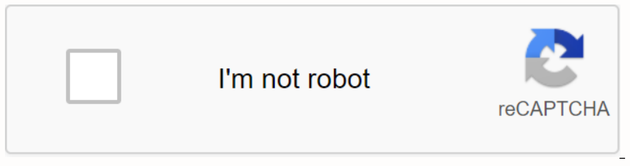

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Enzymes are reusable and specific what does that mean

Understanding:• Enzymes have an active place to which specific substrates are bound An enzyme is a globular protein that acts as a biological catalyst by accelerating the rate of a chemical reactionEnzymes are not altered or consumed by the reactions they catalyze and can therefore be reusedENzims are usually named after the molecules with which they react (called substrate) and end with the suffix -aseFor example , lipids are broken down by the enzyme lipaseActive Site The active site is the region on the surface of the enzyme that binds to the substrate molecule The active site and the substrate complement each other both in terms of form and chemical propertiesWhy only a specific substrate is able to link to the active site of a particular enzymeEnzyme and substrates Learning results Discuss how enzymes function as molecular catalysts Figure 1. Enzymes reduce reaction activation energy, but do not alter the reaction free energy. A substance that helps with a chemical reaction is called a catalyst, and molecules that catalyze biochemical reactions are called enzymes. Most enzymes are proteins and perform the critical task of reducing the activation energies of chemical reactions inside the cell. Most of the critical reactions to a living cell happen too slowly at normal temperatures to be of any use to the cell. Without enzymes to accelerate these reactions, life could not persist. Enzymes do this by binding to reaction molecules and holding them in such a way that the chemical processes of breaking and forming take place more easily. It is important to remember that enzymes do not change if a reaction is exergonic (spontaneous) or endergonic. This is because they do not change the free energy of reactants or products. They only reduce the activation energy required for the reaction to go forward (Figure 1). In addition, an enzyme itself is unchanged by the reaction it catalyzes. Once a reaction has been catalyzed, the enzyme is able to participate in other reactions. Chemical reactants to which an enzyme binds are called enzyme substrates. There may be one or more substrates, depending on the chemical reaction. In some reactions, a single reaction substrate is broken down into several products. In others, two substrates can come together to create a larger molecule. Two reactants could also enter a reaction and both become modified, but leave the reaction as two products. The localization inside the enzyme where the substrate binds is called the active site of the enzyme. The active site is where the action happens. Because enzymes are proteins, there is a unique combination of lateral chains of amino acids within the active site. Each lateral chain is different properties. They may be large or small, weakly acidic or base, hydrophiles or loaded positively or negatively or neutrally. The unique combination of side chains creates a very specific chemical environment within the active site. This specific medium is suitable to bind to a specific chemical substrate (or substrates). Active sites are subject to the influence of the local environment. Increasing environmental temperature generally increases reaction rates, enzymatic-catalyzed or otherwise. However, temperatures outside an optimal range reduce the speed at which an enzyme catalyzes a reaction. Warm temperatures will eventually cause the enzymes to distort, an irreversible change in the three-dimensional shape and therefore the function of the enzyme. Enzymes are also suitable to function best within a certain range of pH and salt concentration, and, as with temperature, extreme pH, and salt concentrations can cause enzymes to distort. For many years, scientists believed that enzyme-substrate binding occurred in a simple and key way. This model stated that the enzyme and substrate fit perfectly in a single instantaneous stage. However, current research supports a pattern called induced matching (Figure 2). The induced-fit model extends to the lock-and-key model by describing a more dynamic link between the enzyme and the substrate. As the enzyme and substrate merge, their interaction causes a slight change in the structure of the enzyme that forms an ideal binding arrangement between the enzyme and the substrate. When an enzyme binds its substrate, an enzyme-substrate complex is formed. This complex decreases reaction activation energy and promotes its rapid progression in one of several possible ways. At the basic level, enzymes promote chemical reactions involving more than one substrate by bringing substrates together in an optimal orientation for reaction. Another way in which enzymes promote the reaction of their substrates is by creating an optimal environment within the active place for the reaction to occur. Figure 2. The induced-fit model is an adjustment of the lock-and-key model and explains how enzymes and substrates undergo dynamic changes during the transition state to increase the substrate affinity for the active site. Figure 3. Have you ever wondered are pharmaceutical drugs