

# Structure and function of a serine protease



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Enzymes have a fundamental role in all metabolic reaction that occurs in all living organisms. Enzymes are proteins which has the ability to bind to substrates and break substrate into products. In most cases, enzymes act as catalyst as they aid the reactants to come closer by using their active sites. In this essay I will be reviewing the structure and function of a serine protease known as subtilisin and I will analyze the different techniques used by different researchers to define the structure or functions of Subtilisin.

Subtilisin is found in various forms such as Carlsberg and 'BNP'. All three enzymes are from different origins, however they have similar primary structure although, their tertiary structures are completely different.

Subtilisin BPN is an extracellular protease enzyme of a spore forming organism *Bacillus Amyloliquefaciens*. This enzyme contains a single peptide chain of amino acid containing 275 residues with no disulphide bridges or SH group. The subtilisin BNP contains 8 right handed  $\alpha$  helical segment these regions are (Alden et al, 1970) . Many distortion has been observed due to the standard helix. The longest helix contains a number of residues such as Ala 223 to Histidine 238 which runs all the way from the top of the molecule through the molecules and to the other end. The other 6 helical segments lie approximately parallel to the longest helix (Alden et al, 1970).

Subtilisins are example of serine protease these are found in every organism that exists. There are several examples of serine protease enzyme and one of them is alpha-Chymotrypsin which is one of the known enzymes in the Trypsin family. The Trypsin and the subtilisin are believed to have evolved as they have similar mechanism of action even though they are not detectably related. Subtilisin is a bacterial protease, however Trypsin including  $\alpha$ -

Chymotrypsin, Trypsin and Elastase are from mammalian origin. These are the known enzymes to have evolved convergent because they have similar active sites and catalytic mechanisms but has no similarities in terms of sequence or conformational homology. The two known types of subtilisin are BPN' and Carlsberg; both have similar polypeptide chain, but there are segments on the chain where a number of residues has either been replaced or deleted. According to Emil (1966), BPN consist of peptide chain of 275 amino acids residues, however Carlsberg enzyme contains only 274 amino acid residues. As mentioned in this paper, the chain is only different by 83 residues which may lead to the question why they are different .

Furthermore, the similarities and differences between BPN and Carlsberg suggest there have been deletions which have caused both of these enzymes to evolve in different ways. These enzymes are believed to be 'serine protease' enzymes which means they all originate from the same source and due to evolution these enzyme has been converging away from each others path and both enzyme adapted in its environment in order to survive. This resulted subtilisin to be present in bacteria and  $\alpha$ -Chymotrypsin is found in the mammalian pancreas. This now brings us to the point that  $\alpha$ -Chymotrypsin and Subtilisins having the same catalytic mechanisms. This suggest that both Trypsin family and subtilisin family originates from source.

Serine proteases are highly specific enzymes as they use His, Ser and Asp part of their active site. The main characteristic of a serine protease is that they have a unique Ser residue of exceptional reactivity that forms a covalent bond with some of the substrates or inhibitors (Creighton J). In both Trypsin and subtilisin shows many similarity and both contains the

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particularly highly reactive serine residue which can be specifically phosphorylated by other substrate such as isopropylfluorophosphate (Alden and Wright, 1970), sulphonated and acylated using different enzymes. Further, research was carried out to find out the protonic equilibria exhibited by both of these enzymes.

The enzymatic similarity between the two different serine protease could be the involvement of a Histidine residue as it has been mentioned in most literature. Previous studies suggested that tertiary structure of  $\alpha$ -Chymotrypsin and subtilisin contains hydrogen bonding involvement in the Enzyme-substrate complex (Polgar and Bender, 1969). The  $K_{cat}$  for hydrolysis of p-nitrophenyl acetate by BPN depends on a group with a  $pK$  of 7.2, which presumably was Histidine (Alden and Wright, 1970).

Studies were carried out to determine the structure of the enzymes by Neidhart et al (1988) and they successfully refined the structure of Carlsberg. The structure was refined at 2.5 Å resolution using X-ray crystallography. Wright et al (1969) found out that subtilisin BPN possesses the 'catalytic triad', arrangement of active site including Aspartate, Histidine, and serine residues characteristic of the Trypsin family. Further study carried out by Robertus et al (1972), demonstrated that the arrangement and characteristics of the active site is identical for both BPN and other member of the Trypsin family, which means the peptide around the active site were highly conserved. In contrast, the overall peptide chain folding of subtilisin BPN reassembles those in the Trypsin family. Thus the relationship between BPN and mammalian Trypsin family of enzymes have been classified as a case of convergent evolution (Robertus et al, 1972). The <https://assignbuster.com/structure-and-function-of-a-serine-protease/>

catalytic triad consist of the residue Asp 32, His 64 and Ser 221. This supports the 'charge relay' hypothesis and the catalytic triad is the most important part of the serine protease as it helps the enzymes to work effectively. Subtilisin also contains calcium binding loops which contains about 75-81 residues which is identical in both type of subtilisin (Neidhart et al, 1988).

Subtilisin and  $\alpha$  Chymotrypsin both are highly specific as they only bind to specific substrates. For example, proflovin is a good competitive inhibitor for  $\alpha$  Chymotrypsin is inefficient against subtilisin. And oppositely subtilisin has a good competitive inhibitor 4-(4'aminophenylazo)-phenylarsonic acid, where as it doesn't interact with Chymotrypsin at all (Alden et al, 1970). Despite the Histidine residue in the active site, some specific inhibitors still bind to the active site by acylation of the Histidine of the Chymotrypsin; however they do not react with subtilisin. There are differences between subtilisin and Trypsin; subtilisin does not contain any disulphide bridges, but Chymotrypsin has five and Trypsin has 6 which suggest that trypsins are more cystine rich. So apart from the surrounding area of the serine 221 in subtilisin, these two classes have no resemblance from each other as far as the primary sequence is concern (Alden et al, 1970).