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Analysis of Enzymatic Transacylase Brønsted Studies with Application to the Ribosome

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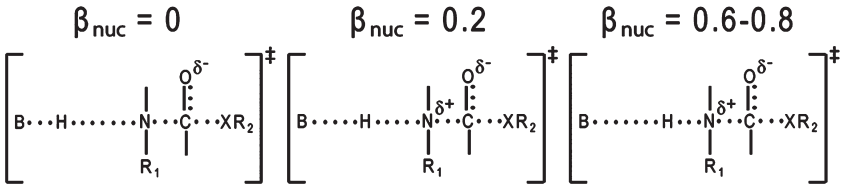

Preferential binding of an enzyme to the transition state relative to the ground state is a key strategy for enzyme catalysis. When there is a difference between the ground and transition state charge distributions, enzymes maximize electrostatic interactions to achieve this enhanced transition state binding. Although the transition state is difficult to observe directly by structural methods, the chemical details of this transient species can be characterized by studies of substituent effects (Brønsted, Hammett, Swain–Scott, etc.) and isotope effects. Brønsted analysis can provide an estimate of transition state charges for the nucleophile and leaving group of a reaction. This Account will discuss the theoretical basis of Brønsted analysis and describe its practical application to the study of transacylase enzyme systems including the peptidyl transferase reaction of the ribosome.

The Brønsted coefficient is derived from the linear free energy relationship (LFER) that correlates the acidity (pK_a) of a reactive atom to the log of its rate constant. The Brønsted coefficient establishes the change in atomic charge as the reaction proceeds from the ground state to the transition state. Bonding events alter the electrostatics of atoms and the extent of bonding can be extrapolated from transition state charges. Therefore, well-defined nucleophile and leaving group transition state charges limit the number of mechanisms that are consistent with a particular transition state. Brønsted results are most informative when interpreted in the context of other mechanistic data, especially for enzymatic studies where an active site may promote a transition state that differs significantly from a prediction based on uncatalyzed solution reactions.

Here we review Brønsted analyses performed on transacylases to illustrate how these data enhanced the enzymatic mechanistic studies. Through a systematic comparison of five enzymes, we reveal a wide spectrum of Brønsted values that are possible for what otherwise appear to be similar chemical reactions. The variations in the Brønsted coefficients predict different transition states for the various enzymes. This Account explores an overriding theme in the enzymatic mechanisms that catalysis enhances commensurate bond formation and proton abstraction events. The extent of the two bonding events in relationship to each other can be inferred from the Brønsted coefficient. When viewed in the context of recent ribosomal studies, this interpretation provides mechanistic insights into peptide bond formation.

Introduction

Brønsted coefficients provide an important piece of experimental transition state data that aid enzyme mechanism studies.[1,2] These measurements provide the apparent charge of an atom as the reaction proceeds from the ground state to the transition state. Related enzymatic mechanisms do not always proceed through similar transition states and lead to varying Brønsted coefficients.[3−8] Transacylase deacylation Brønsted coefficients for the nucleophile (β_{nuc}) range from slightly negative to about one. Although the predicted transition states differ from each another, an overriding theme exists for the transacylase deacylation reactions; namely, catalytic enhancement of nucleophilic bond formation is coupled to varying degrees of proton abstraction,[9] and this accounts for the differences in transacylase β_{nuc} coefficients (Figure 1). Brønsted coefficients closer to zero reflect transition states

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where bond formation and proton abstraction are commensurate and cancel each other's effect upon charge.[3−5] Alternatively, positive Brønsted values near unity are consistent with advanced nucleophilic bond formation with minor proton abstraction.[6,7] These positive Brønsted values and their mechanistic interpretation are comparable to the values measured in related nonenzymatic reactions.[10−12] The absolute Brønsted value represents the extent to which the enzyme promotes proton removal relative to nucleophilic attack during catalysis.

