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The Hydrophobic Effect in Chemistry, Biology and Medicine: An Update

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

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Opinion Article

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ABSTRACT

The roots of the hydrophobic effect (HE) apparently lie in certain chemical principles, although it has far-reaching implications across a swath of chemical biology. In particular, the HE drives the formation of micelles, the catalytic properties of which are believed to model the action of enzymes. The HE is also believed to be the key to drug-receptor interactions as captured in the Hansch equation and enshrined in QSAR.

Yet, the HE remains paradoxical: The attributed medium effects apparently contravene normal experience, as apolar solvents are rarely (if ever) the preferred choice in practice! Indeed, the HE is complex and varied in its manifestations, affecting both structure and reactivity. The latter "kinetic hydrophobic effect" itself exists as "Types 1 and 2", referring to reactions in water (e.g., Diels Alder) and non-aqueous media (e.g., micelles) respectively.

Micellar reactivity also remains mysterious as, apparently, the HE only facilitates the formation of the micelles. The idea that micellar reactivity arises from concentration effects, in fact, appears simplistic for several reasons. Furthermore, reactions may well occur on the micellar surface, particularly with ionic reagents that would be poorly soluble in the core.

There are important differences between micelles and enzymes: Enzymes are pre-configured with catalytic groups and charge-relay systems, although the contribution of the HE to activity remains uncertain. Micelles also form a separate phase, hence defining the thermodynamic ground state of the reactants (contrasting with enzymes).

Certain aspects of the Hansch equation, particularly the decline of drug activity beyond the

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"Goldilocks zone" peak, are intriguing. However, a kinetic model of drug-receptor interactions based on the rates of drug binding and release, offers an explanation for the observations, also leading to further insights into the nature of the binding itself.

Keywords: Enzyme catalysis; Hansch equation; micelles; QSAR; receptors.

1. INTRODUCTION

The "hydrophobic effect" refers to the scientific study of the common-sense notion that oil and water are immiscible. The recognition that the hydrophobic effect could be the key to a better understanding of a wide swath of chemical and biological phenomena apparently dates back to 1945, when the term itself was coined [1]. Currently, the hydrophobic effect is widely accepted as a critical conceptual bridge between chemistry and biology that–despite elements of confusion and controversy–brings together diverse experimental observations under its unifying ambit.

Thus, the hydrophobic effect is the key to understanding protein folding [2-4], which apparently permeates every aspect of modern chemical biology. The hydrophobic effect is also believed to be the key to biological molecular recognition processes, particularly enzyme-substrate [5] and drug-receptor interactions [6]. It is also likely that the hydrophobic effect is involved in several aspects of nucleic acid chemistry and biology [7-9]. Hence, the hydrophobic effect is unsurprisingly the key to a range of biochemical phenomena–from genetic expression to metabolic processes–that perforce occur in an aqueous environment.

All the same, the hydrophobic effect has not been without controversy [1]. In particular, the origins of the hydrophobic effect have been confounded by the terminology employed, as it seems to imply a repulsive effect in a rather absolute sense. However, it is more likely that the hydrophobic effect is a relative effect by which non-polar solutes (or moieties) prefer a non-polar environment to an aqueous one. The reason for this choice is also interestingly complex, being almost certainly based in the need to perturb the structure of water minimally.

The hydrophobic effect influences both structure and reactivity, structural effects being particularly manifest in the formation of micelles and in the laws governing protein folding. The kinetic manifestation of the hydrophobic effect, however, is less easy to pin down, being dependent on the way the claimed rate enhancements are interpreted. When clear-cut kinetic cases do exist, the fundamental basis still remains unclear.

The foundations of the hydrophobic effect and its perhaps colorful history have been reviewed previously [1]. This paper attempts to extend those arguments to newer domains with a more practical significance, in particular micellar reactivity and drug-receptor interactions. Micellar reactivity has often been compared with enzymic reactivity, although important contrasting features exist. Drug-receptor theory, as enshrined in the famous Hansch equation, defines modern medicinal chemistry, yet apparently with a few loose ends. Other relevant topics are also touched upon to place the review on a contemporary footing.

2. DISCUSSION

2.1 General Considerations

2.1.1 Previous works

The comprehensive critique of the hydrophobic effect presented previously will serve as the background to this paper [1]. It is particularly important to bear in mind that the hydrophobic effect is largely entropy driven, apparently derived from the perturbation of the structure of water upon the introduction of a non-polar solute. In fact, enthalpy changes indicate a small but discernible attractive interaction between a non-polar solute and water in many cases. (Hence, the hydrophobic effect is by no means the result of a repulsive interaction.)

Another rather confusing problem concerns the fact that key kinetic studies of the hydrophobic effect have been carried out in water, so there is no partitioning of the reactant from a non-polar phase. This apparently negates the definition of the hydrophobic effect as derived from the partitioning of a solute between non-polar and aqueous phases. These departures apparently need certain assumptions to substantiate the idea of hydrophobic rate enhancements [1,10].

Thus, the rate accelerations are explained as arising from enhanced bimolecular association that favors the transition state: However, the accelerations pertain to rate constants rather than rates, as the hydrophobic effect correspondingly minimizes solubility. Also, a repulsive hydrophobic interaction that minimizes the surface area of contact between solute and water is assumed as the basis of the observed accelerations. (The reactants are transferring to a possibly less hydrophobic micro-environment at the transition state, although the overall environment remains aqueous.)

Therefore, despite a wealth of ingenious experimental studies, the status of "the kinetic hydrophobic effect" (as the accelerations may be termed) remains tantalizingly unclear. All the same, the concept is apparently viable under certain conditions, although with surprising twists and turns concerning the fundamental basis of the effect (vide infra)!

