Solutions to problems for Chapter 9

9.1 The substrate (histidine) could be synthesized with a ^{14}C atom in the carboxylate group. The reaction will yield $^{14}CO_2$, which can be trapped by an appropriate base and its radioactivity measured (Fleming, J.V. and Wang, T.C. (2000) *Mol. Cell. Biol.* **20**, 4932–47).

9.3 The formation of ADP can be coupled via the action of pyruvate kinase (PK) and lactate dehydrogenase (LDH) to the consumption of NADH, which can be monitored continuously at 340 nm. It is essential that PK, LDH, and coupling substrates (phosphoenolpyruvate and NADH) are highly purified and are added in sufficient excess so that it is the reaction of interest (i.e. the creatine kinase reaction), which is rate-limiting. This can be verified by checking that the measured rate is proportional to the amount of creatine kinase added. In addition, the general precautions concerning enzyme assays listed in section 9.2.5 should be taken.

9.5 AMP acts as a non-competitive inhibitor of fructose bisphosphatase. In each case the K_m is 2.7 μM, but V_{max} is reduced from 125 μmol min^{-1} mg^{-1} (no AMP) to 66.7 μmol min^{-1} mg^{-1} (+70 μM AMP) and 45.5 μmol min^{-1} mg^{-1} (+140 μM AMP). From the reduction in V_{max} the value of $K_{EI} = 80$ μM. High concentrations of AMP are an indicator of low energy status in cells and thus that glucose breakdown rather than synthesis should be promoted.

9.7 The primary plot (e.g. 1/v vs 1/[3PGA] for each fixed value of 1/[2,3BPG]) gives a series of parallel lines, indicating that the enzyme follows an enzyme substitution mechanism. From the secondary plot (intercept vs 1/[3PGA]) and the slope of the lines in the primary plot the following parameters can be derived: $V_{max} = 1190$ μmol min^{-1} mg^{-1}; $K_{3PGA} = 1230$ μM; $K_{2,3BPG} = 4.2$ μM. It is likely that the function of 2,3BPG is to prime the enzyme for catalysis by transferring a phosphoryl group to an amino acid side chain at the active site. This phosphoenzyme can react with 3PGA to form 2PGA and regenerate 2,3BPG. An additional experiment to confirm this is described in section 9.9.1. It should be noted that a second class of PGAM enzymes found in certain bacteria, fungi, and insects has no requirement for 2,3BPG and hence must follow a quite different mechanism.

9.9 The side chain of an arginine residue (pK_a 12.5) remains protonated up to very high pH values (around 12). When considering the equilibrium Tyr–OH $\rightleftharpoons$ Tyr–O$^-$ + H$^+$, it is clear that the presence of a neighbouring positive charge will stabilize the anion, relative to the protonated form due to favourable electrostatic interactions. This will favour the dissociation of the Tyr–OH and lead to a lowering of the pK_a.

The relationship between the standard free energy change and the equilibrium constant for a reaction is $-\Delta G^0 = RT \ln K$. For the free amino acid, pK_a = 10.5, thus $K_a = 10^{-10.5}$, i.e. 3.16×10^{-11}. Putting $T = 298$K and $R = 8.31$ J K^{-1} mol^{-1}, $\Delta G^0 = 59.9$ kJ mol^{-1}. In the presence of the Arg side chain, pK_a = 8.0, i.e. $K_a = 10^{-8}$. Hence, $\Delta G^0 = 45.6$ kJ mol^{-1}. Thus, the free energy contribution of the electrostatic stabilization of the anion is –14.3 kJ mol^{-1}.

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9.11 The Arrhenius plot shows two distinct linear regions. In the range from 5 to 20°C, the slope is −16 100K, giving an E_a of 134 kJ mol^{-1}; in the range from 20 to 37°C, the slope is −7 600K, giving an E_a of 63 kJ mol^{-1}. There is a discontinuity in the plot at about 19°C; it is thought that this transition arises from structural changes in the phospholipid molecules, which are tightly associated with the enzyme.

9.13 The ratio (trypsin/chymotrypsin) of rates for BAPNA is 1 580/1; for SAAPFPNA the ratio is 1/480. It is clear that trypsin has a marked preference for BAPNA, which possesses a positively charged side chain (Arg) adjacent to the cleavage site, typical of protein substrates (Lys or Arg). By contrast, chymotrypsin has a very marked preference for SAAPFPNA, which has a Phe side chain adjacent to the cleavage site, consistent with the specificity of the enzyme for protein substrates (cleavage adjacent to bulky or aromatic side chains, such as Met, Leu, Phe, Tyr, or Trp). Note the degree of specificity is much higher with the less reactive amide substrates compared with esters.

9.15 The replacement of Tyr 28 by Phe has dramatically lowered k_{cat} (by a factor of 5 850-fold) but has had no effect on the K_m. This indicates that Tyr 28 is unlikely to play any significant part in binding the substrate, but is very likely to be involved in the catalytic mechanism. As indicated in problem 9.9, it is thought that the Tyr is involved in abstracting a proton from the substrate. Support for these conclusions would be gained from showing that the mutant had the same overall structure as the wild-type enzyme (using X-ray crystallography or circular dichroism) and obtaining structural information on the enzyme complexed with substrate or substrate analogues.

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