



Enzymes are specific?

As students, we are taught many things, and quite naturally we tend to believe what we are taught. Unfortunately because we accept what we are taught, sometimes it becomes dogma, it is never challenged and there is a danger that it becomes a self-perpetuating myth. One of the things we are taught, and which I would like to challenge is the idea that enzymes are specific.

I think this idea really is a self-perpetuating myth. A biological activity is observed, an enzyme (a biological catalyst), typically a protein, is isolated and found to be responsible for that activity. This protein is not tested for any other activities and thus it enters the literature as having only one function. The literature is full of hundreds, if not thousands, of examples of enzymes which have only one known activity. Thus a literature search will support the idea that enzymes are specific, but it does so not because the case is proven, but simply because no-one has looked for any other activity.

It is, of course, rather difficult to look at the structure of a protein and decide what its function is, or indeed what its other functions may be. With the advent of informatics based searching on sequences it ought to be easier to assign possible functions, but I suspect this is rarely done. A classical example is chloramphenicol acetyltransferase (CAT), a bacterial enzyme which detoxifies chloramphenicol and known since the 1960s. This enzyme was present in the organism before chloramphenicol was used as an antibiotic and no-one suggests that the natural function of this enzyme is to detoxify antibiotics. It must have another, as yet unidentified, natural function. If so, it would be hard to classify CAT as a *specific* catalyst.

Classifying enzymes is an interesting subject. Consider Subtilisin. It is classified as a non-specific protease! Proteases digest proteins, the cannibals of the protein world, and are enzymes, which of course we classify as specific. Thus Subtilisin is a non-specific specific biological catalyst.

Even the more specific proteases are quite flexible. Trypsin will cleave proteins at the basic amino acids lysine and arginine. Chymotrypsin is less specific, cleaving at the large hydrophobic residues of phenylalanine, tyrosine, and tryptophan. It also hydrolyses methionine and leucine peptide bonds at a lower rate. So one could be a little less rigid in the definition of specificity and say these enzymes are specific for cleaving peptide (amide) bonds. Except of course that if you look in the literature they also have esterase activity – how far do we loosen the definition of specificity? How far do we look for other activities?

The aliphatic amidase from *Pseudomonas aeruginosa*, hydrolyses acetamide. It also hydrolyses propionamide less well, but in addition to its amidase activity it also possesses esterase activity and transferase activity – the assay for the enzyme is based on the very efficient transfer of the acyl moiety of acetamide to hydroxylamine; not exactly hydrolysis. In the 1960's and '70's it was the subject of a very elegant directed evolution study; in fact all directed evolution studies rely on the fact that enzymes are not specific, but have other functionality which can be enhanced by mutation.

One of the most intriguing lacks of specificity is the enzyme pyruvate kinase, a glycolytic enzyme, from cod muscle. Creighton & Rose (J Biol Chem 251 69-72, 1976) reported that this enzyme is also responsible for oxaloacetate decarboxylase activity, a citric acid cycle reaction. Of course the protein may have been impure, it could have been a fusion protein, or it could be a prime example of evolutionary conservatism, which does not recognise the so-called specificity of enzymes.