2.1.2 "Hydrophobic acceleration" as a non sequitur of sorts

As mentioned above, the hydrophobic effect has both structural and reactivity consequences. The latter "kinetic hydrophobic effect", however, is inherently contradictory and a non sequitur of sorts. This is because non-polar environments are rarely known to accelerate reactions, as the vast majority of transition states are more polar than the ground states [11].

In fact, non-polar media such as hexane are rarely solvents of choice for conducting organic reactions, indeed for several related reasons [11-13]. Prime among these is poor solubility of most reactants that would also affect the polar transition state to a greater extent (relative to the ground state). On the other hand, water is also rarely employed as a solvent for organic reactions, intriguingly because of poor solubility despite its high polarity (the hydrophobic effect per se)!

Thus, preferred media for organic reactions are polar solvents such as THF and ether that define a "Goldilocks zone" in terms of polarity. These arguments are clearly intriguing vis-à-vis the very idea of hydrophobic acceleration and certainly demand deeper enquiry, as attempted further below.

2.2 Micelle Formation and Reactivity

2.2.1 The nature of the micellar phase

Micelles were initially proposed to explain the action of soaps and detergents, and were apparently based on earlier ideas on colloidal suspensions [11,14]. Thus, micelles were an important addition to the middle ground between monophasic and biphasic systems, which apparently paved the way for the modern era of nanoscience. Over a century or so since its inception, the micelle concept has evolved in sophistication beyond the original aims concerning surfactants and detergency, with recent applications [15-18] being directed towards vesicles, drug delivery systems, etc. Theoretically, micelles are also key to understanding self-organization at the molecular level, hence fundamental to understanding molecular recognition phenomena. (The area is well served by several textbooks and monographs [11], and recent trends are briefly highlighted herein as relevant to this paper.)

The hydrophobic effect is the major driving force for the formation of normal micelles (as opposed to inverse micelles). Normal micelles (henceforth "micelles") are formed in aqueous media by the association of long chain hydrocarbon molecules that possess a polar head-group (Fig. 1). It is currently believed that the long hydrocarbon "tails" associate to minimize the contact area between the hydrocarbon moieties and water. This occurs only at and beyond the critical micelle concentration ("cmc"), prior to which the system behaves like a solution. (In fact, the formation of micelles also places polar groups in contact with water, a stabilizing feature that also occurs in the case of protein folding.).

The need to attain the cmc is believed to be a consequence of entropy changes, with the entropy loss resulting from the association being compensated by the entropy gain resulting from the release of water molecules, upon desolvation of the hydrocarbon tails. However, that this occurs only at the cmc and beyond implies a non-linear dependance between the association and resulting entropy changes, with an exponential gain in entropy that sets in at the cmc.

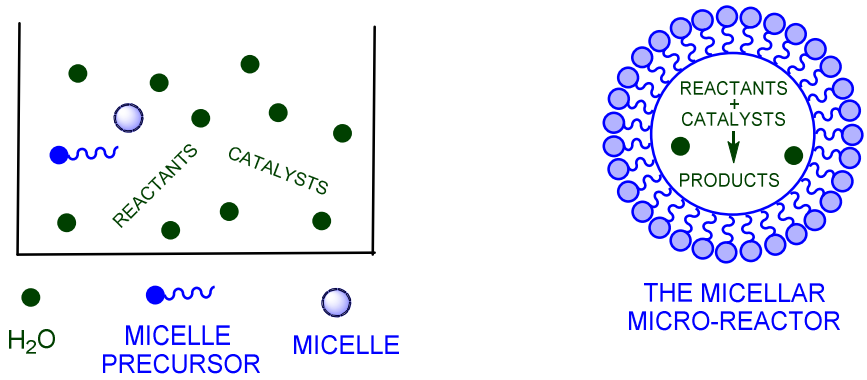

Fig. 1. The formation of micelles (left) and their application (right)

An alternative model, however, invokes the structure of water as driving micelle formation [1]. Thus, the structure of water is defined by microdomains that are rapidly fluxional, but also provide interdomain spaces for accommodating solute molecules. These "holes" are apparently filled up at the cmc, beyond which the structure of water is severely perturbed by solutes. The formation of micelles likely minimizes this perturbation by forming a separate phase, although as a suspension.

2.2.2 Micelles as reaction media

As noted above, the preferred solvents for performing organic reactions apparently strike a compromise between hydrophobicity and polarity. This is unsurprising as the vast majority of organic compounds also fall into this compromise zone. Conversely, water–the hydrophilic solvent by definition–is naturally ruled out as a reaction medium for organic reactions.

Despite these limiting considerations, there has been continuing interest in water as a medium for organic reactions, for diverse reasons. Although environmental concerns have recently come to the fore, a fundamental curiosity about how biological reactions occur–perforce under aqueous conditions–and the hope of mimicking these processes, have spurred theoretical and experimental studies across chemical biology in recent decades.

It is in this context that micelles have assumed importance, apparently by providing hydrophobic pockets that can serve as in situ reaction media, although in a largely aqueous environment. Indeed, there is now an accumulation of evidence that justifies the idea that micelles can be employed for a variety of synthetic purposes [19,20]. Intriguingly, however, this impressive success in the practical application of micelles has been built on the rather nebulous concept of hydrophobic acceleration.

Thus, it remains unclear why organic reactions should be facilitated in an apparently hydrophobic environment, as this is in conflict with the laws of solvent effects on organic reactions (vide supra) [13]. Clearly, a renewed approach to understanding this key phenomenon is indicated, as attempted below.

2.2.3 The possible origins of micellar reactivity and the role of the hydrophobic effect

Interestingly, micellar reactivity is often attributed to proximity effects, implying that high concentrations of the reactants are attained within the micellar core. Indeed, multicomponent reactions with higher order rate laws can be accelerated exponentially by an increase in the concentration. In general, if the overall reaction order is n, an increase in the concentration of all the reactants by a factor of m would lead to an acceleration of m^n.

