Review Article

RNA as ENZYMES and comparison of its properties with PROTEINS as ENZYMES

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Abstract

Background: Enzymes /ˈɛnzaɪmz/ are both proteins and biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. RNA- The chemically versatile molecule : of The information content of the cell is contained in DNA, which codes for proteins that carry out the majority of cellular functions. However, the discovery of catalytic RNA led to the RNA world hypothesis: at one point, RNA could have been both the information carrier and the functional molecule . It is necessary but not sufficient for the RNA world to be self-perpetuating: it must also have given rise to current biology featuring protein enzymes. The details of this transition are purely speculative, but it may have included the recruitment of amino acids or short peptides as enzymatic cofactors. Clearly, RNA is capable of specifically binding these ligands and could use them to assist in chemistry. Enzymes are generally globular proteins, acting alone or in larger complexes. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme . Dynamics of Protein –Enzymes : These are not rigid, static structures; instead they have complex internal dynamic motions – that is, movements of parts of the enzyme's structure such as individual amino acid residues, groups of residues forming a protein loop or unit of secondary structure, or even an entire protein domain. Types of inhibition-Competitive, Non-competitive, Uncompetitive, Mixed, Irreversible. **Conclusion:** Only two reactions are observed to be catalysed by RNA in nature. However, the diversity of catalytic mechanisms for these reactions implies that RNA may be capable of much more. This is supported by the success of in vitro selection to develop ribozymes for RNA ligation.

Key Word: RNA – Enzymes , Protein Enzymes , ribozymes, properties of enzymes

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INTRODUCTION

Enzymes /ˈɛnzaɪmz/ are both proteins and biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.1:8.[1] Metabolic pathways depend upon enzymes to catalyze individual steps. The study of enzymes is called enzymology and a new field of pseudoenzyme analysis has recently grown up, recognising that during evolution, some enzymes have lost the ability to carry out biological catalysis, which is often reflected in their amino acid sequences and unusual 'pseudocatalytic' properties.[2,3] Enzymes are known to catalyze more than 5,000 biochemical reaction types.[4] Other biocatalysts are catalytic RNA molecules, called ribozymes. Enzymes' specificity comes from their unique three-dimensional structures. Like all catalysts, enzymes increase the reaction rate by lowering its activation energy. Some enzymes can make their conversion of substrate to

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product occur many millions of times faster. An extreme example is orotidine 5'-phosphate decarboxylase, which allows a reaction that would otherwise take millions of years to occur in milliseconds.[5,6] RNA- The chemically versatile molecule : of The information content of the cell is contained in DNA, which codes for proteins that carry out the majority of cellular functions. However, the discovery of catalytic RNA led to the RNA world hypothesis: at one point, RNA could have been both the information carrier and the functional molecule [7]. At first the main function would be self-replication, but as more complex RNAs evolved, they would need to synthesize their precursors, store and use energy, and isolate their reaction products from the environment. Eventually, RNA would have to transition towards modern biology, where proteins play most of the catalytic roles. This would involve the increasingly complex task of message-directed protein synthesis, including the specific activation of amino acids to be incorporated. It is now known that natural ribozymes use a variety of catalytic mechanisms, even for phosphoryl transfer. Furthermore, RNA regulatory elements, called riboswitches, have been shown to bind a variety of cofactors, small molecules which expand the repertoire of modern protein enzymes. In addition to phosphoryl transfer ribozymes, the RNA component of the ribosome is responsible for catalysing protein synthesis. This is the key reaction required to transition from RNA to protein as functional molecule. These observations indicate the versatility of RNA in catalysing chemical reactions, an essential characteristic for the RNA world. The RNA components of other phosphoryl transfer enzymes—RNase P and the spliceosome—also bind divalent metal ions important for catalysis [8,9]. Clearly, RNA is suited for recruiting divalent metal ions, which can be used for a variety of catalytic roles. The large ribozymes are all metalloenzymes, but surprisingly, the hairpin, Varkud Satellite (VS) and hammerhead ribozymes are active in the absence of divalent metal ions [10]. Each of these ribozymes, along with the hepatitis delta virus ribozyme (HDV), catalyse self-cleavage and ligation reactions as part of processing genome replicants. The hammerhead ribozyme is perhaps the most extensively studied small ribozyme, and the wealth of biochemical and structural data support a catalytic mechanism dependent on RNA functional groups. Although early crystal structures of a minimal hammerhead construct conflicted with biochemical data, this has been reconciled by the structure of a full-length, fully active construct.[11] This appears to be a common strategy for the small ribozymes. These experiments implicate nucleobases in acid–base catalysis for the hairpin, HDV and VS ribozymes [13,14]. This is unexpected because no RNA functional groups have unperturbed pKas near neutrality. It is possible that some of these bases are participating in hydrogen bonds instead of full acid–base catalysis. Alternatively, the bases could be functioning as alternate tautomers, especially for guanine, which has been repeatedly observed in position for acid–base catalysis [13]. Metal ions may also play an indirect role in altering the pKa of a nucleobase so it may act as a general acid or base [14,15,16,17,18,19]. Protein enzymes commonly have pKa shifts of several units; it appears that RNA is capable of the same. A third strategy for catalysis is demonstrated by the glmS riboswitch. In response to high concentrations of glucosamine-6-phosphate, this riboswitch catalyses its own cleavage, which results in subsequent nuclease degradation of the mRNA. The pKas of glucosamine-6-phosphate and several analogues track closely to the observed reaction pKas of the ribozyme, suggesting a direct role in catalysis.[17] Glucosamine6-phosphate binds in the active site, in position to donate an amino proton to the leaving group.[20] It is also possible that the positively charged amino group stabilizes the negatively charged transition state. In either case, recruitment of a small molecule cofactor expands the functional groups available for catalysis. Aided by this cofactor, glmS can reach rates as high as 5s 21[18]

