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Form and Function in Biological Macromolecules: Kinetic Stability is Key, with Oblique Roles for Intramolecularity and Hydrophobicity in Enzyme Catalysis

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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Opinion Article

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ABSTRACT

Certain structure-reactivity aspects of biological macromolecules, with particular emphasis on protein folding and enzyme catalysis, are discussed herein. Furthermore, the role played by the hydrophobic effect and intramolecularity in enzymic reactivity are evaluated afresh, with new insights of much importance in chemical biology.

Thus, the sum of the energies of the hydrogen bonds constituting the tertiary structures of proteins, determines the overall Gibbs energy of activation for loss of conformational integrity. As protein molecules of even modest size consist of a relatively large number of intramolecular hydrogen bonding interactions, the activation barrier to even partial unfolding of the α-helices and β-sheets forming the tertiary structure would be prohibitively high under normal conditions.

The resulting kinetic stability conserves the natural conformation of a protein molecule established at the ribosomal site of synthesis, carrying the molecule through the thick-and-thin of a range of metabolic pathways during its 'journey of life'. However, protein molecules also acquire flexibility via 'strain delocalization' (Ramachandran plots being relevant), thus enabling stabilization of multiple transition states along a pathway (particularly in case of covalent enzyme-substrate complexes).

Two mechanistic features of enzyme catalysis that have been exhaustively studied are intramolecularity and the hydrophobic effect. Although intramolecularity has for long been touted as the origin of enzymic reactivity, this can be challenged on fundamental physical-organic grounds. Intriguingly, however, the collapse of the classical Michaelis-Menten mechanism for enzyme

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catalysis leads to a reconsideration of the role of intramolecularity, although not as hitherto envisaged. Thus, a majority of enzymes apparently form covalent enzyme-substrate complexes—possibly also exergonically—so the subsequent reactions at the active site may well benefit from the traditional propinquity effect: The critical caveat would be the highly exergonic formation of final products.

It is argued that the hydrophobic effect—although intuitively reasonable—is difficult to pin down quantitatively, model systems (including micelles) leading to inconsistent and debatable results. However, the hydrophobic effect likely contributes to enzymic reactivity along with charge-relay via the proteinic backbone.

Keywords: Hydrogen bonding; Michaelis-Menten; micelles; propinquity effect; protein folding; Ramachandran plots.

1. INTRODUCTION

1.1 General Background

A fundamental understanding of the structure and function of enzymes is of critical importance in the evolving field of chemical biology [1-5]. Thus, enzymes represent a key nodal point in the manifestation of genetic information as the myriad of metabolic processes that constitute life. This derives essentially from the one gene-one enzyme hypothesis, which intriguingly delegates to the enzymes an awesome importance as the key to the efflorescence of life itself. It is thus hardly surprising that enzyme function and reactivity have served as a focus of fundamental enquiry and debate, with chemistry—the 'central science'—leading the way in this greatest of reductionist adventures.

Progress in mechanistic enzymology has been predicated on advances in protein science—in theory and practice—and understandably so. Pioneering work, by Buchner on yeast-mediated fermentations (1897) and kinetic studies by Michaelis and Menten (1913), represented the first stirrings of the nascent science of enzymology [4]. However, advances in protein isolation, purification and characterization, not to mention the founding of molecular biology in the ensuing decades, subsequently laid the foundations of modern enzymology.

The near-miraculous catalytic capabilities of enzymes also began to engage the attention of chemical science, which was itself increasing in sophistication in parallel with advances in biology. A synergistic approach between chemical and biological science thus emerged and has continued to evolve since the de facto founding of 'chemical biology' ~ 1950. The realization that enzyme catalysis was in a class of its own elicited the Pauling theory of transition state stabilization (1948) [5], followed by the determination of the structure of DNA (1953) and the founding of molecular biology [6]. The elucidation of the genetic code in terms of transcription (to mRNA) and translation (to protein) indicated that the proteinic enzymes are destined to hold the key to the mystery of dynamic life itself.

However, although the urgent need to reach a fundamental understanding of enzyme catalysis appears self-evident, the complex nature of enzymes and theoretical uncertainties of chemical science have conspired to envelop enzymology in controversy (if not shrouding it in more mystery)! Thus, the classical Michaelis-Menten equation that served as a bed-rock of enzyme kinetics for a century has been challenged [4]; the Pauling hypothesis remains to be elucidated in mechanistic detail; and physical-organic models particularly based on intramolecularity [7] and hydrophobic effects [8]—however ingenious—have apparently floundered on fundamental grounds.