The problem with this approach, however, lies in explaining how the putative high concentrations are attained in the hydrophobic interior of a micelle, as (again) non-polar media are notoriously poor solvents. Thus, is the interior of a micelle considerably different from (say) hexane? If the answer is yes, it implies that the interior of a micelle may not be as hydrophobic as presumed, most likely because of the presence of traces of water. (This is unavoidable in the aqueous environment of the micelle.)

It is also noteworthy (again) that organic substrates are themselves only partly hydrophobic, thus being poorly soluble in hydrocarbon solvents, with polar organic solvents being the best (THF, ether, etc.). All this points to the possibility that the core of a micelle is only partly hydrophobic (perhaps like THF or even DMF in polarity).

In fact, a conventional proximity effect, in the sense that the reactants are brought together in the core of the micelle, is likely ruled out as the origin of micellar reactivity. This is because a similar proximity effect should be possible in any solvent in which the reactants are highly soluble. (In any case, intramolecular models have failed to reproduce enzymic levels of rate enhancements, so proximity effects are inherently dubious!)

Furthermore, the fact that the substrate is far more soluble in the micellar core than in the aqueous phase, indicates that the micelle needs to be considered as a separate phase altogether, although the individual units are well dispersed. Kinetic and thermodynamic analysis of the reaction, therefore, needs to take this into account, particularly considering the ground state of the reactants. Indeed, considering the poor solubility of the reactants in the aqueous phase, the aqueous phase cannot be considered to represent the reactant ground state. In this sense, micellar reactivity differs fundamentally from enzymic reactivity, as discussed further below.

These arguments, therefore, indicate that conflating micellar reactivity with (particularly) the kinetic hydrophobic effect raises more questions than it answers. This is because both micelles and the hydrophobic effect are per se complex and poorly understood. Many a time, in fact, a presumed hydrophobic acceleration is likely due to the enhancement of the solubility of the reactants in water by hydrophobic additives (even in a pre-micellar regime). Also, claimed accelerations may be dubious as the reference taken is the plain water reaction, although the catalytic reaction is performed at rather high pH!

Clearly, micellar reactivity remains both fascinating and mysterious, being entangled in the complexities of the hydrophobic effect itself. An attempt is made below to sift through the available evidence to arrive at a sensible (if qualitative) resolution of this apparent paradox.

2.2.4 How real is the hydrophobic effect itself?

The ambiguities concerning the hydrophobic effect may be summarized as below.

(i) The kinetic hydrophobic effect remains unsubstantiated for several reasons [1,10].

Firstly, studies in pure water on the kinetics of the Diels-Alder reaction have evidenced only an enhanced rate constant, but this does not necessarily imply an enhanced rate. This is because the enhanced rate constant is due to a raised ground state energy of the reactants, which also leads to a correspondingly low concentration.

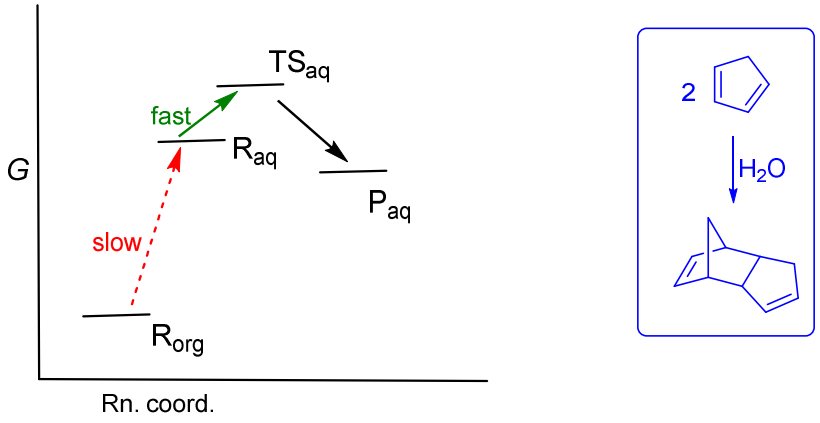

Fig. 2. Gibbs energy (*G*) diagram of the kinetic hydrophobic effect with exemplary reaction (box): R (reactant), TS (transition state), P (product), "org" (organic) and "aq" (aqueous)

An enhanced rate would require that the reaction be performed in an aqueous-organic biphasic medium, with the reactants being transferred from the organic to the aqueous phase rapidly, relative to the rate of the reaction in the aqueous phase. However, not only has this not been demonstrated but is also unlikely, given the poor solubility of the reactants in water (Fig. 2). (Thus, the hydrophobic effect nullifies itself, in a way!)

(ii) The accelerations attributed to micellar systems are manifestly real as having been experimentally observed. However, serious ambiguities remain. Thus, even assuming that the reactions occur in the core of the micelle, the observed enhancements are almost impossible to reconcile with the fact that non-polar media are generally extremely poor solvents [11-13]. This naturally precludes conventional proximity effects, which are ambiguous in themselves.

Thus, the view that "high local concentrations" of reactants are attained in the micellar core implies that said concentrations are relative not only to the overall micellar medium, but also to the saturating concentrations in normal non-polar solvents. (Else the same reactivity should be replicated in these solvents.) There is indeed no justification currently for this assumption.

There is also no reason why highly ionic reagent species should be found in high concentrations within the hydrophobic core of a micelle. This, again, rules out the idea that high local concentrations in the micellar core are the origin of the observed reactivity. The possibility remains, however, that the micellar core is only partly hydrophobic, thus approximating in polarity to the commonly employed organic solvents (THF, ether, etc.).

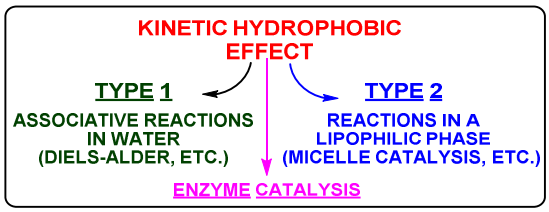

Fig. 3. The two types of hydrophobic effect that influence chemical reactivity with enzyme catalysis representing the middle ground

Traces of water within the micelle–inevitable in the aqueous environment–may well play a critical role, both in enhancing polarity and in acting as catalyst. All the same, the enigma concerning the origin of the reactivity remains!

