Hayashi *et al.* 1973, and Kuhn *et al.* 1973)

Active Site Residues:

- Serine (S146)
- Aspartate (D338)
- Histidine (H397)

Inhibitors:

- Diisopropyl fluorophosphate (DFP)
- PMSF
- APCK
- 4-Hydroxymercuribenzoate
- Aprotinin
- Sensitive to thiol-blocking reagents
- A high affinity inhibitor encoded by the TFSI gene has been characterized and shows homology to a family of lipid binding proteins (Bruun *et al.* 1998)

Applications:

- C-terminal sequencing (Hayashi 1977)
- C-terminal modification/labeling of peptides and proteins (Remington and Breddam 1994)