This paper briefly reviews this essential background and argues that enzymology is indeed emerging from the state of flux, although a bold and frank assessment of previous inadequacies is needed to connect the dots and forge ahead to a new era of chemical biology.

1.2 Thermodynamic Considerations

Modern theories of enzyme catalysis and action are based on the assumption that, despite their biological origins and often forbidding molecular complexity, enzymes are subject to the same laws as apply to in vitro catalysis by small inorganic and organic molecules. These theories essentially derive from the classical law of mass action that is the basis of chemical equilibrium and reaction rate. The thermodynamic versions

of these theories relate to the Boltzmann equation and its formulation in terms of the Gibbs energy, itself composed of enthalpy and entropy contributions [9]. The key idea that a catalyst is regenerated during the reaction—and does not change the equilibrium constant either—is also retained in the case of the enzyme reaction.

These approaches—shorn of all traces of vitalism—thus hold the key to the reduction of sentient life to the laws governing the properties of inanimate matter.

2. DISCUSSION

2.1 Uniqueness of Enzyme Catalysis. Recalcitrant Problems

2.1.1 The dual role of enzymes: As 'evolutionary fulcrum' and as metabolic workhorse

Enzymes are generally medium-sized proteins that are direct products of genetic expression, representing the first stage in the conversion of the genetic code to a non-nucleic acid information system. Thus, enzymes serve as an 'evolutionary fulcrum' that, however, has apparently obfuscated the fact that their proteinic nature is also critical to their catalytic power! In other words, smaller non-protein catalysts are unlikely to rival the catalytic powers of natural enzymes, as is becoming increasingly apparent.

However, the full significance of enzymes can only come to light if the origins of their catalytic powers are fully understood. This is much more than the mere cataloguing of the various catalytic groups and their mechanistic interplay, which have indeed been replicated in model systems [10]. Yet, even the most ingeniously designed models have failed to approach the catalytic powers routinely observed in natural enzymes: As it stands, the whole remains greater than the sum of its parts.

Thus, enzyme catalysis is characterized by enormous rate enhancements (typically > 10^{10}) that are brought about under the mildest of aqueous conditions [1-5]. Hence, they serve as the metabolic work-horses of life. Their remarkable features have elicited various mechanistic theories as explanations, noting that an important practical goal is the design of artificial catalysts that can rival the catalytic powers of enzymes. Indeed, this remains the holy grail of physical organic chemistry, in the service of chemical biology and science itself.

2.1.2 Physical organic models: Intramolecularity and the hydrophobic effect

Two important mechanistic approaches to enzyme catalysis invoke intramolecularity and the hydrophobic effect [11]. These have been implicated in enzyme catalysis essentially because they have led to enhanced rates in model systems, but also because they apparently possess some of the characteristics of enzyme catalysis. Thus, enzyme catalysis occurs via an enzyme-substrate complex, its further 'turnover' to product being necessarily intramolecular; and enzyme active sites are hydrophobic pockets that are apparently complementary to the rate-determining transition state of the reaction.

2.1.2.1 Intramolecularity

However, the intramolecularity model has been challenged as enzyme catalysis is bimolecular (considering only enzyme and substrate) [7], whereas intramolecular reactions are unimolecular. Thus, the enhanced rates of intramolecular reactions are due to a raised ground state (often entropic in origin but also enthalpic in certain cases), whereas enzyme reactions must involve transition state stabilization in some manner.

It is also possible that catalysis involves the energy of the catalyst itself in some form, although this is not so apparent as the catalyst is regenerated. Thus, the catalyst could be a source of potential energy, but problems remain with the exact interpretation of transition state theory as to whether the Gibbs energy or the potential energy is to be employed [12].

In fact, a raised ground state ostensibly justifies the intramolecularity model, the conundrum itself possibly indicating the limitations of chemical theory in explaining complex biological phenomena! All the same, the intramolecularity model is intriguingly resuscitated in light of the recent reinterpretation of enzyme kinetics, as discussed further below.