developed? (credit: Deborah Austin) Enzymes are key components of metabolic paths. Understanding how enzymes work and how they can be regulated are key principles behind the development of many of the pharmaceutical drugs on the market today. Biologists working in this field are working with other scientists to design drugs. Consider statins, for example, statins is the name given to a class of medicines that can reduce the level of These compounds are inhibitors of the enzyme HMG-CoA reductase, which is the enzyme that synthesizes cholesterol in lipids in the body. By inhibiting this enzyme, enzymes, cholesterol synthesized in the body can be reduced. Similarly, acetaminophen, popularly marketed under the brand name Tylenol, is an inhibitor of the enzyme cyclooxygenase. While it is used to provide relief from fever and inflammation (pain), its mechanism of action is not yet fully understood. Are the drugs discovered? One of the biggest challenges in drug discovery is the identification of a drug target. A drug target is a molecule that is literally the target of the drug. In the case of statins, HMG-CoA reductase is the target of the drug. Drug targets are identified by thorough laboratory research. Identifying the target itself is not sufficient; scientists must also know the target acts inside the cell and which reactions go wrong in the case of the disease. Once the target and path are identified, then the actual drug design process begins. At this stage, chemicalists and biologists work together to design and synthesize molecules that can block or activate a particular reaction. However, this is just the beginning: If and when a prototype drug is successful in performing its function, then it is subjected to several tests from in vitro experiments to clinical trials before it can obtain approval from the U.S. Food and Drug Administration to be on the market. Many enzymes do not work optimally, or not at all, unless related to other specific non-protein aid molecules. They can be bonded either temporarily by ion or hydrogen bonds or permanently by stronger covalent bonds. Linking to these molecules promotes the optimal form and function of their respective enzymes. Two examples of these types of aid molecules are cofactors and coenzymes. Cofactors are inorganic ions, such as iron and magnesium ions. Coenzymes are aiding organic molecules, those with a basic atomic structure made up of carbon and hydrogen. As enzymes, these molecules participate in reactions without having changed themselves and are ultimately recycled and reused. Vitamins are the source of coenzymes. Some vitamins are the precursors of coenzymes, and others act directly as coenzymes. Vitamin C is a direct coenzyme for several enzymes that take part in building important connective tissue, collagen. Therefore, the enzyme function is, in part, regulated by the abundance of different co-enzymes and coenzymes, which can be provided by the diet of an organism or, in some cases, produced by the body. Help! Did you have an idea for improving this content? We'd like your opinion. Improve this pageLearn more An enzyme is a protein that acts as a catalyst in biochemical reactions. The substrates are linked to its active place and converted into a product. If you see message, means that we have problems loading external resources on our website. If you're behind a web filter, make sure that *.kastatic.org and *.kasandbox.org domains are unlocked. Unlocked. kinetics is the study of factors that determine the speed of enzymatic catalyzed reactions. It uses some mathematical equations that can be confusing for students when they first meet them. However, kinetic theory is both logical and simple and it is essential to develop an understanding of this subject in order to be able to appreciate the role of enzymes in both metabolism and biotechnology. Tests (measurements) of enzyme activity may be carried out either in a discontinuous or continuous manner. Discontinuous methods involve mixing the substrate and enzyme together and measuring the product formed after a set period of time, so that these methods are generally easy and fast to perform. In general, we would use such discontinuous tests when we know little about the system (and do preliminary investigations), or, alternatively, when we know a lot about the system and are sure that the time frame we choose is appropriate. In continuous enzyme tests, we generally study the rate of an enzyme catalyzed reaction by mixing the enzyme with the substrate and continuously measuring the appearance of the product over time. Of course, we could measure the reaction rate just as well by measuring the disappearance of the substrate over time. Apart from the actual direction (one rising and one decreasing), the two values would be identical. In enzymatic kinetic experiments, for convenience we often use an artificial substrate called chromogen that produces a brightly colored product, making the reaction easy to trace using a colorimeter or spectrophotometer. However, we could actually use any available analytical equipment that has the ability to measure the concentration of the product or substrate. In