Transition State Characterization

The Brønsted linear free energy relationship (LFER) correlates changes in reaction rate created by isosteric substituents that have varying basicities.[1,2,13] Upon close examination, it may seem counterintuitive that a linear relationship exists between a kinetic (rate constant, logarithmic) and equilibrium (pK_a) constant. However, according to transition state theory, the rate constant of a reaction depends on the exponential function of negative one times the activation free energy ($\Delta G^{\ddagger}$), which is dependent upon the logarithm of the pseudo-equilibrium between the ground state and the transition state.[1] Fersht explains that the basis for the Brønsted relationship is the observation that the ratio of $\Delta\Delta G^{\ddagger}/\Delta\Delta G$ is approximately constant for structural changes that result in minimal changes in the free energy equilibrium of the substrate ($\Delta\Delta G$).[2]

The Brønsted relationship for nucleophilicity explains how the LFER can be used to elucidate transition state data. Experimentally, it has been observed that nucleophilicity generally correlates with basicity.[11] This association makes intuitive sense because both nucleophiles and Lewis bases donate an electron pair in their respective reactions.[1,13] Therefore, the Brønsted LFER relates the transition state of a nucleophile attacking an electrophile to the transition state for the addition of a proton to a Lewis base that is accompanied by the development of a positive charge on the base.[1] Other factors such as solvation, polarization, and sterics also modulate the reactivity of different nucleophiles.[11] However, when these factors are minimized, a linear correlation is observed between nucleophilicity and basicity over a range of reactivity. The correlation is described by eq 1. The constant k is the measured rate constant for the nucleophilic reaction, β_{nuc} (Brønsted coefficient) describes the relative sensitivity of the examined electrophile substrate to the reactivity of the nucleophile, and the pK_a is the acidity constant of the nucleophile, which is a measure of its reactivity. Deviations from linearity result from breakdown in the Brønsted relationship, that

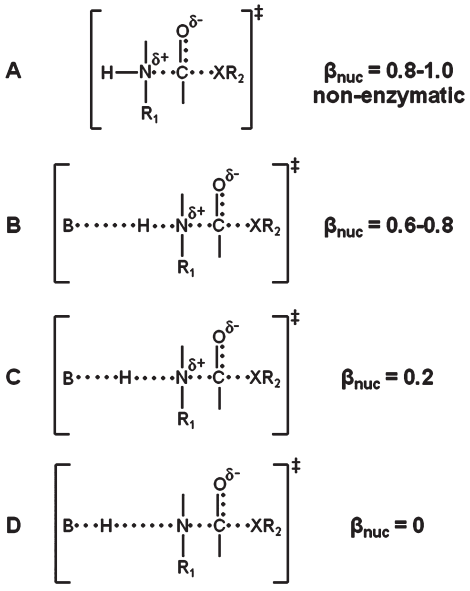


FIGURE 1. Proposed transition states predicted by acyltransferase deacylation and nonenzymatic model reaction Brønsted values. X represents the leaving group atom. Adapted from ref 9.

is, no correlation exists for the transition state of the measured reaction and the transition state of the reference reaction. Such deviations are usually interpreted as a change in the transition state or a change in the rate-determining step.[14−16]

In addition to the relationship described for the nucleophile, a similar equation (eq 2) correlates the relative sensitivity of a reaction to the nucleofugality of the leaving group. Nucleofugality is a measure of an atom or group to act as a leaving group. Each constant describes the same parameter as in eq 1 but in terms related to leaving group reactivity. This second Brønsted relationship is based on the observation that nucleofugality is often correlated to acidity.[13]

$$\log(k) = \beta_{nuc} pK_a + \log(C) \quad (1)$$

$$\log(k) = \beta_{lg} pK_a + \log(C) \quad (2)$$

The Brønsted coefficient (β_{nuc} or β_{lg}) is obtained from the slope of log(k) versus the nucleophile or leaving group pK_a, respectively. This measurement requires a series of sterically similar reactants with varying pK_as for either the nucleophile or leaving group. The pK_as are perturbed by electron-withdrawing or -donating groups substituted near the reactive atom. Appropriate isosteric substituents are required to ensure that the free energy surface is not perturbed by large variations in $\Delta\Delta G$ unrelated to the reactive group's pK_a. This analysis also requires the measurement of reaction rate constants under conditions where chemistry is rate-limiting

and at a pH sufficiently above the reactive group pK_a to ensure the atom is in the reactive protonation state.