2.1.2.2 The hydrophobic effect

The view that the hydrophobic effect plays a major role in enzyme catalysis has also been debated [8], the results of model studies being difficult to interpret. Although the studies demonstrate enhanced rates of certain reactions

(particularly cycloadditions) in water, the results are stymied by the low solubility of the hydrophobic reactants in water! The results are thus valid in select cases, apparently, with water catalysis being a viable option that cannot be easily ruled out. Generally, in fact, a hydrophobic medium is non-polar and thus not conducive to enhanced reactivity: Unsurprisingly, enhancements have been observed in the case of concerted reactions, polar reactions likely requiring supplementary charge relay effects as may occur in enzymes (and possibly micellar systems too, vide infra).

Thus, the hydrophobic effect observed in certain model systems is possibly misleading. The results evidence the intuitively reasonable idea that a reaction in which the transition state is less hydrophobic than the ground state is accelerated in water (relative to an organic medium). However, the greater hydrophobicity of the substrate implies a correspondingly lower solubility, so the rate enhancements are not practically significant. In fact, substantial accelerations are also apparently observed in organic-water emulsions, which likely implies interfacial catalysis by water that cannot be separated from the hydrophobic effect per se. Also, the hydrophobic effect is not a general accelerating effect applying to all reactions in aqueous media, as is often assumed.

2.2 Enzymes as Proteins

2.2.1 Macromolecular nature of enzymes: Protein folding and activity

Biological macromolecules manifest overall three-dimensional shape as biological function [13-16], thus apparently representing a twilight zone: between synthetic high polymers that are characterized by their mechanical properties (e.g., polyethylene [17]), and smaller oligomeric fragments of no defined form or function. In fact, a complex and intimate relationship between form and function, particularly among the ubiquitous proteins (enzymes, wool, skin, etc.), distinguishes biological macromolecules from their smaller and larger congeners.

Furthermore, among the three major classes of biological macromolecule—nucleic acids, proteins and carbohydrates—proteins are the warp and woof of the central dogma of biological information flow [6]: DNA – RNA – protein – organism. However, whilst the proteinic enzymes control and regulate every aspect of life's complex machinery, they also possess a myriad of conformational states. Clearly, only one among these states is apparently enforced at the ribosomal site of biosynthesis [18]: but what helps retain the overall shape thus conferred? The answer holds the key to the mystery of life's sustenance, based in the unerring consistency of an organism's biochemical complexity [19].

And intriguingly, the overall conformation conferred need not be the thermodynamically most stable one, else both function and stability would need to be optimized by evolution! Indeed, the degeneracy of the genetic code could imply—via codon-specific t-RNA-synthetases—changes in the ribosomal micro-environment, which lead to different tertiary structures being adopted by the same polypeptide primary sequence! Furthermore, a subtle balance between flexibility and rigidity is of critical importance to macromolecular function and enzyme action in particular, as argued below.

2.2.2 Protein dynamics and enzyme action: Ramachandran plots

2.2.2.1 Polypeptide flexibility: Mechanisms and catalytic role

As noted above, the mystery of enzyme catalysis is predicated on an understanding of protein structure and dynamics. Of particular importance to enzyme catalysis is the possibility of conformational changes that would lead to a flexible active site, which can stabilize several different transition states along a reaction pathway. The possibility of charge relay along the protein backbone has also been proposed as a way of stabilizing polar transition states in a hydrophobic environment [4]. (Indeed, the presence of the catalytic triad in proteases indicates the importance of charge-relay in a hydrophobic environment [4].) That proteins strike a balance between rigidity and flexibility via 'strain delocalization' has been proposed as being critical to the reactivity of catalytic groups around the active site (vide infra).

2.2.2.2 The concept of strain delocalization

An intriguing and novel mechanism by which a polypeptide backbone can acquire flexibility is the possibility of strain delocalization [4]. This is possible as bond angle strain increases exponentially with decreasing angle, so the distribution of angle strain over several angles is thermodynamically favored over the

concentration of the same angle strain in a single or fewer angles. The consequent 'delocalization of strain' is indeed feasible in macrocyclic and pseudo-macrocyclic systems, e.g., in a relatively long polypeptide chain. Strain delocalization, in fact, would enable precise reaction trajectories to be attained by interacting catalytic groups in the active site. Indeed, this possibly explains the macromolecular nature of enzymes, as smaller molecules fail to show similar catalytic powers.