almost all cases, I also add a buffer solution to the mixture. After we see, the enzyme activity is strongly influenced by pH, so it is important to set the pH at a certain value and to maintain constant throughout the experiment. Our first enzyme kinetic experiment may therefore involve mixing a substrate solution (chromogen) with a buffer solution and adding the enzyme. This mixture would then be placed in a spectrophotometer and measure the appearance of the coloured product. This would allow us to follow a rapid reaction that, after a few seconds or minutes, could begin to slow down, as shown in Figure 4.A common reason for this slowing of the speed (speed) of the reaction is that the substrate in the mixture is used and thus becomes limiting. Alternatively, the enzyme may be unstable and distort during the experiment or change the pH of the mixture, as many reactions consume or release protons. For these reasons, when we are asked to specify the rate of a reaction, we do so from the beginning, ce enzyme enzyme and where none of the above limitations apply. We refer to this initial rapid rate as the initial speed (v0). Measuring the reaction rate at this early stage is also quite simple, because the rate is actually linear, so we can simply draw a straight line and measure the gradient (dividing the concentration variation by the time interval) to assess the reaction rate during this period. We can now perform a series of similar enzymatic tests to assess how the initial speed changes when the substrate or enzyme concentration is altered or when the pH is changed. These studies will help us characterize the properties of the enzyme studied. The relationship between the enzyme concentration and the reaction rate is usually a simple one. If we repeat the experiment described, but add 10% more enzyme, the reaction will be 10% faster, and if we double the enzyme concentration the reaction will continue twice as fast. Thus, there is a simple linear relationship between the reaction rate and the amount of enzyme available to catalyze the reaction (Figure 5). This relationship applies to both in vivo enzymes and those used in biotechnology applications, where regulation of the amount of enzymes present can control reaction rates. When we perform a series of enzymatic tests using the same enzyme concentration, but with a series of different substrate concentrations, a slightly more complex relationship occurs, as shown in Figure 6. Initially, when the concentration of the substrate is increased, the reaction rate increases considerably. However, as the substrate concentration is further increased, the effects on the reaction rate begin to decrease until a stage is reached when the increase in substrate concentration has a slightly greater effect on the reaction rate. At this time, the enzyme is considered to be close to substrate saturation, and demonstrating the maximum speed (Vmax). Note that this maximum speed is, in fact, a theoretical limit that will not really be reached in any experiment, although we could come very close to it. The relationship described here is a fairly common one, which a mathematician would immediately identify as a rectangular hyperbole. The equation describing such a relationship is as follows: The two constants a and b thus allow us to describe this hyperbolic relationship, as in a linear relationship (y = mx + c), which can be expressed by the two constants m (slope) and c (interception). In fact, I've already defined the constant of - it's Vmax. Constant b is a little more complex because it is the value on the x axis that gives half the maximum value of y. In enzymology we refer to this as the Michaelis constant (Km), which is defined as the concentration of the substrate that gives maximum speed. Our final equation, usually called the equation therefore becomes: The initial reaction rate(v0)=Vmax×Substrate allocation Substrate concentration+KmIn 1913, Leonor Michaelis and Maud Menten showed for the first time that it was actually possible to obtain this mathematical equation from the first principles, with some simple hypotheses about how an enzyme reacts with a substrate to form a product. At the heart of their derivation is the concept that the reaction occurs by forming an ES complex which, once formed, can either dissociate (productively) to release the product or dissociate in the reverse direction without the formation of the product. Thus, the reaction can be represented after the following, k1, k−1 and k2 being the rate constants of the three individual reaction steps: the Michaelis-Menten derivation requires two important hypotheses. The first hypothesis is that we take into account the initial reaction speed (v0) when the concentration of the product will be non-ligible low (i.e. [S] ≫ [P]), so that we can ignore the possibility of any product returning to the substrate. The second hypothesis is that the concentration of the substrate far exceeds the concentration of the enzyme (i.e. [S]≫[E]). The derivation begins with an equation for expressing the initial rate, the product formation rate, as the rate at which the ES complex dissociates itself to form the