(iii) A particular problem concerns the standard for comparison for estimating the reactivity effect in micellar catalysis. In many (if not most) cases, this appears to be the plain water reaction: In this case, the hydrophobic effect is only relevant to the formation of the micellar phase, which serves as a dispersed organic microreactor in which the observed reaction occurs. This is essentially no different from conducting the reaction in an organic solvent, considering the rates (but retaining the environmental advantages of micellar systems).

(iv) In fact, it would be useful to define two types of kinetic hydrophobic effect, termed "Type 1" and "Type 2", although with a common basis (Fig. 3). These refer to the putative rate enhancements in a purely aqueous medium (Type 1) or in a largely non-aqueous medium (Type 2). Thus, the Type 1 effect would pertain to reactions in water that are driven by the aggregation of the reactants (e.g., Diels-Alder); the Type 2 effect would pertain to reactions occurring in a largely non-aqueous medium (e.g., micellar reactions). The latter is essentially a medium effect that enhances the solubility of the reactants in the core of a micelle, etc. (Enzyme reactions apparently fall in between, vide infra.)

Thus, apparently, whereas the Type 1 effect is stymied by the inherently low concentration of the reactant, the Type 2 effect–in principle–would be stymied by the low polarity of the medium. Hence, the former is not consummated as a rate enhancement, and the latter would be viable only in the presence of charge-relay possibilities.

2.2.5 New approaches to micellar reactivity: Tweaking the hydrophobic effect

The enigmatic, will-o'-the-wisp quality surrounding the twinned micellar-hydrophobic effect clearly calls for a departure from conventional conceptual norms! A few interesting possibilities need serious consideration, as discussed below.

(i) There is a likelihood that the interior of the micelle is less densely packed than currently believed, because of steric repulsions between the long chains. This may well allow high concentrations of the reactants to be attained in the core, substantiating the proximity effect hypothesis discussed above. However,

there is some evidence that the density of the micellar interior is not all that different from that of a normal organic solvent [21], which serves as a caveat to these ideas.

On the other hand, intriguingly, it is possible that the long chain moieties within the micellar core are far more constrained in their motions than the molecules in a normal solvent. Liquids are known to possess a rapidly fluxional internal structure that possibly makes them very different from the interior of a micelle [1]. On this basis, constrained molecular motions within the micellar core–but allowing the reactants to diffuse–could lead to higher reactivity of included guest reactants. This, rather than conventional proximity effects may well be the key to micellar reactivity.

In fact, the constrained motions in the micellar core correspond to low entropy, hence high Gibbs energy. This assumes importance in light of the above proposal that micelles constitute a separate phase per se, as now the high Gibbs energy can be considered as the ground state of the guest reactants. (In this, micelles would differ fundamentally from enzymes, in which the reactant ground state remains in the aqueous phase.)

(ii) It is known that pK_a orders may be inverted in a hydrophobic environment (as evidenced by theoretical calculations in the gas phase) [22]. Thus, the moderate general acids and bases normally employed in micellar reactions, may well be considerably enhanced in the core. Furthermore, hydrogen-bonded chains of water molecules present in traces in the interior, may relay charges to the aqueous exterior. This charge dispersal would stabilize transition states, which generally develop high levels of charges because of bonding changes. (These mechanisms are possibly employed by enzymes, in particular [5].

(iii) All micellar reactions are not created equal, in the sense that some may not even occur within the core at all! This is particularly plausible in the case of charged nucleophiles and electrophiles, which would be poorly soluble in the hydrophobic core. Examples would be Bronsted acids and bases, metal ions, etc. Thus, these may well be electrostatically attached to the micellar exterior, where high charge densities would be the norm because of the proximity of an enormous number of charged head-groups.

Thus, the high local concentrations of negative charges (anionic micelle) or positive charges (cationic micelle) would render them highly reactive, despite the possibility of stabilization by water molecules. The charged head-group moieties may themselves function as super-nucleophiles and super-electrophiles, along with the added catalytic agents. The anchoring of these agents at the head-groups would also enhance reactivity, assuming that the reaction does occur on the exterior of the micelle.

Indeed, considering that proximity effects cannot be justified as the basis of micellar reactivity, the possibility that many micellar reactions occur at the micellar surface now demands serious consideration. In general, the micellar outer surface defines a congested environment in which the acidic and basic head groups can become highly reactive. This may well be the basis of micellar reactivity in the majority of cases as, apparently, there is scant evidence that the reactions do occur in the interior core in all cases!

These proposals are meant to foster a critical reassessment of the basis of micellar reactivity, which remains tantalizingly unclear despite a wealth of experimental studies supporting it. Currently, micelles enjoy unparalleled growth and attention as favored media for a variety of synthetic reactions, many of industrial and commercial importance. The urgent need for reaching a fundamental understanding of the micelle phenomenon, therefore, can hardly be overstated.

2.3 Enzymes and the Hydrophobic Effect

2.3.1 The enigma of enzymic reactivity

Enzymes are Nature's catalysts par excellence, evolved over the millennia to sustain and propagate life. In their mildness, selectivity and efficiency, enzymes indeed remain unrivalled. Understanding the secrets of their catalytic power would not only unlock the mysteries of life but could also lead to the design of non-natural catalysts perhaps of equal power.

Following their discovery over a century ago, the isolation and characterization of an enormous number of varied enzymes over recent decades has led to increasing understanding of their mechanism of action. Despite this explosive growth, however, the fundamental basis of enzymic reactivity has remained elusive, thus

posing a continuing challenge at the frontiers of modern science [5,23].