Interpreting Brønsted Data

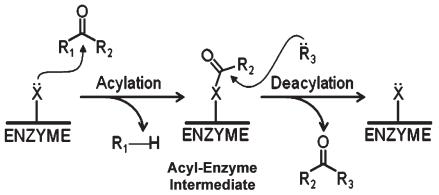
Three general values are possible for the Brønsted coefficient. A positive β value signifies that positive charge is localized upon the reactive atom during the rate-determining transition step. A positive β value would also be observed for an atom that experienced a loss in negative charge character as the reaction progressed from the ground state to the transition state. A negative value is interpreted as a localization of negative charge on the reactive atom or that a ground state positive charge is reduced in the transition state. Finally, a zero β value indicates that there is no change in charge on that atom as the reaction proceeds to the transition state. A Brønsted coefficient of zero could also signify that there were no net changes in bond order in the transition state or that the chemical step is not rate-limiting. These Brønsted coefficients reflect the apparent change in charge and include solvent equilibrium effects.[17] The Brønsted coefficient for the desolvation of an amine (β_{desolv}) is -0.2.[18]

In addition to providing an estimate of the apparent transition state charge, Brønsted data can also provide an indication of bond order.[1,2] Transition state charges for reactive atoms can be moderated by simultaneous proton abstraction (or bond cleavage and proton donation). Fersht commented that small Brønsted values can be easily misinterpreted as a minimal change in bond order when the data are reporting a small change in charge resulting from large but compensating bond changes.[19] Therefore, only in the absence of concerted or coupled bonding events can the value of the Brønsted data be interpreted as a quantifiable measure of heavy atom bonding.

For mechanisms with concerted bonding events, the degree of coupling is reflected in the Brønsted data. A Brønsted coefficient near one indicates that the two events are not significantly coupled and one dominates over the other. At the other extreme, a near-zero Brønsted value implies that the two bonding events are extensively concerted and neither event governs the reaction. For values between zero and one, the extent of each bonding event is determined by comparing the Brønsted data to the possible transition states at the two extremes.[6,20]

Brønsted analysis is used extensively in physical organic chemistry to probe nonenzymatic chemical reaction mechanisms. It has only been applied to a limited number of enzymes because the enzyme must have sufficiently low substrate specificity to accept the chemical modifications necessary to perturb a reactive group pK_a and the rate-limiting step must be chemistry. Furthermore, several substrate derivatives that cover a broad range of pK_as are required for the analysis. Sometimes this is not possible because chemical modifications introduce secondary steric effects that perturb binding or alter the rate-determining step. In the next section, we describe a series of enzymatic Brønsted studies that demonstrate the feasibility of using this physical organic chemistry technique to study biologically catalyzed chemical mechanisms.

SCHEME 1. Simplified Transacylase Chemical Reaction

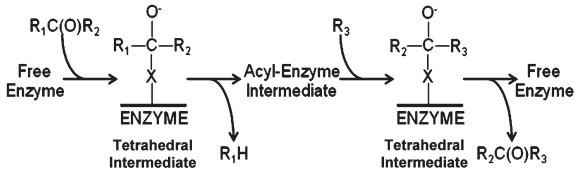


Brønsted Studies for Enzymatic Transacylase Reactions

Several Brønsted studies have been performed on transacylases, enzymes that catalyze the transfer of acyl groups (RC═O) from one functional group to another. Acyl transfer enzymes can catalyze either the alcoholysis or aminolysis of chemical groups such as amides (RC(O)NR′), esters (RC(O)OR′), or thiolesters (RC(O)SR′). In biological systems, they play important roles in cellular detoxification, protein modification, and bioactivation of compounds.[6,7,20] The most notable transacylase enzymes are the serine and cysteine proteases. Many acyl transfer enzymes tolerate some variation in the structure of their substrates, which makes them amenable to the chemical modifications required for Brønsted studies.