2.2.2.3 Ramachandran plots: Infinite thermodynamic degeneracy

Intriguingly, in the absence of any kinetic barriers, protein molecules are infinitely flexible, as is apparent in the well-known Ramachandran plots [15,16,18]. These contour diagrams essentially imply that an infinite range of dihedral angles (φ, ψ) around the peptide bonds in a polypeptide are acceptable. Thus, to the extent that the contours are not discontinuous, they indicate that an amino acid residue can adopt an infinite range of conformations—within an allowed subset—that are thermodynamically degenerate. These arguments can be extended to whole molecules with due caveats, leading to 'impressionistic' diagrams displaying smudged distributions of nearly overlapping data points, indicating a high level of thermodynamic degeneracy.

2.2.2.4 Kinetic stability of polypeptide conformers: Additivity of hydrogen bond strengths

Whilst Ramachandran plots imply infinite thermodynamic flexibility, they also indicate the importance of kinetic barriers to the maintenance of the tertiary structures of proteins! A major part of the kinetic stability of a polypeptide would undoubtedly be provided by the network of intramolecular hydrogen bonds—literally the molecular scaffolding—that forms the α-helices and β-sheets, but also arranges them in molecular space [13-16].

Indeed, notwithstanding the mildness of biological conditions—and the strength of the peptide bond—non-covalent forces control protein folding, its retention through thick and thin during a cell's life cycle thus representing a remarkable feat of molecular endurance.

The key to this "conundrum of protein folding", in fact, lies not just in the network of hydrogen bonds that maintains a polypeptide conformation, but also in its cumulated thermodynamic effect (cf. Fig. 1). Thus, a network of *n* hydrogen bonds is stabilized by the strength of the first (*n*-1) hydrogen bonds, and by the barrier to the cleavage of the last hydrogen bond: The sum of all these represents the activation. barrier to the dismantling of the overall polypeptide conformation, i.e., its kinetic stability [20]. (In Fig. 1, $n = 4$, $E_1 - E_4$ being the stated hydrogen bond energies).

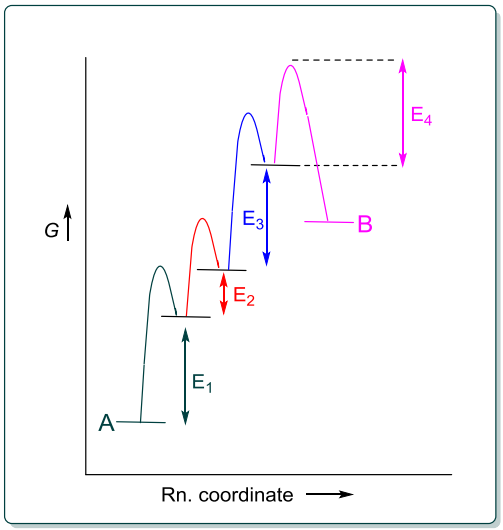

Fig. 1. Gibbs energy (*G*) changes during the unfolding of a polypeptide fragment

Thus, a single turn of the *α*-helix would be stabilized by a sizable barrier > 20 kcal mol^{-1} (based on 7 hydrogen bonds worth 3 kcal mol^{-1} each [13-16]). Clearly, even moderately sized proteins composed of several *α*-helix and *β*-sheet units would possess forbidding barriers to unfolding. Little wonder that the denaturation of proteins requires particularly harsh conditions and that controlled unfolding of proteins is catalysed by ATP-dependent enzymes [21].

2.3 Enzymes as Catalysts

2.3.1 Enzyme kinetics: Collapse and renewal

2.3.1.1 The Michaelis-Menten equation and its aftermath

The pioneering studies of enzyme kinetics by Michaelis and Menten (1913) represent an intrepid foray into the then evolving fields of biological catalysis and chemical kinetics [11]. Early observations indicated that enzyme catalysis followed 'saturation kinetics', wherein the reaction rate (v) initially increased linearly

with the substrate concentration ([S]), but levelled off asymptotically at high [S]. This led to the proposal that the reaction occurred via the rapid pre-equilibrium formation of an enzyme-substrate complex (ES), which 'turned over' relatively slowly to products, enshrined in the Michaelis-Menten rate law (Eq. 1), $[E_o]$ being the (constant) initial enzyme concentration, k_{cat} the turnover number and K_M the Michaelis constant.

$$v = k_{cat}[E_o][S]/(K_M + [S]) \quad (1)$$

$$v = k_{cat}[E_o] \quad (2)$$

$$K = k_{cat}/k_{cat}' \quad (3)$$

$$K = [P]/[S] \quad (4)$$

$$(k_{cat}/k_{cat}') \neq [P]/[S] \quad (5)$$

2.3.1.2 Inadequacies of the Michaelis-Menten equation

Interestingly, Eq. 1 reduces to Eq. 2 at high [S], apparently reproducing the experimentally observed 'saturation kinetics'. This ostensibly indicated the gradual saturation of the pre-equilibrium that was nearly complete at high [S].