Current views have been inspired by the early (1948) proposal by Pauling that enzymes accelerate reactions by stabilizing the key transition state. Ever since, this idea has served as leitmotif, although it is being subjected to critical analysis in recent times. All the same, the Pauling hypothesis is far from being supplanted, rooted as it is in transition state theory, the generally accepted paradigm of chemical reactivity.

The Pauling hypothesis is based on the idea of an enzyme active site, wherein the ephemeral transition state is generated during the course of bonding changes in the bound substrate (Fig. 4). Stabilizing the transition state would lower the activation energy and accelerate the reaction. Although the requirement that the substrate enter and bind within the confines of the active site seems trite, it is noteworthy that formation of the enzyme-substrate complex results in considerable loss of entropy. And this is where the hydrophobic effect enters the picture!

Thus, it is currently believed that the substrate is "lured" into the active site by the hydrophobic effect (noting that enzyme catalysis generally occurs in water). This results in the formation of a weakly bound enzyme-substrate complex that is the precursor for the ensuing transition state. The Pauling hypothesis implies the complementarity of the active site cavity to the transition state, in their steric and electronic characteristics.

These proposals are, in fact, predicated on the key assumption that the active site in the enzyme interior itself defines a hydrophobic zone. This is essentially substantiated by the principles of protein folding, according to which the relatively hydrophobic amino acid residues are tucked away in the protein interior, thus minimizing contact with the aqueous environment.

Whilst these broad contours of enzyme action are not in question–despite recent controversy surrounding the Pauling hypothesis–the manner in which the observed accelerations accrue, however, remains elusive.

2.3.2 The role of the hydrophobic effect in enzyme action

Although the hydrophobic effect was implicated in early theories of enzyme action, an accurate assessment of its role is still evolving. The problem is that the Pauling hypothesis implies that the active site cleft is complementary to the transition state, which was also taken to imply that the substrate ground state needed to be bound weakly, to avoid the risk of a "thermodynamic pit". However, recent developments have indicated the formation of relatively stable covalent enzyme-substrate intermediates, particularly among the most efficient enzymes.

The current view is thus veering away from the possibly fallacious thermodynamic-pit hypothesis [23]. In fact, this implies that the hydrophobic effect can act equally on the substrate both in the ground and transition states. However, it is crucial that the ground state binding is carried over to the transition state, and also that the overall reaction is exergonic, to avoid the buildup of enzyme-substrate intermediates. Otherwise, as in an enzyme-catalyzed drive toward equilibrium, the thermodynamic-pit problem would be real, so weak binding of the substrate ground state is indicated.

Furthermore, the hydrophobic effect does not act alone at the active site, being supplemented by other binding modes, notably van der Waals forces and hydrogen bonding. Disentangling the transition state hydrophobic effect from this skein of forces is clearly daunting, hence assessing the exact contribution of the hydrophobic effect to enzymic reactivity remains a challenge.

The hydrophobic association of the enzyme and substrate was also presumed to lead to a proximity effect, considered for long to be the basis of the enzymic reactivity. The study of intramolecular models was essentially based on this idea, although this has been challenged. The problem with the intramolecularity approach is that the enzyme reaction is overall bimolecular and hence subject to the Pauling hypothesis, with intramolecular reactivity arising rather out of ground state effects.

Interestingly, this also leads to a key distinction between enzymic and micellar reactivity. Thus, in the enzyme reaction the substrate ground state rests in the aqueous solution phase, so the stabilization of the transition state at the enzyme active site is the key to the observed reactivity. In the micellar case, however, the micelle defines a separate phase, so the hydrophobic environment is common to both the ground and transition states of the reacting substrate (vide supra).

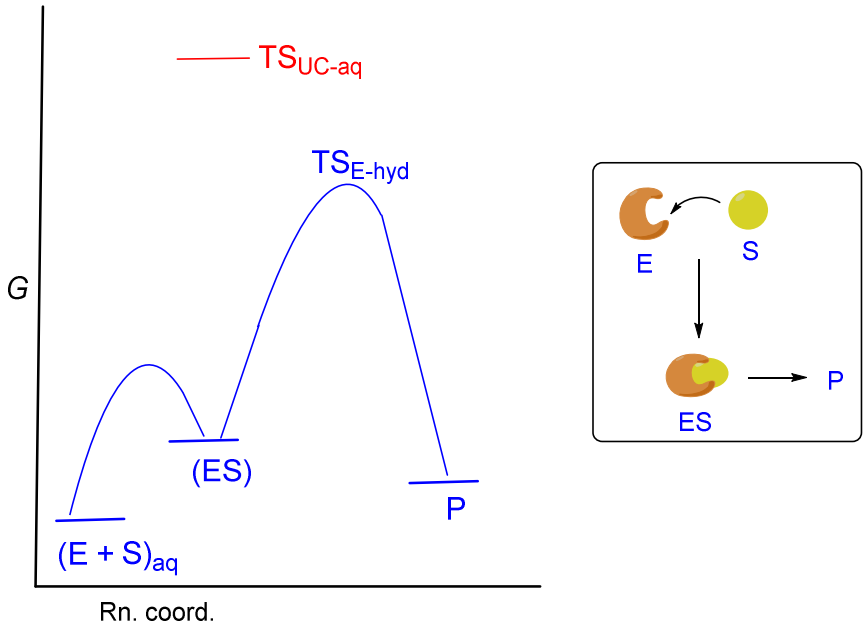

Fig. 4. Gibbs energy (*G*) profile of enzyme catalysis (left) with cartoon (box) of the reaction between enzyme (E) and substrate (S) via enzyme-substrate complex (ES) and transition states (TS): uncatalyzed-aqueous (UC-aq), enzyme-hydrophobic (E-hyd)

This apparently makes micellar systems more enigmatic than the enzymes they are meant to model!