For the transacylase examples that we will consider for this Account, the overall chemical reaction proceeds through an acyl−enzyme intermediate and can be subdivided into two reactions (Scheme 1).[3,4,6−8,20,21] Acylation occurs when a catalytic residue from the enzyme nucleophilically attacks the carbonyl carbon of an acyl-donor substrate to form the acyl−enzyme intermediate. Subsequent deacylation is promoted when an acyl-acceptor substrate nucleophilically attacks the carbonyl carbon of the acyl−enzyme intermediate to produce the free enzyme product. Deacylation is promoted by either a water molecule, another nucleophilic substrate, or both, depending on the enzymatic system. For experimental purposes, donor and acceptor substrate reactivity dictates whether the acylation or deacylation step

SCHEME 2. Transacylase Tetrahedral Intermediates



limits the overall reaction. Therefore it is possible to study each reaction independently from the other when the appropriate substrates are selected. Both the acylation and deacylation reactions are assumed to proceed through a tetrahedral intermediate (Scheme 2).[22,23]

This Account will focus solely on acyl−enzyme intermediate breakdown in order to simplify the comparison of Brønsted results (Scheme 1). Unless otherwise stated, conclusions about transition states are made without taking a position on whether formation of the tetrahedral intermediate or the breakdown of the tetrahedral intermediate is rate-determining for the reactions studied.[3,21]

Nonenzymatic Model Reactions

In solution, the chemical mechanism of ester aminolysis is well-defined and provides a model for comparison of enzymatic transacylase Brønsted results (Figure 2).[10,16] For the nonenzymatic aminolysis of an ester, nucleophilic attack of the free amine on the ester follows a mechanism with two tetrahedral intermediates. The first is the zwitterionic T± intermediate. The second is the anionic T− intermediate that occurs upon deprotonation of the amine. In the final step, the C−O bond is cleaved and the leaving group is protonated. The net result of the reaction is the transfer of the acyl group from the ester oxygen to the amine to form an amide bond.

The data obtained from Brønsted analysis was essential for establishing the rate-determining transition state of the solution aminolysis reaction. Jencks and others performed several Brønsted studies measuring the β_{nuc} for different model ester aminolysis reactions. Their work established that reactivity is strongly correlated to basicity (β_{nuc} = 0.8−1.0) (Figure 3) and that the nucleophile thus has significant positive charge in the rate-determining transition state in solution (Figure 1A).[10−12,14,24] The positive charge buildup results from the nucleophile retaining both of its protons as the nitrogen−carbon bond forms the tetrahedral intermediate.[16] After establishing the contributions of proton transfer in the reaction, it was established that the rate-determining step of the non-enzyme-catalyzed reaction was the breakdown of the T± intermediate.[16] The large positive Brønsted coefficient suggests that

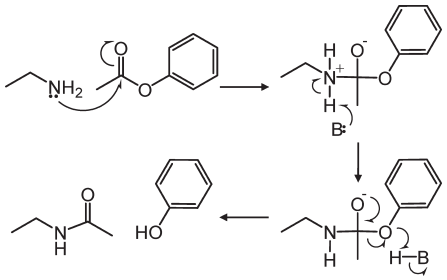


FIGURE 2. Nonenzymatic ester aminolysis reaction mechanism.

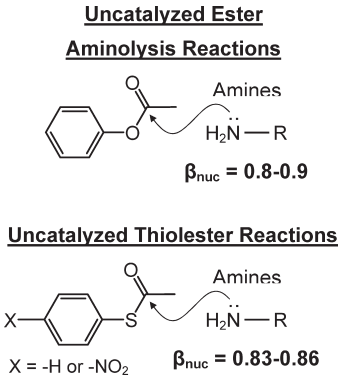


FIGURE 3. Nonenzymatic ester and thiolester reaction Brønsted values.

the transition state for the overall reaction resembles the T± intermediate (Figure 2).[10] This trend holds for all but the most reactive nucleophiles and leaving groups. At these extremes, there was a break in linearity (β_{nuc} = 0.1−0.2), which suggested a change in reaction mechanism.[14]