However, a serious problem with Eq. 2 is that it leads to Eq. 3 under conditions of overall equilibrium between substrate and product (P) (at high [S] and [P], K is the overall equilibrium constant and k_{cat}' the turnover number for the reverse reaction).

Clearly, Eq. 3 is in conflict with the natural equilibrium constant of the reaction (K, Eq. 4). Indeed, this led to the proposal of 'one-way' enzymes although this idea contravened a fundamental tenet of thermodynamics that the equilibrium constant depends only on the Gibbs energy change in the reaction (at constant temperature, Eq. 5 representing the general case).

2.3.2 Alternatives to the Michaelis-Menten scheme: Inhibition at high [S] via secondary binding

In view of the above anomalies, the Michaelis-Menten formulation of enzyme kinetics is fundamentally invalid and needs to be abandoned. In fact, a viable alternative that has been proposed is based on the idea of a gradual inhibition of the enzyme at high substrate concentrations that leads to the observed levelling of the rate [4]. Such inhibition is almost certainly likely to occur via the binding of a second molecule of substrate at the active site, which would prevent the release of product formed and regeneration of enzyme. Accordingly, Eq. 6 was proposed as an alternative to the Michaelis-Menten equation (k is the overall reaction rate constant, K_S the equilibrium constant for the secondary binding of substrate and E_F the free enzyme).

$$v = k[E_o][S] - kK_S[E_F][S]^3 \quad (6)$$

In Eq. 6, the first term on the right-hand side refers to the linear increase in rate with increasing [S], whereas the second term refers to the inhibition via secondary binding. Thus, K_S is low as secondary binding is weak, hence the second term becomes significant at high [S], increasing exponentially to 'rein in' the first term. An analogous equation can be written for the reverse reaction, leading to the thermodynamic equilibrium constant, noting that the forward and reverse reactions are catalyzed only by E_F.

Thus Eq. 6 captures the observed levelling effect of the rate at high [S] and also leads to the thermodynamically valid equilibrium constant. It is also noteworthy that, although Eq. 6 was originally derived for a pre-equilibrium formation of the enzyme-substrate complex, this assumption is strictly not necessary as the rate constant k is per se not based on the pre-equilibrium assumption. Therefore, Eq. 6 is generally valid regardless of the relative stability of the enzyme-substrate complex, thus leading to various mechanistic possibilities for further consideration.

2.3.3 Exergonic formation of the enzyme-substrate complex and intramolecularity

2.3.3.1 Endergonic and exergonic enzyme-substrate binding; covalent bond formation

As noted above, the Michaelis-Menten equation was derived on the basis of a pre-equilibrium mechanism, in which a weakly-bound enzyme-substrate complex was formed endergonically. (The observed levelling of rate was believed to arise from the putative saturation of the pre-equilibrium.) However, in view of the collapse of the Michaelis-Menten regime in toto, alternative mechanisms can now be considered.

An interesting possibility is the exergonic formation of the enzyme-substrate complex from enzyme and substrate. In fact, this mechanism acquires particular significance in light of findings that a substantial number of enzymes form covalent enzyme-substrate complexes [5]. An interesting advantage of covalent enzyme-substrate binding is that it allows for conformational changes in the enzyme, so the active site would be flexible enough to stabilize all possible transition states in the reaction pathway.

The covalent complex, however, may also be formed endergonically. Whilst this indicates that the strength of the covalent bond linking enzyme and substrate is carried over to the transition state, this can contribute to the catalysis only if the binding is stronger in the transition state relative to the ground state. This implies that the ground state complex is strained in some manner, the strain being relieved on reaching the transition state.

However, a more likely possibility is that the covalently linked complex is formed exergonically, considering the strength of a covalent bond. In the earlier Michaelis-Menten scheme, however, such exergonic formation of the enzyme-substrate complex was ruled out, essentially because the turnover step would be slower rather than faster (as would also be release of enzyme). (This was the 'thermodynamic pit' problem that apparently negates catalysis [11].) However, this mechanism now merits serious consideration for several reasons, not least of which is the intriguing involvement of intramolecularity (vide infra).