product. This is based on the constant rate k2 and the concentration of the ES complex, as follows: Since ES is an intermediate, its concentration is unknown, but we can express it in terms of known values. In an approximation in a stable state we can assume that although the concentration of the substrate and product changes, the concentration of the ES complex itself remains constant. The rate of formation of the ES complex and its breakdown rate must therefore be balanced, where: Es = k1[E][S] and ES Complex Breakdown Rate = (k−1 + k2)[ES]Therefore, in stable condition: This equation can be rearranged to produce [ES] following: Km michaelis constant can be defined as follows: : Equation 2 can thus be simplified to : Since the substrate concentration far exceeds the concentration of the enzyme (i.e. [S] ≫ [E]), the concentration of the non-combined substrate [S] is almost equal to the total concentration of the substrate. The concentration of the non-combined enzyme [E] is equal to the total enzyme concentration [E]T minus that combined with the substrate [ES]. The introduction of these terms to Equation 3 and the resolution for ES gives us the following: We can then enter this term in Equation 1 to give: The term k2[E]T actually represents Vmax, the maximum speed. Thus, Michaelis and Menmen were able to obtain the final equation as: A more detailed derivation of the Michaelis-Menmen equation can be found in many biochemistry manuals (see section 4 of the Recommended Reading section). There are also very useful web-based tutorials available on the subject. Michaelis constants have been determined for many commonly used enzymes and are usually in the lower millimolar range (Table 5). The typical range of Michaelis constant values. EnzymeKm (mmol l−1)Anhydrase carbonic26Chymotripsin15Ribonuclease8Tyrosyl-tRNA synthesis0.9Pepsin0.3 It should be noted that enzymes that catalyze the same reaction, but which are derived from different organisms, can have very different km values. In addition, an enzyme with several substrates can have quite different km values for each substrate. A low value km indicates that the enzyme requires only a small amount of substrate to become saturated. Therefore, the maximum speed is reached at relatively low substrate concentrations. A high value of km indicates the need for high substrate concentrations to reach the maximum reaction speed. Thus, we generally refer to Km as a measure of the enzyme's affinity for its substrate — in fact it is a reverse measure, where a large Km indicates a low affinity and vice versa. The value of Km tells us several important things about a particular enzyme. An enzyme with a low value km in relation to the physiological concentration of the substrate will probably always be saturated with substrate and will therefore act at a constant rate, regardless of variations in substrate concentration in the physiological range. An enzyme with a high value km in relation to the physiological concentration of the substrate will not be saturated with substrate, and its activity will therefore vary depending on the concentration of the substrate, so the rate of formation of the product will depend on the availability of the substrate. If an enzyme acts on several substrates, the substrate with the lowest Value Km is frequently assumed to be the natural substrate of that enzyme, although this may not be true in all cases. If two enzymes (with

similar Vmax) in different metabolic paths compete for the same substrate, then if we know the Km values for the two enzymes we can predict the relative activity of the two paths. Essentially, the path that has the enzyme with the lower value Km is likely to be the preferred path, and more substrate will flow through this path under most conditions. For example, phosphofructokinase (PFK) is the enzyme that catalyzes the first stage engaged in the glycolytic pathway, which generates ATP energy for the cell, while glucose-1-phosphate uridylyltransferase (GUT) is an early enzyme in the path that leads to the synthesis of glycogen (an energy storage molecule). Both enzymes use hexosis monophosphates as a substrate, but Km of PFK for its substrate is smaller than that of GUT for its substrate. Thus, at lower cell concentrations of hexosis phosphate, PFK will be active and GUT will largely inactive. At higher phosphate concentrations both paths will be active. This means that the cells store glycogen only in times of plenty, and always give preference for the ATP production pathway, which is the most essential function. Very often it is not possible to estimate the values km from a direct parcel of speed in relation to the concentration of the substrate (as shown in Figure 6), because we have not used concentrations high enough to approach even the estimate of the maximum speed and therefore we cannot evaluate half the maximum speed and therefore Km. Fortunately, we can trace our experimental data in a slightly different way to obtain these values. The most commonly used alternative is the Lineweaver-Burk plot (often referred to as the double-reciprocal plot). This parcel linearizes the hyperbolic curved relationship, and the line