Chymotrypsin

The Brønsted results for the deacylation of acyl−chymotrypsin demonstrate that the rate constant of this aminolysis reaction is not significantly correlated with basicity.[3,4] Chymotrypsin (EC 3.4.21.1) is a serine protease and is one of several digestive peptidases that are vital for the catabolism of proteins. The mechanism of chymotrypsin is well established and reviewed elsewhere.[22,23,25−28] Briefly, the γ-oxygen of serine-195 acts as the nucleophile and is covalently modified in the acyl−enzyme intermediate.[29] Chymotrypsin forms a variety of stable acyl−enzyme intermediates with natural peptide-like acyl donor substrates,[4,30] non-native activated acyl compounds such as acyl chlorides, *p*-nitrophenyl esters, alkyl esters of cinnamic acid, and *N*-acetyltryptophan.[31−33] In addition to water, chymotrypsin can use a variety of alcohols and amines as acyl acceptor substrates to cleave the acyl−enzyme intermediate and regenerate the free enzyme.[3,34] The relatively broad acyl acceptor substrate

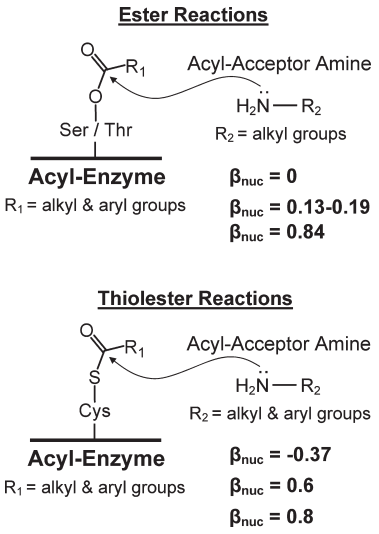


FIGURE 4. Enzymatic reaction Brønsted values.

specificity for chymotrypsin made it possible to probe the deacylation catalytic mechanism by Brønsted analysis.

Inward and Jencks demonstrated that substituent effects for the furoyl–chymotrypsin deacylation reaction were not significant for aminolysis. Nucleophilic amines (pK_a range 4.6–11.2) were monitored for reactivity with furoyl–chymotrypsin by the disappearance of the characteristic acyl–enzyme intermediate absorbance. There was a minor correlation between substrate basicity and the furoyl–chymotrypsin deacylation rate constant when corrected for steric hindrance and the alpha effect. The β_{nuc} values for the amines varied from 0.13 to 0.19 over the entire pK_a range (Figure 4, Table 1). This substrate series showed no break in linearity suggesting that the rate-determining step was the same for all substrates.[3]

Zeeberg and Caplow reported Brønsted results for the acetyltyrosyl–chymotrypsin aminolysis by alkyl amines (pK_a range 3.9–11.8). Compared with furoyl–chymotrypsin, the nonaromatic amines displayed an even more pronounced lack of correlation between reactivity and basicity that was evidenced by a $\beta_{nuc} \approx 0$ (Figure 4).[4] The minor variation between furoyl– and acetyltyrosyl–chymotrypsin β_{nuc} values most likely represents steric variation due to structural differences in the acyl portions.[35] The β_{nuc} values near zero indicate proton abstraction and bond formation are concerted during acetyltyrosyl–chymotrypsin deacylation and a $\beta_{nuc} = 0.13–0.19$ suggests that bond formation proceeded slightly further than proton abstraction in the case of furoyl–chymotrypsin.

