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PROTEIN STRUCTURE AND FUNCTION

REVIEW THE CONCEPTS

1. The primary structure of a protein is the linear arrangement or sequence of amino acids. The secondary structure of a protein is the various spatial arrangements that result from folding localized regions of the polypeptide chain. The tertiary structure of a protein is the overall conformation of the polypeptide chain, its three-dimensional structure. Secondary structures, which include the alpha (α) helix and the beta (β) sheet, are held together by hydrogen bonds. In contrast, the tertiary structure is primarily stabilized by hydrophobic interactions between non-polar side chains of the amino acids and hydrogen bonds between polar side chains. (The quaternary structure describes the number and relative positions of the subunits in a multimeric protein.)

2. Despite the fact that folded proteins adopt conformations that are energetically favorable, the amount of time required for a particular protein to arrive at this conformation on its own can vary significantly. This is especially true if there are other "quasi-stable" conformations available to the polypeptide. Molecular chaperones function to protect an unfolded protein from participating in interactions that will take it off the "pathway" to its native, functional conformation. Chaperonins provide similar support to an unfolded protein. However, chaperonins can also use encapsulation and ATPase activity to give energetic "kicks" to misfolded proteins and get them back on the pathway toward their native folded state.

3. The active site of an enzyme is the region within which the substrate binds and is converted into product. The turnover number (k_{cat}) is the rate constant that can be used to calculate the rate at which a product is formed (V). The Michaelis constant

(K_m) is equal to the substrate concentration at which an enzyme will generate product at precisely one-half its potential maximal velocity. This maximal velocity (V_{max}) is the theoretical limit to the rate at which product can be formed by a particular preparation of enzyme. A rate constant never changes for a particular enzyme. However, the actual measured rate of the chemical reaction will change in response to many variables including the concentration of enzyme, its affinity for substrate, the concentration of substrate, the potential of product to inhibit substrate binding, etc. To calculate a rate (V), one would multiply the turnover number (k_{cat}) and [ES] (the concentration of enzyme-substrate). The rate (V) will equal the maximal rate (V_{max}) when all of the enzyme in a reaction is bound with substrate ([ES] = $[E]_{Total}$).

4. The addition of enzyme does not affect the free energy of either the substrate or the product. Therefore, the difference in free energy (ΔG) for a chemical does not change as a consequence of adding enzyme. The free energy of the enzyme-substrate complex (ES) is lower for E2. Therefore, E2 binds substrate with greater affinity than E1. The transition state ($X^{\ddagger}$) is stabilized equally well by both E1 and E2. Although both E1 and E2 stabilize $X^{\ddagger}$ equally well, E2 binds more tightly to S than E1. This results in a greater activation energy barrier for the reaction catalysed by E2 than by E1 and, therefore, E1 is the better catalyst. In fact, the reaction should proceed at the uncatalyzed rate in the presence of E2 because the height of the barrier between the lowest energy state of the substrate and its transition state is unchanged. By contrast, in the presence of enzyme E1 that barrier is significantly smaller resulting in a more rapid transition between substrate and product.

5. In order for an antibody to catalyze a chemical reaction it should show preferential binding affinity for the transition state of a chemical reaction. As transition states are, by definition, high-energy intermediates of chemical reactions rather than stable molecules, one would first have to synthesize a stable molecule with chemical properties similar to the transition state and use this "transition state analog" as an antigen to promote an adaptive immune response in a test animal.

6. Ubiquitin is a 76–amino acid protein that serves as a molecular tag for proteins destined for degradation. Ubiquitination of a protein involves an enzyme-catalyzed transfer of a single ubiquitin molecule to the lysine side chain of a target protein. This ubiquitination step is repeated many times, resulting in a long chain of ubiquitin molecules. The resulting polyubiquitin chain is recognized by the proteasome, which is a large, cylindrical, multisubunit complex that proteolytically cleaves ubiquitin-tagged proteins into short peptides and free ubiquitin molecules. Proteasome inhibitors would be useful to treat cancers if they blocked the degradation of proteins (e.g., tumor suppressors) required to halt the progression of uncontrolled cell growth. In the case of the proteasome inhibitor Velcade, which is used to treat patients with multiple myeloma, cells undergo apoptosis (programmed cell death), and because a protein serving as a pro-survival factor called NFκB cannot be activated when proteasome activity is blocked (reviewed in A. Fribley and C. Y. Wang, *Cancer Biol. Ther.*, 2006 July 1; 5(7):745–8).

7. Cooperativity, or allostery, refers to any change in the tertiary or quaternary structure of a protein induced by the binding of a ligand that affects the binding

of subsequent ligand molecules. In this way, a multisubunit protein can respond more efficiently to small changes in ligand concentration compared to a protein that does not show cooperativity. The activity of many proteins is regulated by the reversible addition/removal of phosphate groups to specific serine, threonine, and tyrosine residues. Protein kinases catalyze phosphorylation (the addition of phosphate groups), while protein phosphatases catalyze dephosphorylation (the removal of phosphate groups). Phosphorylation/dephosphorylation changes the charge on a protein, which typically leads to a conformational change and a resulting increase or decrease in activity. Some proteins are synthesized as inactive propeptides, which must be enzymatically cleaved to release an active protein.

