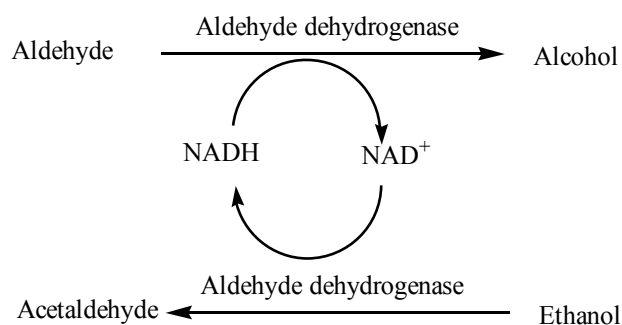


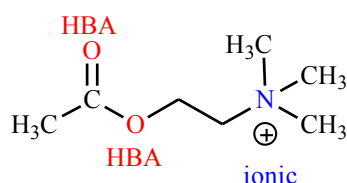
### Answers to end-of-chapter questions

1) The enzyme-catalysed reduction of an aldehyde requires one equivalent of the cofactor NADH, which is oxidised to  $\text{NAD}^+$ . However, if ethanol is added to the reaction, aldehyde dehydrogenase can catalyse its oxidation to acetaldehyde. In the process,  $\text{NAD}^+$  is converted back to NADH. As a result, only a catalytic quantity of NADH is required.

It is important to appreciate that enzymes can catalyse reactions in either direction until an equilibrium is reached. In this case two separate reactions involving the same enzyme are coupled together to recycle the cofactor.



2) Acetylcholine contains an ester functional group and a quaternary nitrogen which is charged. Thus hydrogen bonds and ionic interactions may be important. There are also methyl groups which might fit into hydrophobic pockets and interact via van der Waals interactions. The actual binding interactions for acetylcholine are discussed in section 22.5 and include the possibility of induced dipole interactions between the quaternary ammonium group of acetylcholine and aromatic/heteroaromatic groups of amino acid side chains.



3) A mechanism similar to that described for the hydrolysis of peptide bonds by chymotrypsin (section 3.5.3) would be feasible, involving a catalytic triad of serine, histidine and aspartate (or glutamate). Serine would serve as a nucleophile, histidine as an acid/base catalyst and aspartate (or glutamate) as an activating and orientating group. The actual mechanism for the hydrolysis of acetylcholine is described in section 22.12.3.2.

4) In general, binding interactions will involve acetylcholine being bound in a particular conformation such that the binding interactions are maximised. As a result,

certain bonds might be placed under strain with the result that they are broken more easily. For example, a hydrogen bond involving the carbonyl oxygen of acetylcholine may result in a weakening of the carbonyl  $\pi$  bond such that it is more easily broken in the first stage of the enzyme-catalysed mechanism. Note also that the strength of this interaction is likely to be more significant for the transition state compared to the substrate itself. In this way, the transition state will be stabilised more significantly than the substrate, resulting in a lowering of the activation energy.

5) The following table shows the values necessary for both the Michaelis Menton and Lineweaver-Burk plots.

Substrate concentration [S] ( $\text{mol dm}^{-3}$ )	Initial rate ( $\text{mol dm}^{-3} \text{ s}^{-1}$ )	1/(substrate concentration) $1/[S]$ ( $\text{mol}^{-1} \text{ dm}^3$ )	1/Initial rate ( $\text{mol}^{-1} \text{ dm}^3 \text{ s}$ )
0.05	28.6	20	0.035
0.10	51.5	10	0.0194
0.25	101	4	0.0099
0.50	141	2	0.0071
1	172	1	0.0058

The Lineweaver-Burke plot has 1/initial rate on the y-axis and 1/substrate concentration on the x-axis. This should give a straight line having an intercept of 0.00400 on the y-axis and a slope of 0.00155

Therefore, the maximum rate of reaction =  $1/\text{intercept} = 250.2 \text{ mol dm}^{-3} \text{ s}^{-1}$

$K_M = \text{slope} \times (\text{maximum rate of reaction}) = 0.387 \text{ mol dm}^{-3}$

The maximum rate of reaction is achieved at high substrate concentration.

$K_M$  is the Michaelis constant and corresponds to the substrate concentration at which the initial rate of reaction is half of  $J_{\text{max}}$ .

A Michaelis Menton plot of the maximum rate of reaction versus substrate concentration shows a curve where the line is approaching a maximum plateau, but has not yet reached it. Therefore, any attempt to define the maximum rate of reaction from this kind of plot can only be an estimate. Since we need to know the maximum rate of reaction in order to define  $K_M$ , the value of  $K_M$  will also be inaccurate.

The Lineweaver-Burke plot gives a straight line and allows us to define the maximum rate of reaction and  $K_M$  more accurately.

6) In the unmutated version of lactate dehydrogenase there is a glutamine residue in the active site which contains a neutral primary amide group at the end of the side chain. In the mutated version of the enzyme, this amino acid has been replaced by an arginine residue which contains a protonated guanidinium functional group. This can form an additional ionic interaction with malate that is not possible with lactate. The additional binding interaction accounts for the selectivity for malate over lactate as the former will have a much stronger affinity for the active site.

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Chapter 3 – Enzymes: structure and function

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