The aminolysis results from two acyl–chymotrypsin Brønsted studies demonstrate that the nitrogen nucleophile is approximately charge neutral in the transition state of this deacylation reaction. The significantly reduced charge of the enzymatic transition state suggests that the nucleophile cannot retain both of its protons if nitrogen–carbon bond formation has occurred to a significant degree in the rate-determining transition state. This suggests that the proton must be abstracted from the nucleophile as the bond forms (Figure 1C,D). His-57 is proposed to act as the general base aiding in this proton abstraction.[3,4]

TABLE 1. Enzymatic Brønsted Values

enzyme	reaction	β_{nuc} value	ref
acetyltyrosyl–chymotrypsin	*O*-ester	0	4
50S and 70S ribosomes	*O*-ester	−0.06 and 0.06	5
furoyl–chymotrypsin	*O*-ester	0.13–0.19	3
rat kidney GGT	*O*-ester	0.84	6
GPL-TGase	thiolester	−0.37	20
PL-NAT	thiolester	0.6	8
rH-NAT	thiolester	0.8	7

Rat Kidney γ-Glutamyltranspeptidase

Aminolytic deacylation by rat kidney γ-glutamyltranspeptidase (GGT) provides an interesting contrast to the chymotrypsin results because the Brønsted coefficient for this enzyme is significantly different. γ-Glutamyltranspeptidase (EC 2.3.2.2) is most abundant in kidneys where it aids detoxification through the formation of mercapturic acids that are excreted in urine. The reported kinetic mechanism is a modified ping–pong mechanism that is reminiscent of chymotrypsin.[36] Recent *Escherichia coli* crystallographic studies confirmed the existence of the acyl-intermediate establishing that the γ-glutamyl moiety was bonded to the nucleophilic Thr-391.[37] The acyl donor for GGT is glutathione (GSH) or any other γ-glutamyl-containing compound, such as glutamine and glutathione disulfide.[36] The γ-glutamyl acceptor substrate can be a water molecule (hydrolysis), an amino group of an L-amino acid, or a small L-peptide (transpeptidation). Dipeptides are better acyl acceptor substrates than simple amino acids.[38]

Keillor and associates performed a Brønsted study for the γ-glutamyl acyl–enzyme deacylation reaction.[6] They synthesized a set of alkyl L-methionine derivatives to act as a dipeptide acceptor mimic. These mimics were designed to bind exclusively to the acceptor site and a D-γ-glutamyl donor substrate (D-γ-glutamyl-*p*-nitroanilide) was used to ensure acceptor binding was not inhibited. The authors confirmed that deacylation was rate-limiting and found that the rate constant correlated well with basicity over the pK_a range of 7.1–7.4 resulting in a β_{nuc} of 0.84 (Figure 4).[6] Significant secondary steric effects precluded a study across a large pH range thus limiting the confidence of the observation; this Brønsted coefficient suggests that significant positive charge is localized

on the nucleophile in the transition state and agrees well with Brønsted values for the nonenzymatic ester aminolysis reactions ($\beta_{nuc} = 0.8-1.0$) (Figure 3).[11,14]

Additional data demonstrate that the GGT mechanism is consistent with competing reactions of nucleophilic attack and proton abstraction. A normal solvent isotope effect measured for the GGT deacylation reaction is consistent with the transfer of a proton in the deacylation rate-determining step.[6] Furthermore, Keillor et al. reported that the rat kidney GGT acylation step is a general-acid-catalyzed mechanism coupled with nucleophilic attack where breakdown of the tetrahedral intermediate limits the reaction rate.[39] According to the principle of microscopic reversibility, the rate-determining transition state for deacylation would be tetrahedral formation by general base catalysis.[6] The Brønsted data therefore are consistent with nucleophilic bond formation that is significantly more advanced than proton abstraction (Figure 1B).

Guinea Pig Liver Transglutaminase

The guinea pig liver transglutaminase (GPL-TGase) Brønsted values predict a transition state that involves a significant level of proton abstraction during carbon−nitrogen bond formation. Transglutaminases (TGase, EC 2.3.2.13) post-translationally modify glutamine-containing peptides by forming protease-resistant isopeptides with a variety of primary amines.[20,40,41] TGases are classified in the same family (amine γ-glutamyltransferases) as cysteine proteases due to the similarity in their catalytic mechanisms and active site organizations.[40,41] In fact, the transamidation reaction has been described as the reverse mechanism of cysteine protease proteolysis.[40,42] The strict TGase acyl donor specificity for peptidyl glutamine residues is not well understood.[41] The primary amines that act as TGase acyl acceptors are either the ε-amino group of a peptide lysine residue or a broad range of low-molecular weight polyamines.[41] In the absence of an amine, water can also act as the acyl acceptor to deaminate glutamine and form glutamic acid.[40,41]