produced is easy to extrapolate, allowing the evaluation of Vmax and Km. For example, if we only get the first seven data points in Figure 6, we would have difficulty estimating Vmax from a direct plot, as shown in Figure 7a. However, after showing in Figure 7b, if these seven points are plotted on a graph of 1/speed compared to the concentration of 1/substrate (i.e. a double-reciprocal plot), the data are linearised, and the line can be easily extrapolated to the left to provide interceptions on both the y and x axis, from which Vmax and Km can be assessed, respectively. These concentrations may be most prone to errors (due to difficulties in making multiple dilutions) and could lead to reaction rates which, as they are slow, may also be the most prone to measurement errors. Often, as shown in Figure 8, such points when converted to the Lineweaver-Burk plot have a significant impact on the line of the best estimated fit in the data and therefore on the extrapolated values of both Vmax and km. The two sets of points shown in Figure 8 are identical, with the exception of the single point on the upper right, which reflects (due to the double reciprocal nature of the plot) a single point derived from a very low substrate concentration and a low reaction rate. However, this single point can have a huge impact on the best match line and accompanying estimates of kinetic constants. In fact, there are other kinetic plots that can be used, including the Eadie-Hofstee plot, the Hanes plot and the Eisenthal-Cornish-Bowden plot, which are less prone to such problems. However, the Lineweaver-Burk plot is still the most described kinetic plot in enzymology textbooks, and thus retains its influence in undergraduate education. Various environmental factors may affect the rate of enzymatic catalyzed reactions by reversible or irreversible changes protein structure. The effects of pH and temperature are generally well understood. Most enzymes have a characteristic optimal pH at which the catalysed reaction speed is maximum and higher and below which the speed decreases (Figure 9). The pH profile depends on a number of factors. As the pH changes, the ionization of the groups at both the active site of the enzyme and on the substrate may change, influencing the rate of binding of the substrate to the active site. These effects are often reversible. For example, if we take an enzyme with an optimal pH (pHopt) of 7.0 and put it in an environment at pH 6.0 or 8.0, the loading properties of the enzyme and substrate can be suboptimal, so that binding and therefore the reaction rate are reduced. If we then re-adjust the pH to 7.0, the optimal loading properties and therefore the maximum activity of the enzyme are often restored. However, if we place the enzyme in a more highly acidic or alkaline environment (e.g. at pH 1 or 14), although these conditions cannot actually lead to changes in the very stable covalent structure of the protein (i.e. its configuration), they may produce changes in the conformation (shape) of the protein, so that, when returned to pH 7.0, the original conformation and , therefore, the complete catalytic activity of the enzyme are not restored. It should be noted that the optimal pH of an enzyme cannot be identical to that of its normal intracellular environment. This indicates that the local pH can exert a controlling influence on the activity of enzymes. The effects of temperature on the activity of enzymes are quite complex and can be considered two forces acting simultaneously, but in opposite directions. As the temperature is high, the rate of molecular motion and therefore the reaction rate increases, but at the same time there is a progressive inactivation caused by the distortion of the enzyme protein. This becomes more pronounced as the temperature rises, so an optimal apparent temperature (Topt) (Figure 10) is observed. Thermal distortion depends on time, and for an enzyme the term optimal temperature has a reduced real significance, unless the duration of exposure to this temperature is recorded. The thermal stability of an enzyme can be determined by first exposing the protein to a range of temperatures for a fixed period of time and subsequently by measuring its activity at a favourable temperature (e.g. 25°C). The temperature at which distortion becomes important varies from enzyme to enzyme. Normally, it is negligible below 30°C and begins to become significantly above 40°C. Enzymes derived from microbial sources usually have much greater thermal stability than those from mammalian sources, and enzymes derived from highly thermophile microorganisms would be (a protease from Bacillus thermoproteolyticus) and Taq polymerase (a DNA from Thermus aquaticus), it could be completely thermostable at 70 °C and still retains substantial levels of activity even at 100 °C.After spending time learning about enzyme kinetics and the Michaelis-Menten relationship, it is often quite disconcerting to find that some of the most important enzymes do not actually display such properties. Alasteric enzymes are key regulatory enzymes that control metabolic path activities by responding to inhibitors and