The exergonic formation of an enzyme-substrate complex, however, would be predicated on the overall reaction itself being highly exergonic, in order to avoid a buildup of the said complex. All the same, this may not be a serious limitation as—for obvious practical reasons—the majority of enzyme catalyzed reactions are perforce exergonic. (Like any catalyst, an enzyme cannot alter the equilibrium constant of a reaction; however, even an endergonic equilibrium can be displaced by further reactions of the product.)

An interesting problem with the exergonic formation of the enzyme-substrate complex, however, is noteworthy. This is the possibility that the initial exergonic pre-equilibrium would be partially reversed with an increase in temperature, leading to a subdued (or even negative) temperature dependence of the overall rate [3,20]. Apparently, however, the positive temperature dependence of the subsequent sub-reactions generally overcomes the reversal of the initial pre-equilibrium. Also, the reversal may not occur if the by-product (not shown) in the formation of ES_1 (Figs. 2 and 3, vide infra) is concomitantly expelled from the active site. (Any reversal, however, would lead to an observed Gibbs energy of activation that would be lower than the real one).

2.3.3.2 Intramolecularity makes a comeback: The ES complex is the ground state!

The energy profile for exergonic enzyme-substrate binding—whether covalent or not—is shown in Fig. 2. In a fascinating mechanistic flip, it is now observed that the enzyme-substrate complex (ES) represents the ground state of the reaction! An intriguing consequence is that the subsequent clutch of catalytic reactions would possess all the virtues of intramolecularity, so the traditional propinquity effect can be invoked as contributing to the overall rate enhancement. The mechanistic reasoning is of considerable subtlety, and only becomes apparent upon comparison with a putative profile in which—hypothetically—intramolecularity does not play a role (dashed lines in Fig. 2).

An important caveat to these arguments, however, is that the formation of the covalent enzyme-substrate complex, as also the final release of product, have become rate-determining. The corresponding transition states, of course, do not benefit from intramolecularity, and are thus subject to the Pauling hypothesis. In other words, the enzyme now needs to stabilize only two extreme transition states in the overall profile, the propinquity effect dealing with the key catalytic reactions occurring within the confines of the active site. (Thus, the enzyme need not necessarily stabilize the several intermediate transition states).

An interesting variation is the case wherein formation of the enzyme-substrate complex is exergonic, but fast (possibly diffusion-controlled) relative to the intermediate intramolecular steps (Fig. 3). (The release of product, likewise, may also be fast.) The intramolecular steps would then be rate-determining with the overall rate being dependent on the stability of the enzyme-substrate complex. Thus, in the (presumed) absence of any stabilization of the intermediate transition states, an excessively stable enzyme-substrate complex would detract from the overall

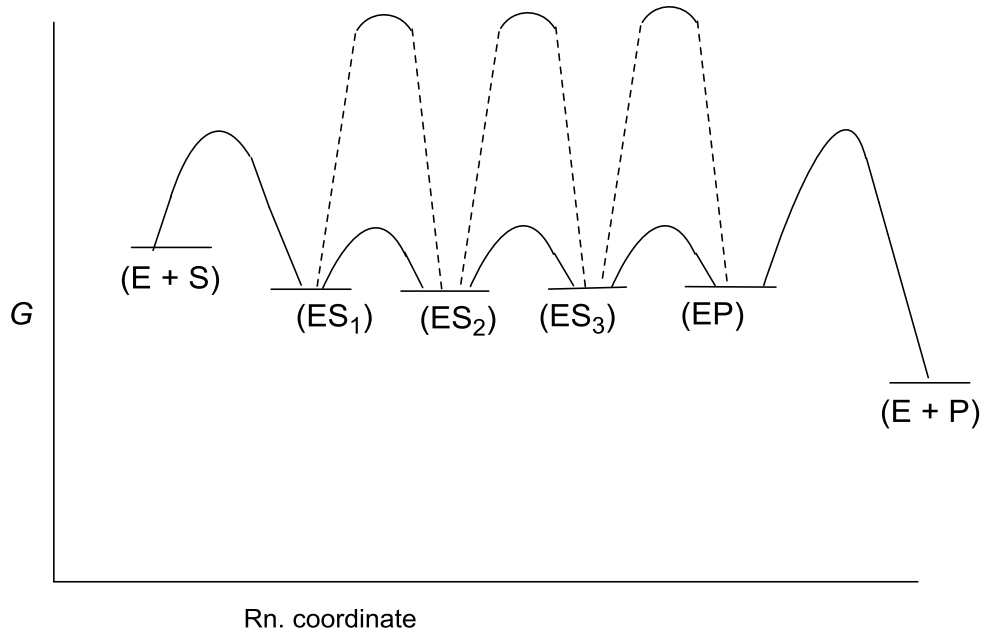

Fig. 2. Gibbs energy (*G*) profile for the rate-limiting exergonic formation of an enzyme-substrate complex (ES)