Unlike the other transacylases, GPL-TGase deacylation rate constants increase with increasing amine acidity. Keillor et al. measured the catalyzed aminolysis of the thiolester acyl-intermediate by alkyl primary amines (pK_a range 5.6−10.5) under steady-state conditions where deacylation is rate-limiting. When $\log(k_{cat}/K_m)$ versus the amine pK_a was plotted, a linear correlation with a negative slope ($\beta_{nuc} = -0.37$) was observed for the entire amine substrate series (Figure 4). The fact that a linear correlation was only obtained for the plot of $\log(k_{cat}/K_m)$ suggests that the deacylation rate is a combination of the rate of amine (acyl-acceptor) binding and nucleophilic attack.[20]

At first glance, a negative β_{nuc} value implies that a negative charge develops on the amine in the transition state, but this is unlikely given the high pK_a of a primary or secondary amine. Keillor et al. interpreted their GPL-TGase Brønsted data as evidence for significant amine deprotonation during rate-limiting nucleophilic attack consistent with a general base deacylation mechanism.[20] This would be consistent with a slope near zero, but does not explicitly explain the observation of a negative slope. While the slope of the k_{cat}/K_m data was negative, a plot of the more relevant k_{cat} data was rather scattered but trended toward a slope of zero. The substituents used to create the reactivity series may have increased binding interactions between the substrate and the enzyme. Such changes in substrate affinity are evidenced by the varying K_m values. While the contribution of these differential binding interactions on the reported negative Brønsted value is difficult to determine, the general observation is the same: slopes near zero can provide evidence of significant deprotonation during the reaction.

Arylamine *N*-Acetyltransferases

Arylamine *N*-acetyltransferase (NAT, EC 2.3.1.5) data demonstrate that negative Brønsted values are not representative of all enzyme-catalyzed thiolester aminolysis reactions. NATs are responsible for the metabolism of xenobiotics including the bioactivation of prodrugs and cellular detoxification in many organisms.[43] Similar to transglutaminases, NATs have a highly conserved Cys-His-Asp catalytic triad that is structurally analogous to the cysteine protease family.[44] NATs catalyze an acetyl group transfer from acetyl-CoA (AcCoA, donor substrate) to the terminal amine for a broad variety of arylamines, arylhydroxylamines and arylhydrazines (acceptor substrates). In vitro, *p*-nitrophenyl acetate and aniline derivatives have also been demonstrated to be an efficient acetyl donor substrate.[45] Many of the details for the NAT acetyltransferase mechanism are reminiscent of the other transacylases reviewed where the active site nucleophile (cysteine) is acetylated to form an acetyl−enzyme intermediate, which is then deacetylated by a second substrate to form a transacetylated product and free enzyme.[8,43,44]

Results from two different deacylation NAT Brønsted studies both displayed reactivities that correlate well with nucleophile basicity for weakly basic anilines (pK_a less than ~5), but displayed no correlation for more basic amines (pK_a greater than ~5). Riddle and Jencks performed the first study with

pigeon liver NAT (PL-NAT) and determined that $\beta_{nuc} = 0.6$ for the arylamine derivative (pK_a range 1.74−3.98) aminolysis of the thiolester acetyl−enzyme intermediate (Figure 4).[8] Thirty-five years later, a $\beta_{nuc} = 0.8$ was reported for the recombinant hamster NAT (rH-NAT) catalyzed deacylation reaction with arylamine derivatives and water (pK_a range −1.7 to 4.67) acting as the donor substrate (Figure 4).[7] A normal solvent kinetic isotope effect established that the rH-NAT deacylation reaction is dependent upon general-base catalysis that was assigned to the histidine residue of the catalytic triad.[7]