It is also noteworthy that the term "hydrophobic binding" is inaccurate as the hydrophobic effect is a relative one. Thus, the selective hydrophobic "binding" of the transition state implies that the transition state (being derived from the hydrophobic substrate) is more stable in the active site than in the free aqueous solution (Fig. 4).

2.3.3 The hydrophobic effect in enzymes and micelles — a comparison

As discussed above, the hydrophobic effect plays a key role in bringing the enzyme and the substrate together, and in keeping the substrate ensconced in the confines of the enzyme active site long enough for the overall reaction to occur. The stabilization of the rate determining transition state may also be attributed to the hydrophobic effect (although supplemented by additional stabilization modes, vide supra).

The implication of the hydrophobic effect in the formation of micelles, and the fact that micelles could also catalyze a wide variety of reactions, led to the view that micelles were enzyme models. This has strongly influenced the development of micellar catalysis, understandably for both theoretical and practical reasons, that also launched the nascent field of chemical biology.

However, as argued above, not only is the hydrophobic effect complex in itself, also, its role in enzyme and micellar catalysis is rather tenuous. In the case of enzyme catalysis, the exact contribution of the hydrophobic effect to the observed rate enhancements is generally unknown. In the micellar case, the presumed role of the hydrophobic effect on observed reactivity is difficult to substantiate. In each of these cases, however, there is little doubt that the hydrophobic effect has a role to play, although a quantitative assessment has proved difficult.

The major difference between enzyme and micellar catalysis lies in the fact that micelles constitute a distinct phase by themselves, thus defining the ground state of the reactants (possibly) trapped in the core. In the enzyme case, the ground state of the substrate remains in the bulk aqueous solution, the enzyme itself being present in catalytic amounts.

Furthermore, it is not always clear, in the micellar case, that reactions are occurring in the core, particularly with highly ionic reagents and

substrates. If indeed the reactions occur on the micellar surface in these cases, the hydrophobic effect is obliquely involved, in only forming the micelle.

All the same, despite these ambiguities, the study of enzymes and their presumed micellar analogs will continue apace for both practical and fundamental reasons, as chemical biology continues to evolve in its pursuit of understanding and harnessing Nature for human welfare.

2.3.4 The kinetic hydrophobic effect in summary

The manifestation of the hydrophobic effect as enhanced reactivity in certain chemical and biological systems has been a continuing theme in recent decades. Whilst this kinetic hydrophobic effect is often taken for granted by the majority of investigators, the facts apparently indicate blurred contours at best. Thus, it is highly unlikely that the enhanced specific rates observed for a few reactions in water imply an enhanced rate. The basis of micellar catalysis also remains unclear, as the nonpolar micellar core cannot be a medium conducive to enhanced reactivity. Enzyme catalysis involves the hydrophobic effect but obliquely, in presumably stabilizing the transition state in the active site, although supplemented by several other modes of electrostatic stabilization.

It is also noteworthy that the putative “Type 1” hydrophobic effect in water is not exactly the same as the putative “Type 2” hydrophobic accelerations in a nonpolar environment (typically, micelles) (Fig. 3), with the enzyme case defining a middle ground (Fig. 4). These considerations indicate due circumspection in dealing with the kinetic hydrophobic effect, as genuine cases are apparently few and far between.

2.4 The Hydrophobic effect in Medicinal Chemistry

2.4.1 Medicinal chemistry and drug design

The design of drugs and medicaments for the alleviation of disease and human suffering is one of the loftiest aims of modern science, and a key pursuit within chemical biology. If medicinal chemistry be defined broadly as the study of the chemical aspects of pathological states thus leading to their remediation, drug design would represent a core activity and the culmination of a process that begins with a particular etiology.

Modern drug design is a highly complex intellectual pursuit that is also capital and labor intensive in its execution [24-27]. Each disease has its own idiosyncratic origins, thus leading to the plethora of strategies for dealing with the afflictions besetting modern societies. Apparently, the only unifying theme is based on the idea of a target for the drug in question, be it an enzyme that needs to be inhibited or a receptor that needs to be activated or deactivated. The key role earmarked for mechanistic and synthetic organic chemistry in this endeavor is obvious, the practitioners of these disciplines indeed stepping up to the plate most enthusiastically.

Although modern drug design strategy is dominated by computational modeling, these in silico approaches have evolved from earlier experimental strategies that were largely empirical. Thus, classical methodologies were essentially based on quasi-random modification of prior molecular structures known to possess activity, whether natural or synthetic. Improvements in potency and minimization of undesirable side-effects were thus achieved at considerable expense in time and money. Systematic drug design, however, was also evolving simultaneously but slowly, essentially based on unravelling the biochemical basis of the disease in question, leading to the identification of the drug target.

Bearing in mind biochemical complexity in general, progress in these approaches was understandably slow, although notable hits included improved antibiotics, as well as treatments for both nuisance ailments (e.g., diabetes and hypertension) as also killer diseases (e.g., cancer and cardiovascular states). From a drug design perspective, however, there was a need for empirical generalizations linking structure and activity.

2.4.2 The Hansch equation, its anomalies and QSAR

In fact, an early development was the intriguing discovery that the activity of a drug was related to its hydrophobicity [6]. And that this could be placed on a quantitative footing adds to the sense of wonderment, given the complexities of biology and drug action itself! The relationship between drug activity and hydrophobicity was

enshrined in the Hansch equation (strictly, the extended Hansch-Fujita equation, Eq. 1):

$$\log(1/C) = a\sigma + b(\log P) + c \quad (1)$$

In this, the minimum concentration (C) of the drug necessary to obtain a certain level of activity is plotted along the y axis as -log$C$, and the hydrophobicity is plotted along the x axis as log$P$, P being the octanol/water partition coefficient for the drug. Whilst a, b and c are constants, σ is the Hammett substituent constant, indicating that the equation can be a linear free energy relationship [28].