activators. These enzymes actually exhibit a sigmoidal (S-shaped) relationship between the reaction rate and the substrate concentration (Figure 11), rather than the usual hyperbolic relationship. Thus, for alosteric enzymes there is an area where the activity is less than that of an equivalent normal enzyme and also an area where the activity is greater than that of an equivalent normal enzyme, with a rapid transition between these two phases. This is rather like a switch that can be quickly changed from off (low activity) to on (full activity). Most allosteric enzymes are polymeric, that is, they are composed of at least two (and often many more) individual polypeptide chains. They also have several active sites where the substrate can bind. Much of the understanding of the function of alosteric enzymes comes from hemoglobin studies which, although not an enzyme, bind oxygen in a similar way of cooperation and thus also demonstrate this sigmoid alrelationship. Alosteric enzymes have an initially low affinity for the substrate, but when a single substrate molecule binds, it can break some bonds within the enzyme and therefore change the shape of the protein so that the remaining active sites are able to bind with greater affinity. Therefore, alosteric enzymes are often described as moving from a tense state or from the T state (low affinity) in which no substrate is bound, to a relaxed state or R-state (high affinity) as the substrate binds. Other molecules can also bind to alisteric enzymes at additional regulatory sites (i.e. not to the active site). Molecules that stabilize the protein in its T state therefore act as alosteric inhibitors, while molecules that move the protein to its R state will act as alosteric activators or promoters. A good example of an alosteric enzyme is transcarbamoylase aspartate (ATCase), a key regulatory enzyme that catalyzes the first step engaged in the sequence of reactions that produce pyrimidine coreotides, which are essential components of DNA and RNA. The reaction is as follows: The final product in the pathway, the pyrimide triphosphate (CTP) pyrimide (CTP) as an allosteric inhibitor enzyme ATCase. Therefore, when there is a high concentration of CTP in the cell, it feeds and inhibits the enzyme ATCase, reducing its activity and thereby reducing the production rate of additional pyrimidine nuleotides. As As the concentration of CTP in the cell decreases, as does the inhibition of ATCase, and the resulting increase in enzyme activity leads to the production of more pyrimidine nuleotides. This negative feedback inhibition is an important element of biochemical homeostasis inside the cell. However, in order to synthesize DNA and RNA, the cell requires not only pyrimidine nutreotides, but also purine collectotides, and these are required in approximately equal proportions. The synthesis of purine takes place by a different, but interesting way, the final product, purine neotide adenosine triphosphate (ATP), is a powerful activator of the enzyme ATCase. This is logical, because when the cell contains high concentrations of purine nuleotides it will require equally high concentrations of pyrimidine neotides for these two types of nucleotides to combine to form the DNA of polymers and RNA. Thus, ATCase is able to regulate the production of pyrimidine nuleotides inside the cell according to cell demand and also to ensure that the synthesis of pyrimidine nuleotides is synchronized with the synthesis of purine nuleotides — an elegant biochemical mechanism for regulating an extremely important metabolic process. There are some rare, though important, cases of monomeric enzymes that have a single substrate binding site, but are able to demonstrate the kinetics of the sigmoid reaction characteristic of alosteric enzymes. Particularly noteworthy in this context is the menomeric enzyme glucokinase (also called hexokinase IV), which catalyzes the phosphorylation of glucose into glucose-6-phosphate (which can be metabolized through the glycolytic pathway or can be used in glycogen synthesis). It has been postulated that this kinetic behavior is the result of individual glucokinase molecules existing in one of two forms — a form with low affinity and a form of high affinity. The low affinity form of the enzyme reacts with its substrate (glucose), is then transformed into the form with high affinity and remains in this state for a short period of time before slowly returning to its original form with low affinity (demonstrating a so-called slow transition). Therefore, at high substrate concentrations, the enzyme is likely to react with a second substrate molecule shortly after the first (i.e. while still in its high affinity form), while at lower substrate concentrations the enzyme may return to its low affinity form before reacting with subsequent substrate molecules. This leads to its characteristic kinetics of sigmoidal reaction. Page 2The turning over time of common enzymes with large variations. Enzymatic roll-over rate (alunit product s−1 mole enzyme−1)Carbonic anhydrase600 000Catalase93 000b-galactosidase200Chymotrypsin100Tyrosinase1 000b–galactosidase200Chymotrypsin100Tyrosinase1