In both NAT Brønsted studies, deacylation rate constants for more basic aniline donor substrates were measured, but both Brønsted plots displayed a nonlinear deviation for compounds above p$K_a \approx 5$.[7,8] The break in linearity was attributed to a change from rate-determining deacylation to enzyme acylation due to the increased reactivity of the anilines. Above p$K_a \approx 5$, the more reactive anilines attack the acetyl−enzyme intermediate faster than the rate of acetylation; therefore acylation becomes limiting.[8] The deacylation transition state predicted by the NAT Brønsted data is more similar to the transition state proposed for RK-GGT. The positive β_{nuc} values (0.6 and 0.8) indicate that significant positive charge is localized on the nucleophile during the rate-determining transition state. Similar to RK-GGT, the authors interpreted these values as evidence for minor general-base catalyzed proton abstraction coupled to significant nucleophilic bond formation.[7,8] The minor variation in the β_{nuc} values demonstrates that PL-NAT has deprotonated the amine in the transition state to a greater extent than rH-NAT(Figure 1B).

Ribosomal Peptidyl Transferase

The ribosome-catalyzed peptidyl transferase (PT) reaction is responsible for protein synthesis in all living organisms.[46,47] The PT reaction is the transfer of an acyl group (RC(O)) from an ester oxygen to an amine or to water. In this context, the P-site substrate (peptidyl-tRNA) represents the acyl−enzyme intermediate and the α-amine of the A-site substrate (aminoacyl-tRNA) is the acyl acceptor. The PT mechanism is still unresolved so a Brønsted analysis provided useful information for resolving this important mechanistic question.[5]

Ribosomal Brønsted studies demonstrate that PT reactivity is not correlated to nucleophile basicity. Puromycin is a nucleotide analog that is used extensively in ribosomal assays in place of the aminoacyl-tRNA because binding of the full tRNA substrate is rate-limiting. We synthesized a series of puromycin derivatives (A-site substrates) with varying α-amine pK_a's (<5 to 7.2).[5,48] With this series of substrates we determined that β_{nuc} on both 50S and 70S ribosomes is near zero ($\beta_{nuc} = 0.06$ and −0.06, respectively).[5] This suggests that the ribosome is promoting a transition state unlike that observed in solution. ^{15}N-KIE results provide evidence for an early PT transition state where N−C bond (A-site α-amine nitrogen−carbonyl carbon of P-site ester) formation is rate-limiting.[49] Such a transition state could be achieved if the degree of N−C bond formation is commensurate with the degree of amine deprotonation[5] similar to the transition state predicted for acetyltyrosyl−chymotrypsin (Figure 1D).[4]

The ribosomal β_{nuc} value suggests that the ribosome-catalyzed reaction is facilitated by amine deprotonation. The ribosome may increase the reactivity of the α-amine nucleophile and the O3′-leaving group by preorganization of the active site.[50−52] The increased reactivity of both groups resulted in a different rate-determining step than the one observed for less reactive nucleophiles and leaving groups.[14,16] Therefore, the ribosome PT active site may lower the energy barrier for proton transfer such that it is no longer rate-limiting. This is consistent with proposed "proton shuttling" mechanisms.[51,53−56] The catalytic importance[57] and physical proximity of the A76 2′-OH of the peptidyl tRNA suggests that it is involved in a proton shuttle between the α-amine and other groups within the active site.[53,54,58]

General Acyltransferase Transition State Features

The unifying theme for all of the reviewed acyltransferase deacylation reactions is that nucleophilic bond formation is coupled with a proton abstraction.[9] Although a common theme exists among the enzymatic mechanisms, the degree of coupling between the two bonding events varies between enzymes and leads to a range of nucleophile charges in the different transition states. The degree to which the proton is abstracted in relationship to nucleophilic bond formation is evidenced in the β_{nuc} data (Figure 1).

BIOGRAPHICAL INFORMATION

David A. Kingery received his B.S. from Rutgers University and his Ph.D. from Yale University in the laboratory of Scott A. Strobel. He is currently a research biochemist at the U.S. Army Medical Research Institute for Infectious Diseases. His research interests include mechanistic enzymology.

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FOOTNOTES

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