PROTEIN SYNTHESIS and RNA: It is necessary but not sufficient for the RNA world to be self-perpetuating: it must also have given rise to current biology featuring protein enzymes. The details of this transition are purely speculative, but it may have included the recruitment of amino acids or short peptides as enzymatic cofactors. Clearly, RNA is capable of specifically binding these ligands and could use them to assist in chemistry. Interestingly, many of the cofactors discussed above contain a nucleic acid element [20], suggesting that molecules like them may have been selected in part for their ability to bind to RNA. As peptides became more complex, the chances of encountering them randomly in solution would drop precipitously, and machinery for specifically synthesizing them would have to be developed. Eventually, this could become the mechanism for protein synthesis. Since the machinery for making proteins would have to predate proteins, the RNA world hypothesis predicts such machinery would be made of RNA. In all extant organisms, protein synthesis is carried out by a large nucleoprotein complex, the ribosome. Strikingly, the active site for peptide bond formation is composed entirely of RNA [21, 22]. This is possibly the single most significant piece of data supporting the RNA world hypothesis. The ribosome manufactures proteins by linking individual amino acids through peptide bonds . As in phosphoryl transfer, this reaction may be catalysed by deprotonating the nucleophile, protonating the leaving group and stabilizing build-up of charge in the transition

state. Substrate positioning is expected to play a large role, as it does for all bimolecular reactions. It is unknown (and perhaps unknowable) what a more primitive ribosome looked like, and at what point aminoacyl adenylates were used as substrates. Although a small ribozyme has been developed by in vitro selection which can form a peptide bond between two aminoacyl adenylates [23], message-directed synthesis requires a more complex system with decoding abilities. Nevertheless, the modern ribosome is a vivid demonstration that RNA has the catalytic properties necessary to begin protein synthesis. A fundamental task of proteins is to act as enzymes—catalysts that increase the rate of virtually all the chemical reactions within cells. Although RNAs are capable of catalyzing some reactions, most biological reactions are catalyzed by proteins. In the absence of enzymatic catalysis, most biochemical reactions are so slow that they would not occur under the mild conditions of temperature and pressure that are compatible with life. Enzymes accelerate the rates of such reactions by well over a million-fold, so reactions that would take years in the absence of catalysis can occur in fractions of seconds if catalyzed by the appropriate enzyme. Cells contain thousands of different enzymes, and their activities determine which of the many possible chemical reactions actually take place within the cell 24.

Properties and characteristics of protein as enzymes :

Enzymes are generally globular proteins, acting alone or in larger complexes. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme.[25] Although structure determines function, a novel enzymatic activity cannot yet be predicted from structure alone.[26] Enzyme structures unfold (denature) when heated or exposed to chemical denaturants and this disruption to the structure typically causes a loss of activity.[27] Enzyme denaturation is normally linked to temperatures above a species' normal level; as a result, enzymes from bacteria living in volcanic environments such as hot springs are prized by industrial users for their ability to function at high temperatures, allowing enzyme-catalysed reactions to be operated at a very high rate. Enzymes are usually much larger than their substrates. Sizes range from just 62 amino acid residues, for the monomer of 4-oxalocrotonate tautomerase,[28] to over 2,500 residues in the animal fatty acid synthase.[29] Only a small portion of their structure (around 2–4 amino acids) is directly involved in catalysis: the catalytic site.[30] This catalytic site is located next to one or more binding sites where residues orient the substrates. The catalytic site and binding site together comprise the enzyme's active site. The remaining majority of the enzyme structure serves to maintain the precise orientation and dynamics of the active site.[31]

