Chapter 6 - Processing Enzymes

End of chapter review questions answer key

Hands-on Exercise

1. Why does one waft?

Smelling can be very dangerous and can cause harm or loss of consciousness. Wafting a chemical vapour towards you with your hand dilutes the concentration of vapour making it safer. You should always know the chemicals you are working with and wether they are dangerous to breathe in.

2. What is the difference between the two negative control plates in the Smell-it Experiment?

One negative control includes cells that do not produce atf1 and therefore should not convert the substrate to the banana-smelling product. The second negative control includes the atf1 expressing strain but does not include any substrate and therefore cannot generate the banana smell. The second control demonstrates the strain itself does not make the smell and the first control demonstrates that the cells don't create atf1 without being engineered.

3. What are the experiment steps to get you to microfacture banana smell molecules?

1. Engineer blank cells with the DNA plasmid that causes expression of atf1 enzyme
2. Create Petri dishes that have the IAA substrate within them
3. Double streak engineered cells on the petri dish containing the IAA substrate
4. Incubate plates and allow the IAA substrate to diffuse into the engineered cells and be catalyzed into isoamyl acetate (banana smell)
5. Open and waft the smell towards to assess the smell.

4. What made it feasible to complete the Blue-it Kit using cell extract rather than in the cells (like the Smell-it Kit)

Cost. In the Smell-it Kit, the second substrate molecule needed to complete the chemical reaction is acetyl-CoA which is very expensive but is made naturally by the cells (free substrate!). Because of this, we use the cells to create this molecule on an ongoing basis for the reaction and therefore do not lyse the cells. In the Blue-it Kit, the X-gal substrates are affordable and the second substrate molecule needed during the reaction with beta-galactosidase is water. This means that the substrates are affordable and with the enzyme from the lysate can catalyze

the reaction outside the cell. If you wanted to and had a lot of money to spend, you could buy acetyl-CoA and do the Smell-it Kit experiment outside of the cells similar to the Blue-it Kit experiment.

5. What is DTT? Why is it used in the lysis and reaction buffers?

Dithiothreitol (DTT) is a small molecule that has the ability to reduce (give electrons to) other molecules. CElls are good at regulating the amount of oxygen inside of them which is important because oxygen is an oxidizing agent (steals electrons from other molecules). Outside of the cell in a tube, the oxygen is more abundant and a reducing agent like DTT will keep the important molecules reduced and functional

Fundamentals

1. Explain the Four B's in relation to a one or two substrate-enzyme reactions.

Bump: the enzyme and the substrate bump around the cell
Bind: the substrates have the right shape and charge to bind to the enzyme and so eventually form a complex.
Burst: the enzyme catalyzes a reaction turning the substrates into products
Bump: the products do not have the right size, shape or charge to remain bonded to the enzyme and bump out. Further substrates can now bump in and the process repeats.

2. How is the classic illustration of an atom incorrect? (Figure 6-10)

The classic planetary model posits that electron orbit in-ring-like patterns. It is now well understood that the electrons follow 3-dimensional balloon-like orbits around the nucleus. So while there was some truth to the original model, the orbital model is much more accurate.

3. What is a mole?

A unit of measurement that is based on a certain number of atoms (Avogadro's number) and is used to determine the atomic or molecular weights of different atoms or molecules.

4. Describe how a single electron can create an orbital.

Like the blades of a fan that are moving very fast and can be perceived as a disk, the very fast-moving electrons can be perceived as a "balloon shaped" cloud. The negative charge of the electron is held in an orbit by the positively charged nucleus.

5. What shapes do s-orbitals and p-orbitals have?

S-orbitals are spherical. P-orbitals are in the shape of dumbells

6. What are the four major types of bonding?

- Covalent bonding: electrons are shared between atoms,
- Ionic bonding: + and - charges of different atoms attract or repulse,
- Hydrogen bonding slightly negative charged atoms such as oxygen or nitrogen can be attracted to slightly positive charge hydrogen atoms
- Van der Waals bonding: when one side of an atom can be slightly more negatively charged and the other side slightly more positively charged and these small charges can interact with neighbouring atoms with these same properties.

7. What type of bonding is used when two strands of DNA zip together to form the double helix? Explain.

Hydrogen bonding: The nitrogenous base of each nucleotide has hydrogens (slightly + charge) and nitrogen or oxygen (slightly - charge). The complementary nucleotide (A-T C-G) lines up in a DNA helix so that the hydrogen can bond to the nitrogen/oxygen on the complementary nucleotide. There are two hydrogen bonds between As and Ts while there are three hydrogen bonds between Gs and Cs.

8. How does chloramphenicol acetyltransferase start the chemical reaction that is important for selection during genetic engineering?

A histidine amino acid in the active site of CAT uses spare electrons to steal one hydrogen from chloramphenicol. This causes a cascade of electron movement that results in chloramphenicol having an acetate group added to it rendering it inactive.

9. What is the difference between a simple enzyme reaction and a complex enzyme reaction?

A simple enzyme reaction involves one catalytic step whereas a complex enzymatic reaction involves many enzyme steps where the product of one enzymatic reaction becomes the substrates for another. This is very common in metabolic reactions.