Plotting Eq. 1 for a particular drug (i.e., keeping σ constant but varying P) yields a parabolic curve, indicating that the activity passes through a maximum before decreasing with further increase in hydrophobicity (Fig. 5). Intriguingly, this implies a "Goldilocks zone" of maximal activity at moderate levels of hydrophobicity (but leading to crucial insights as argued below).

The Hansch equation, with its predictive power, introduced a welcome sense of order to a field perhaps largely dependent on happenstance! Furthermore, the Hansch equation was the harbinger of a revolutionary approach to drug design based on the linear free energy relationships (LFER). This introduced additional terms based on electronic and steric effects, alongside the original hydrophobicity terms. The electronic effects were estimated by the Hammett σ substituent constants, thus enhancing the predictive capabilities of Eq. 1 enormously and in a rigorous mathematical framework. In fact, these ideas led to a general strategy for understanding biological activity in molecular-structural terms, the "Quantitative Structure Activity Relationship" (QSAR) approach.

The Hansch equation has been traditionally explained by assuming that the targeted receptor site is a hydrophobic pocket, so increasing hydrophobicity of the drug leads to correspondingly better binding at the receptor. However, the dip in the curve at high levels of hydrophobicity (Fig. 5) was explained as being due to the decreasing solubility of the drug in the largely aqueous biological media. However, a problem with this idea is that not only is it untested but also that the nature of the plot itself indicates that the hypothesis is flawed.

This is because the plot shows that decreasing levels of the drug are needed up to the maximum point, and importantly, increasing levels of the drug are needed beyond the maximum point: However, this implies that the drug is indeed dissolving even in the higher hydrophobicity region, as otherwise this part of the curve would not exist! (It is futile to load a saturated solution with more solute; and conversely, continued dissolution of the solute implies that the solution is not saturated.) Hence, an alternative explication of the Hansch plot (Fig. 5) is necessary to deal with these ambiguities.

2.4.3 Alternative interpretations of the Hansch equation: A kinetic approach

Current models of drug action are apparently based on a thermodynamic approach to drug-receptor binding. Thus, stronger drug-receptor binding is believed to lead to higher activity. This model manifestly fails in the case of the Hansch equation, particularly in the high hydrophobicity regions (vide supra). However, a dynamic model of drug-receptor interactions, apparently, leads to a comprehensive rationale for the Hansch plot (Fig. 5). ("Receptor" is defined broadly herein to imply the targeted site of biological activity, including enzymes marked for inhibition, etc.).

In this dynamic model, the rate of binding of the drug to the receptor is the key determinant of the drug's activity. Thus, the biological response elicited by the drug is assumed to be much faster than the rate of its binding to the receptor. Hence, the overall strength of the biological response is directly proportional to the concentration of the drug-receptor complex, and the intensity of the response becomes a function of the rate of formation of the drug-receptor complex.

Furthermore, for a continued biological response, the release of the drug from the receptor is necessary. This is regardless of whether the drug is degraded by its interaction with the receptor or not (as in suicide inhibition of an enzyme), and also assuming that every binding event can lead to a biological response but only once. In other words, every response needs the binding of a new drug molecule. Under these conditions, the release of the drug from the receptor becomes important.

Thus, it may be envisaged that at moderate levels of hydrophobicity, the binding of the drug to the receptor is slow and rate determining, hence increasing hydrophobicity leads to a more intense response in this regime (via faster

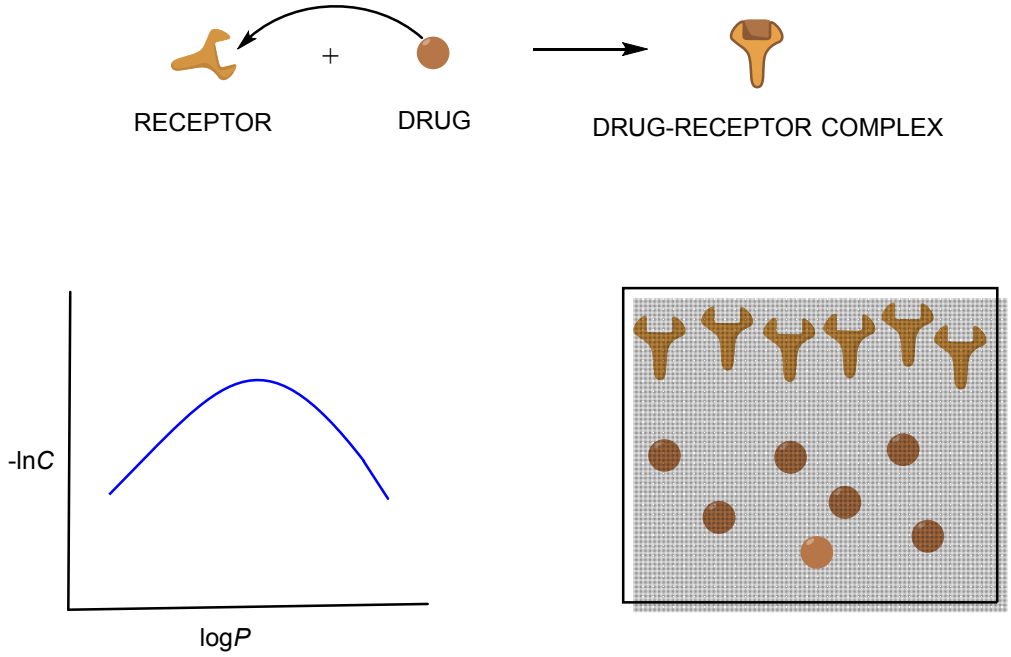

Fig. 5. Cartoon representation of drug-receptor binding (upper) with comparable numbers of drug molecules and receptors (box) and Hansch plot (left, for *C* and *P* see text)

binding). However, at higher levels of hydrophobicity–beyond the maximum in the Hansch plot (Fig. 5)–the release of the drug from the receptor may well become rate determining. Hence, the intensity of the biological response decreases with hydrophobicity in this regime.

