

# Mechanism of Enzyme Action of Chymotrypsin

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## Experiment Overview

Chymotrypsin is a proteolytic enzyme which is synthesised in the pancreas and released into the digestive tract as an inactive proenzyme or zymogen. Once in the gut, it is activated by the removal of part of the peptide chain. Its biological role is to hydrolyse dietary protein in the small intestine. It cleaves the peptide bond on the C (carbonyl) side of the aromatic residues, phenylalanine, tyrosine and tryptophan, and some large hydrophobic residues e.g. methionine and leucine.

When studying chymotrypsin's mechanism of action, an artificial substrate is usually used. There are a number of such substrates such as the methyl esters of amino acid analogs. These substrates are similar to some of chymotrypsin's biological substrates (it works on esters as well as proteins) and these artificial substrates have the added advantage of producing a coloured product. This enables us to monitor reactions spectrophotometrically.

The students will explore the two-step reaction mechanism, the enzyme dependence of this reaction, the activation of the zymogen, chymotrypsinogen by trypsin.

In this laboratory session the students are going to investigate the mechanism of action of chymotrypsin using the artificial substrate *p*-nitrophenylacetate (*p*-NPA).

## Learning Experience

This experiment gives students at intermediate level studying Protein Biochemistry an opportunity to study the mechanism of enzyme reaction they learn about in the lectures. The experiment is constructively aligned to their lectures.

## Aims and Objectives

The objective of this experiment is to give students an experience at designing and performing enzyme assays. This experiment will help students understand the mechanism of the action of chymotrypsin and how it relates to the experimental results. The students will also investigate the structure of chymotrypsin in the light of their experimental data. In the second part of the experiment the students will be introduced to the concept of zymogens and the activation of enzymes.

## Level of Experiment

Intermediate level biochemistry

## Keyword Descriptions of the Experiment

### Domain

Protein Chemistry

### Specific Descriptors

Enzyme kinetics, mechanism of action of an enzyme, structure function relationship of an enzyme.

Activation of zymogens.

## Course Context

This practical is normally in a lab session in Protein Biochemistry. Because of the fundamental nature of the mechanism of action of an enzyme, the students will be presented with the principles in the lectures. These principles will be reinforced in the experiments carried out in the lab session.

## Prerequisite Knowledge and Skills

Students require prior knowledge of some kind of enzyme assay, some molecular biology and biochemistry techniques. The students will have already covered the theory on enzymes and the kinetics and mechanism of action in the lectures. They will also have become familiar with setting up and performing an enzyme kinetics assay in the laboratory session prior to this one.

## Time Required to Complete

**Prior to Lab:** 1 hour

**In Laboratory:** 3 - 5 hours

**After Laboratory:** 2 hours

## Experiment History

This experiment was modified and worked on by Dr Dale Hancock and the technical staff in SMB in 1993 and has become an integral part of the labs for Protein Chemistry since then.