Enzymes must bind their substrates before they can catalyse any chemical reaction. Enzymes are usually very specific as to what substrates they bind and then the chemical reaction catalysed. Specificity is achieved by binding pockets with complementary shape, charge and hydrophilic/hydrophobic characteristics to the substrates. Enzymes can therefore distinguish between very similar substrate molecules to be chemoselective, regioselective and stereospecific.[32] Some of the enzymes showing the highest specificity and accuracy are involved in the copying and expression of the genome. Some of these enzymes have "proof-reading" mechanisms. Here, an enzyme such as DNA polymerase catalyzes a reaction in a first step and then checks that the product is correct in a second step.[33] This two-step process results in average error rates of less than 1 error in 100 million reactions in high-fidelity mammalian polymerases. Similar proofreading mechanisms are also found in RNA polymerase,[34] aminoacyl tRNA synthetases[35] and ribosomes.[36] To explain the observed specificity of enzymes, in 1894 Emil Fischer proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another.[37] This is often referred to as "the lock and key" model.[1]:8.3.2 This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.[38] In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme.[39] As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site.[40] The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined.[41] Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism.[42]

Enzymes can accelerate reactions in several ways, all of which lower the activation energy (ΔG‡, Gibbs free energy)[43]

1. By stabilizing the transition state:
   - Creating an environment with a charge distribution complementary to that of the transition state to lower its energy[44]
2. By providing an alternative reaction pathway:

- Temporarily reacting with the substrate, forming a covalent intermediate to provide a lower energy transition state[45]

3. By destabilising the substrate ground state:
   - oDistorting bound substrate(s) into their transition state form to reduce the energy required to reach the transition state[46]
   - By orienting the substrates into a productive arrangement to reduce the reaction entropy change[47] (the contribution of this mechanism to catalysis is relatively small)[48]

Enzymes may use several of these mechanisms simultaneously. For example, proteases such as trypsin perform covalent catalysis using a catalytic triad, stabilise charge build-up on the transition states using an oxyanion hole, complete hydrolysis using an oriented water substrate.[49,50,51,52,53]

Dynamics:

Enzymes are not rigid, static structures; instead they have complex internal dynamic motions – that is, movements of parts of the enzyme's structure such as individual amino acid residues, groups of residues forming a protein loop or unit of secondary structure, or even an entire protein domain. These motions give rise to a conformational ensemble of slightly different structures that interconvert with one another at equilibrium. Different states within this ensemble may be associated with different aspects of an enzyme's function. For example, different conformations of the enzyme dihydrofolate reductase are associated with the substrate binding, catalysis, cofactor release, and product release steps of the catalytic cycle,[53] consistent with catalytic resonance theory.

Types of inhibition

Competitive

A competitive inhibitor and substrate cannot bind to the enzyme at the same time.[56] Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, the drug methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate.[54] The similarity between the structures of dihydrofolate and this drug are shown in the accompanying figure. This type of inhibition can be overcome with high substrate concentration. In some cases, the inhibitor can bind to a site other than the binding-site of the usual substrate and exert an allosteric effect to change the shape of the usual binding-site.[57]

Non-competitive

A non-competitive inhibitor binds to a site other than where the substrate binds. The substrate still binds with its usual affinity and hence Km remains the same. However the inhibitor reduces the catalytic efficiency of the enzyme so that Vmax is reduced. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration.[55,56–64]

Uncompetitive

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex; hence, these types of inhibitors are most effective at high substrate concentration. In the presence of the inhibitor, the enzyme-substrate complex is inactive.[55,64] This type of inhibition is rare.[58]

Mixed

A mixed inhibitor binds to an allosteric site and the binding of the substrate and the inhibitor affect each other. The enzyme's function is reduced but not eliminated when bound to the inhibitor. This type of inhibitor does not follow the Michaelis-Menten equation.[62–64]

Irreversible

An irreversible inhibitor permanently inactivates the enzyme, usually by forming a covalent bond to the protein.[73] Penicillin[74] and aspirin[75] are common drugs that act in this manner.