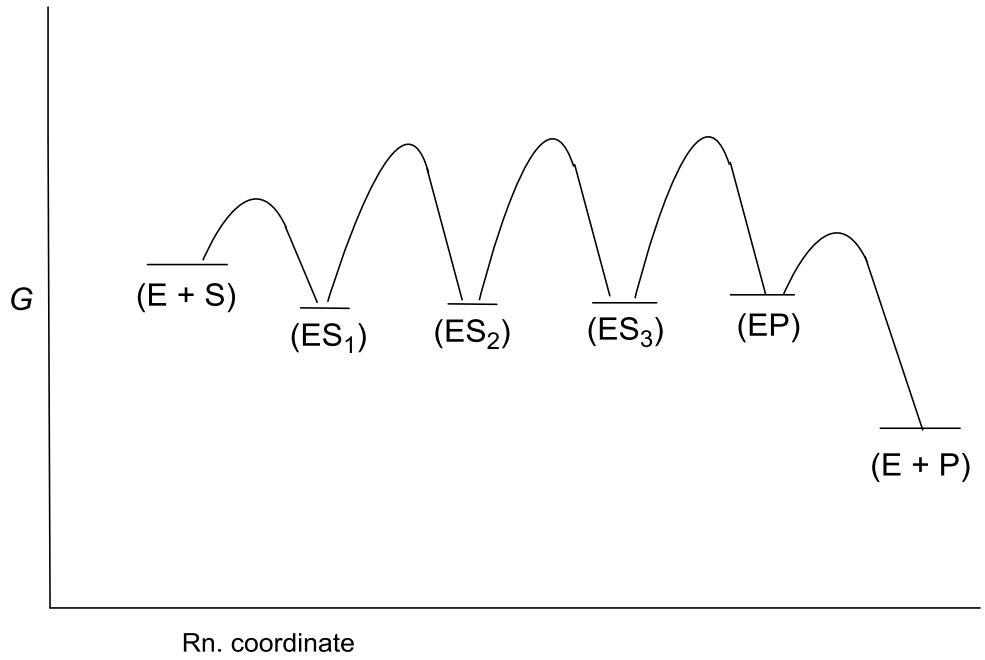

Fig. 3. Gibbs energy (*G*) profile for the rapid and exergonic formation of an enzyme-substrate complex (ES)

rate enhancement. It is noteworthy that intramolecular reactions show an enormous range of effective molarity (EM) values that reflect—inter alia—correspondingly raised ground states.

It is particularly noteworthy that these arguments were impossible in the framework of the Michaelis-Menten scheme, as it was based on the endergonic formation of the enzyme-substrate complex, with the ground state being represented by free enzyme and substrate. Intramolecularity was hence indefensible in that context, with transition state stabilization being sine qua non for all steps.

Furthermore, the alternative kinetic proposal (Eq. 6) is broadly applicable to both cases, i.e., endergonic and exergonic formation of the enzyme-substrate complex (ES). This is apparent from the presence of the unitary rate constant *k* in Eq. 6: In the case of exergonic formation of ES, *k* would refer to the formation of ES itself; in the case of endergonic formation of ES, *k* would also refer to the breakdown of ES to products. Both cases would be valid without prejudice to the observed levelling of rate, as inhibition via secondary binding would apply equally.

3. CONCLUSIONS

The above discussion has briefly reviewed an extensive range of topics of fundamental significance to the origins of enzyme catalysis. The inevitable conclusion, apparently, is that mechanistic enzymology has entered into a state of flux, from which it is indeed beginning to emerge. The major conclusion is that the classical theory based on the Michaelis-Menten equation stands discredited and needs to be replaced by a credible alternative.

Currently, a viable mechanism based on secondary binding of substrate at high concentrations appears reasonable. This is in accord with the essential theory of chemical equilibrium and kinetics, and can also explain the levelling of the rate at high substrate concentrations. Although the Pauling theory of transition state stabilization remains paramount, alternatives also may now be considered, in particular the intramolecularity concept. The hydrophobic effect also plays a role, although along with a charge-relay mechanism possibly involving the protein backbone.

