Chapter 10 Odd Solutions

1. The original and still pretty much the best evidence is that free indole cannot be detected in solution. For example, all efforts to trap indole proved unsuccessful. The crystal structure is also good evidence, as is kinetics, showing that indole gets from one active site to the other at a rate of more than $1000\,s^{-1}$, which is not consistent with its diffusing through solvent (estimated to be a rate of only $40\,s^{-1}$). Rather more sophisticated kinetic data show that indole is not generated by the α subunit until serine has bound at the β subunit: this is not evidence for channeling but does indicate tight allosteric control between the two subunits. Steady-state kinetics is not very helpful, because the reaction rate is limited by the slow release of tryptophan from the active site. However, stop-flow kinetics experiments such as those described by K.S. Anderson et al. (*J. Biol. Chem*. 266:8020–8033, 1991) have been used to show that the rate of formation of tryptophan at the β site is identical to the rate of synthesis of indole-3-glycerol-phosphate at the α site. Together with kinetic data on the mutant enzyme, this provides clear evidence of channeling.

3. Most probably yes. These two enzyme activities catalyze the first and third steps of methionine and threonine biosynthesis. In evolutionarily lower organisms, AK and HSDH are present as separate enzymes with varying feedback inhibition loops, affected by methionine and threonine. However, in higher plants the enzymes are present as a bifunctional enzyme with sensitivity only to threonine. The binding of threonine to a regulatory domain affects the activities of both catalytic domains (see, for example. S. Paris et al., *J. Biol. Chem*. **278**:5361–5366, 2003). Therefore the enzyme fulfills the requirements for a MEC as defined here.

5. Pyruvate dehydrogenase kinase phosphorylates E1 and deactivates it; this is reversed by pyruvate dehydrogenase phosphatase. Deactivation of PDH slows down pyruvate oxidation in the mitochondrion, which makes more pyruvate available for reduction to lactate in the cytoplasm. As one might expect, the kinase is activated by ATP, NADH, and acetyl-CoA (effectively the end products of the PDH complex) and inhibited by ADP, NAD^+, coenzyme A, and pyruvate. There are four human isozymes of the kinase, showing the complexity of regulation of PDH complex in humans.

7. Rather little is known about the organization and structure of nonribosomal peptide synthases (NRPSs) in comparison with polyketide synthases for example. A review (A. Koglin and C.T. Walsh, *Nat. Prod. Rep.* 26:987–1000, 2009) describes recent progress and is the basis for much of what follows. Amino acids are added on by a basic three-domain module, consisting of a condensation domain, which makes the peptide bond, an adenylation domain, which activates the peptide in preparation for condensation, and a thiolation domain, which is covalently attached to the growing chain in much the same way as the acyl carrier protein in polyketide synthases

or fatty acid synthases. Thus, the basic architecture has a set of these three domains for each amino acid added, which seem to be organized in fairly fixed relative positions appropriate for passing the peptide chain from one active site to another. As in polyketide synthases and fatty acid synthases, there is a termination domain at the end, to remove the mature chain. There are structures known for some individual subunits and even of entire modules, but no structure of an overall assembly. If the assembly is merely 'beads on a string' and the growing peptide chain is passed hand over hand from one unit to the next, then no larger-scale structure is required, and one would for example not expect to be able to solve a crystal structure for larger-scale units. There are, however, some indications that the structure is indeed more organized. At least some NRPSs have short communication-mediating domains that connect together individual peptide-adding units. This would be particularly important where the growing peptide chain has to pass from one protein chain to another, a frequent occurrence. A crystal structure has been obtained in 2007 of two sequential modules. Unfortunately it does not have the phosphopantetheine carrier arm, and the active sites of the relevant domains of the two adjacent modules do not seem to be close enough to pass the peptide from one to the other. It is thus not yet clear how organized the full-length protein may be. So far no structures have been obtained of complexes containing any of the additional tailoring enzymes such as hydroxylases, oxidases, or methyltransferases. If we define an MEC as requiring some communication between the component parts, then so far there is little indication that NRPSs could really be called MECs.

N1. From BRENDA, the turnover number of *E. coli* carbamoyl phosphate synthetase is roughly $9\,s^{-1}$. If the ammonium ion has a concentration of $150\,\mu M$, then at pH 7 ammonia has a concentration of $150\,\mu M \times 10^{-(9.2-7)}$ or $1\,\mu M$. Following the argument in Section 10.1.1, the rate of diffusion of ammonia into the active site (if unhindered) is expected to be about $10^9 \times 10^{-6}$ or $10^3\,s^{-1}$. This is 100 times faster than the enzyme turnover rate of about $10\,s^{-1}$, implying that the enzyme rate is unlikely to be slowed by diffusion—that is, one cannot use rate enhancement as an argument as to why this enzyme should use substrate channeling.