8. Proteins can be separated by mass by centrifuging them through a solution of increasing density, called a density gradient. In this separation technique, known as rate-zonal centrifugation, proteins of larger mass generally migrate faster than proteins of smaller mass. However, this is not always true because the shape of the protein also influences the migration rate. Gel electrophoresis can also separate proteins based on their mass. In this technique, proteins are separated through a polyacrylamide gel matrix in response to an electric field. Because the migration of proteins through a polyacrylamide gel is also influenced by shape of proteins, the ionic detergent sodium dodecyl sulfate is added to denature proteins and force proteins into similar conformations. During rate-zonal centrifugation, a protein of larger mass (transferrin) will sediment faster during centrifugation, whereas a protein of smaller mass (lysozyme) will migrate faster during electrophoresis.

9. Gel filtration, ion exchange, and affinity chromatography typically involve the use of a bead consisting of polyacrylamide, dextran or agarose packed into a column. In gel filtration chromatography, the protein solution flows around the spherical beads and interacts with depressions that cover the surface of the beads. Small proteins can penetrate these depressions more readily than larger proteins and thus spend more time in the column and elute later from the column; larger proteins do not interact with these depressions and elute first from the column. In ion-exchange chromatography, proteins are separated on the basis of their charge. The beads in the column are covered with amino or carboxyl groups that carry a positive or negative charge, respectively. Positively charged proteins will bind to negatively charged beads, and negatively charged proteins will bind to positively charged beads. In affinity chromatography, ligand molecules that bind to the protein of interest are covalently attached to beads in a column. The protein solution is passed over the beads and only those proteins that bind to the ligand attached to the beads will be retained, while other proteins are washed out. The bound protein can later be eluted from the column using an excess of ligand or by changing the salt concentration or pH.

10. Proteins can be made radioactive by the incorporation of radioactively labeled amino acids during protein synthesis. Methionine or cysteine labeled with sulfur-35 are two commonly used radioactive amino acids, although many others have also been used. The radioactively labeled proteins can be detected by autoradiography. In one example of this technique, cells are labeled with a radioactive compound and then overlaid with a photographic emulsion sensitive to

radiation. The presence of radioactive proteins will be revealed as deposits of silver grains after the emulsion is developed. A Western blot is a method for detecting proteins that combines the resolving power of gel electrophoresis, the specificity of antibodies, and the sensitivity of enzyme assays. In this method, proteins are first separated by size using gel electrophoresis. The proteins are then transferred onto a nylon filter. A specific protein is then detected by use of an antibody specific for the protein of interest (primary antibody) and an enzyme-antibody conjugate (secondary antibody) that recognizes the primary antibody. The presence of this protein-primary antibody-enzyme-conjugated secondary antibody complex is detected using an assay specific for the conjugated enzyme.

11. X-ray crystallography can be used to determine the three-dimensional structure of proteins. In this technique, x-rays are passed through a protein crystal. The diffraction pattern generated when atoms in the protein scatter the x-rays is a characteristic pattern that can be interpreted into defined structures. Cryoelectron microscopy involves the rapid freezing of a protein sample and examination with a cryoelectron microscope. A low dose of electrons is used to generate a scatter pattern that can be used to reconstruct the protein's structure. In nuclear magnetic resonance (NMR) spectroscopy, a protein solution is placed in a magnetic field and the effects of different radio frequencies on the spin of different atoms are measured. From the magnitude of the effect of one atom on an adjacent atom, the distances between residues can be calculated to generate a three-dimensional structure.

    X-ray crystallography can provide extremely high-resolution structural information on molecules and molecular complexes of any size. The principal disadvantage of x-ray crystallography is the challenge of producing samples in the form of single crystals suitable for diffraction experiments. NMR spectroscopy gives high-resolution information on protein structures in solution. It also is ideal for monitoring protein dynamics. However, NMR spectroscopy is limited in its ability to conclusively determine the structures of very large proteins and symmetrical macromolecular assemblies. The principal advantage of electron microscopy is the relative ease of sample preparation. However, structural resolution is generally not so high as with the other methods, especially for asymmetric assemblies. NMR is better for small proteins. Electron microscopy and x-ray crystallography, so long as a suitable crystal can be obtained, are ideal for large proteins and macromolecular assemblies.

12. The four features of a mass spectrometer are 1) the ion source, 2) the mass analyzer, 3) the detector, and 4) a computerized data system. Basically, the investigator would collect protein samples from the cancerous cells and from the normal healthy cells, the latter serving as a control. Samples would be prepared for 2D PAGE and after electrophoresis the gels would be dyed and the profiles compared. If a protein "spot" were present in the sample from the cancer cell and not the control, it would be isolated out of the gel, protease-digested using trypsin to generate peptides that are mixed with a matrix, and applied to a metal target. A laser is used to ionize the peptides, which are vaporized into singly charged ions. In the case of a time of flight (TOF) mass analyzer, the time it takes the ions to pass through the analyzer before reaching

the detector is inversely proportional to its mass and directly proportional to the charge they carry, generating a spectrum in which each molecule has a distinct signal, allowing the investigator to calculate each ion's mass. The fourth essential component is a computerized data system that acquires and stores the data, which are then compared to information in databases. The mass and charge signature, or fingerprint, of the unknown is compared to that of peptides in a database and the best match protein is identified.