Interestingly, the activity of the drug can be restored to a desired level by increasing the concentration of the drug, in this regime. This implies that the concentration of the free receptor is very low, clearly because of the slow decomposition of the drug-receptor complex. Thus, the rate of drug-receptor binding is retarded by a concentration effect, despite the high hydrophobicity of the drug. Hence, both the rate of binding and the rate of release have become slow although for different reasons: the former due to a concentration effect and the latter to high hydrophobicity per se! However, the rate of binding determines drug activity, hence the efficacy of higher drug concentrations.

These arguments also imply that the number of receptor sites (*n*) is of the same order as the concentration of the drug employed. If *n* were relatively small, drug release would always be rate limiting, but increasing the drug concentration would not help. If *n* were relatively large, slow release of the drug would be immaterial as there would always be free receptor sites available for binding. In either case, therefore, the Hansch plot (Fig. 5) would not result.

2.4.4 Broader implications for the theory of drug action

The above analysis of the Hansch plot (Fig. 5) culminating in an alternative dynamic model of drug-receptor interaction, in fact, leads to fascinating insights into the theory of drug action in general [6]. In particular, the dependence of drug activity on a narrowly defined set of properties of the drug indicates that electronic rather than structural effects dominate the drug-receptor interaction. This can be understood with further analysis of the Hansch equation (Eq. 1).

The dependence of drug activity on hydrophobicity, in particular, is indeed remarkable. (A wide range of structures can possess similar hydrophobicity values.) A possible explanation would be that the receptor is far more flexible and accommodating in terms of molecular shape and size than currently believed. Also, the unfolding of the receptor may well be the rate-determining step, possibly initiated by the prior weak binding of the drug at the receptor surface. This is reminiscent of the

formation of a Michaelis complex in enzyme-substrate binding, although in the drug-receptor case geometrical aspects may be secondary.

The possibility that drug-receptor interaction is similar to the induced-fit mechanism in enzyme catalysis is also noteworthy, as the receptor can then adapt itself to the shape of the drug molecule. All these possibilities indicate that drug-receptor interactions are only tenuously guided by the geometrical features of the drug molecule. Thus, the Hansch equation leads to intriguing (if speculative) insights into the nature of drug-receptor interactions in general.

3. CONCLUSION

The hydrophobic effect occupies a fascinating crossroads between not only fundamental and applied concerns but also between chemistry and biology! The hydrophobic effect refers to the observed association of nonpolar solutes and molecular moieties in an aqueous environment. As such, the effect is of fundamental importance in the study of biological phenomena, but also in the burgeoning area of green chemistry which emphasizes the use of water as a reaction medium (out of environmental concerns).

Although it is widely recognized that the hydrophobic effect has consequences for both structure and reactivity, these are often difficult to pin down even qualitatively. It is particularly noteworthy that the hydrophobic effect is essentially a relative effect that compares a nonpolar medium with water. However, whereas the structural consequences of the hydrophobic effect are perhaps less tendentious, its influence on reactivity ("kinetic hydrophobic effect") is generally enigmatic.

Thus, the idea of hydrophobic acceleration conflicts with the common observation that nonpolar solvents are not preferred media for most reactions. Mechanistic studies purporting to evidence and measure the hydrophobic effect on reactivity are of dubious generality, as they do not demonstrate enhanced "real rates" of practical significance. These considerations (inter alia) raise serious questions about the validity of current models based on the hydrophobic effect. (In fact, two types of the kinetic hydrophobic effect may be defined: Types 1 and 2, referring to aqueous and non-aqueous media, respectively.)

In particular, micellar catalysis has for long been touted as a model for enzyme reactivity, and is also gaining increasing application in synthetic methodologies. Whilst the formation and practical importance of micelles are not in doubt, the fundamental basis of these effects remains unclear. Thus, the idea that micelles accelerate reactions via the hydrophobic effects seems simplistic for the above reasons, with additional effects currently but poorly identified and understood being likely contributors to a complex skein.

Prime among these is the possibility that the micellar core is only partly hydrophobic because of traces of water, so polar enough to facilitate reactions. It is also unclear what the comparison standard is for the claimed reactivity, but appears to be the plain water reaction, so micellar media just combine the best of the aqueous and organic extremes. Another possibility is that some of the micellar reactions occur at the surface of the micelle, where the sterically congested environment renders the headgroups highly reactive. This may be particularly relevant to cases involving highly ionic reagents and catalysts, which would be poorly soluble in the micelle core.

Although much has been made of the hydrophobic effect in enzyme catalysis, its exact contribution is difficult to estimate. Whilst there is no doubt that the hydrophobic effect drives the formation of the enzyme-substrate complex thus also stabilizing the transition state, the problem of stabilizing the developing charges remains. This indicates the critical importance of charge-relay systems within the enzyme interior, including the polypeptide backbone itself. This also implies that hydrophobic acceleration cannot occur by itself, but must act in tandem with other polar effects known to enhance reactivity.

The hydrophobic effect has played a fundamental role in the development of the modern theory of drug action that is based on the quantitative structure activity relationships (QSAR). Earlier work on the Hansch equation laid the foundations of these studies, also indicating the importance of the hydrophobic effect in drug-receptor interactions. However, the steep decline in drug activity beyond the "Goldilocks maximum" has been perplexing, but can be explained by a dynamic theory of drug-receptor interaction.

In this, the activity of the drug is proportional to its rate of binding to the receptor, leading to a

nearly linear relationship between hydrophobicity and activity, although up to moderate levels of drug hydrophobicity. Beyond this, apparently, the release of the drug becomes slow and rate determining, thus depleting the concentration of the free receptor. Generally, the QSAR model apparently indicates that drug receptors are essentially flexible and that their binding to the drug is dominated by electronic rather than steric effects.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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