Functions of inhibitors

In many organisms, inhibitors may act as part of a feedback mechanism. If an enzyme produces too much of one substance in the organism, that substance may act as an inhibitor for the enzyme at the beginning of the pathway that produces it, causing production of the substance to slow down or stop when there is sufficient amount. This is a form of negative feedback. Major metabolic pathways such as the citric acid cycle make use of this mechanism. Since inhibitors modulate the function of enzymes they are often used as drugs. Many such drugs are reversible competitive inhibitors that resemble the enzyme's native substrate, similar to methotrexate above; other well-known examples include statins used to treat high cholesterol,[62] and protease inhibitors used to treat retroviral infections such as HIV.[77] A common example of an irreversible inhibitor that is used as a drug is aspirin, which inhibits the COX-1 and COX-2 enzymes that produce the inflammation messenger prostaglandin.[61] Other enzyme inhibitors are poisons. For example, the poison cyanide is an irreversible enzyme inhibitor that combines with the copper and iron in the active site of the enzyme cytochrome c oxidase and blocks cellular respiration.[64]

General characteristics Catalytic RNAs: are broadly separated into two classes based on their size and reaction mechanisms (reviewed by [65-69]). The large catalytic RNAs consist of RNase P, and the group I and group II introns. These molecules range in size from a few hundred nucleotides to around 3000. They catalyze

reactions that generate reaction intermediates and products with 3P hydroxyls and 5P phosphates . The small catalytic RNAs include the hammerhead, the hairpin (or paperclip), hepatitis delta and VS RNA. These molecules range in size from V35 to V155 nucleotides. They use the 2P hydroxyl of the ribose sugar as a nucleophile, and they generate products with a 2P,3P-cyclic phosphate and a 5P hydroxyl . The relationship between the size and the reaction mechanism of these molecules has raised intriguing questions about their origins and evolution. It may be that the reaction mechanism and the size of the large ribozymes are needed to bring often very distal elements of the substrate into close proximity. The small, self-cleaving, RNAs are not faced with this constraint and perhaps this permitted them to evolve smaller catalytic centers. It remains possible, however, that the relationship between the size and reaction mechanism is simply fortuitous. With one exception, all these RNAs catalyze reactions that modify themselves. Hence, they cannot be considered true enzymes or catalysts. The exception is RNase P, which processes the 5P end of tRNA precursors. It is the only known example of a naturally occurring RNA-based enzyme. However, all these molecules can be converted, with some clever engineering, into true RNA enzymes that modify other RNAs in trans without becoming altered themselves. Ribozymes increase reaction rates by up to 1011- fold and have reaction e¤ciencies, kcat/Km, up to 108 M31 min31, which is in the range for di¡usion-controlled duplex formation between oligonucleotides [65]. While impressive, the rate enhancements provided by ribozymes are still V103-fold less than those provided by protein enzymes catalyzing comparable reactions [70]. Moreover, ribozymes cannot compare with proteins as multiple-turnover enzymes, mostly because product release is so slow that the catalytic site of the ribozyme is easily saturated. This may be an inherent limitation of RNA enzymes, but it could also re£ect evolutionary constraints, since ribozymes generally catalyze intramolecular, single-turnover, reactions in nature. An exhaustive comparison of the enzymatic mechanistics of protein and RNA enzymes has recently been made [70]. All known ribozymes have an absolute requirement for a divalent cation, which is generally Mg2 . Some, notably within the large catalytic RNAs, require divalent cations for proper assembly of the tertiary structures as well. On this basis, catalytic RNAs are considered to be metalloenzymes, and a general two-metal-ion reaction mechanism has been proposed for the large catalytic RNAs, based on analogy with the properties of protein metalloenzymes [71].

CONCLUSION

Only two reactions are observed to be catalysed by RNA in nature. However, the diversity of catalytic mechanisms for these reactions implies that RNA may be capable of much more. This is supported by the success of in vitro selection to develop ribozymes for RNA ligation [72], carbon–carbon bond formation [73], glycosidic bond formation [74]and other activities. Furthermore, in vitro-selected ribozymes can use catalytic cofactors, demonstrated by an alcohol dehydrogenase [75]. If the RNA world hypothesis is correct, then almost all ribozymes have been replaced by protein counterparts. Why then do some ribozymes persist in the presence of proteins, while others do not? First, the lower reaction rate could be compensated for by the increased effective concentration, since most ribozymes exist in nature as cis-acting elements, and not true trans-acting enzymes. In fact, the deficiency of many ribozymes compared with protein enzymes is not in the catalytic rate, but in binding the engineered substrate. Further, some ribozymes are mobile genetic elements, which benefit from requiring as little exogenous protein as possible (and, in fact, introns often contain their own open reading frames) [76].

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