The proteinic nature of enzymes also needs to be accorded more serious consideration henceforth. In particular, the kinetic stabilization of the tertiary structure afforded by the network of hydrogen bonds plays foil to the inherent flexibility of a polypeptide chain. The classical theory of protein structure, particularly based on the Ramachandran plots, may be augmented by newer ideas (e.g., strain delocalization). These lead to novel insights into the dynamics of polypeptide molecules likely of key significance to enzyme catalysis.

In summary, an integrated approach involving ideas from both mechanistic chemistry and polypeptide dynamics is indicated for a comprehensive understanding of enzyme catalysis to be reached. (Indeed, a palette of mechanistic options is now apparent, with each enzyme employing the most appropriate mechanism to fulfill its metabolic purpose).

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Agarwal PK. A biophysical perspective on enzyme catalysis. Biochemistry. 2019; 58(6):438–449.
   DOI: 10.1021/acs.biochem.8b01004
2. Kirby AJ, Hollfelder F. From enzyme models to model enzymes. Cambridge (UK): Royal Society of Chemistry; 2009.
3. Chandrasekhar S. The origins of enzyme catalysis and reactivity: Further assessments. Asian Journal of Chemical Sciences. 2021;9(3):38-47.
   DOI: 10.9734/AJOCS/2021/v9i319075
4. Chandrasekhar S. Understanding enzymic reactivity – new directions and approaches. Asian Journal of Research in Biochemistry. 2020;7(2):1-13.
   DOI: 10.9734/ajrb/2020/v7i230133
5. Zhang X, Houk KN. Why enzymes are proficient catalysts: Beyond the Pauling paradigm. Acc Chem Res. 2005:38(5): 379-385.
   DOI: 10.1021/ar040257s
6. Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R. Molecular Biology of the Gene. 7th Ed. Boston: Pearson; 2013.
7. Chandrasekhar S. Intramolecularity and enzyme modelling: A critique. Res Chem Intermed. 2003;29(1):107-123.
   DOI: 10.1163/156856703321328451
8. Chandrasekhar S. The hydrophobic effect in chemistry, biology and medicine: An update. Asian Journal of Chemical Sciences. 2021;9(4):22-36.
   DOI: 10.9734/AJOCS/2021/v9i419078
9. Atkins P, de Paula J. Atkins' physical chemistry. 8th Ed. New York: W. H. Freeman; 2006.
10. Silverman RB. The organic chemistry of enzyme-catalyzed reactions. San Diego: Academic Press; 2002.
11. Fersht A. Structure and mechanism in protein science. 2nd Ed. Cambridge (UK): Kaissa Publications; 2017.

12. Chandrasekhar S. The Gibbs energy–potential energy conundrum and chemical reactivity: Implications for catalysis and enzyme action. Asian Journal of Chemical Sciences. 2021;9(4):1-7.
DOI: 10.9734/AJOCS/2021/v9i41907
13. Dill KA, MacCallum JL. The protein folding problem, 50 years on. Science. 2012; 338(6110):1042-1046.
DOI: 10.1126/science.1219021
14. Kuriyan J, Konforti B, Wemmer D. The molecules of life: Physical and chemical principles. New York: Garland Science; 2013.
15. Kessel A, Ben-Tal N. Introduction to proteins structure, function and motion. 2nd Ed. Boca Raton: CRC Press; 2018.
16. Buxbaum E. Fundamentals of protein structure and function. New York: Springer Science; 2017.
17. Koltzenburg S, Maskos M, Nuyken O, Hughes K. Polymer chemistry. Berlin-Heidelberg:Springer-Verlag; 2017.
18. Rosenberg AA, Marx A, Bronstein AM. Codon-specific Ramachandran plots show amino acid backbone conformation depends on identity of the translated codon. Nat Commun. 2022;13:2815.
DOI: 10.1038/s41467-022-30390-9
19. Nelson DL, Cox MM. Lehninger principles of biochemistry. 7th Ed. New York: Freeman; 2017.
20. Anslyn EV, Dougherty DA. Modern physical organic chemistry. Sausalito CA: University Science Books; 2006.
21. Olivares AO, Baker TA, Sauer RT. Mechanical protein unfolding and degradation. Annu Rev Physiol. 2018;80: 413-429
DOI:10.1146/annurev-physiol-021317-121303

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