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A Thermochemical Study of the Hydrolysis of Urea by Urease

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Abstract: Hydrolysis products of the urea–urease system have been deduced from thermochemical measurements. Two distinct sets of products are found which depend upon the buffer used. Classical products (HCO_3^- and NH_4^+) are found in phosphate (pH 7.5 and 6.7) and maleate (pH 6.7) buffers. Ammonium carbamate in virtually quantitative yields is obtained in citrate (pH 6.7) and Tris (pH 7.5) buffers. The possible effect of these two sets of products on previous kinetic and thermodynamic studies is discussed in terms of buffer effects.

"Buffer effects" on the hydrolysis of urea catalyzed by crystalline urease (EC 3.5.1.5) are well known. These effects include the variation of the pH of maximum activity,[1] differing relative maximum activities,[1] varying susceptability to substrate inhibition,[2,3] and differences in activation energy data.[2,4] To date, the exact nature of the buffer contribution to these effects is not completely understood. Indeed, it is thought by some[5] that these effects arise from the method of enzyme preparation, or the presence of several isoenzymes.

This report concerns a thermochemical study of the hydrolysis products of urea. The results show that the product formed is dependent upon the buffer used, and this information is helpful in elucidating some of the above "buffer effects."

Theory

Heats of reaction may be determined calorimetrically by measuring the temperature change due to a chemical process. A knowledge of the moles of product formed (n_p) and the heat capacity of the system (k) yields ΔH via eq 1.

$$\Delta H = -\Delta T(k)/n_p \quad (1)$$

For the hydrolysis of urea, there is sufficient thermochemical data available to calculate theoretical heats of reaction, at standard state ($\Delta H°$). Reactions 2–4 summarize the processes involved in forming the classical products in approximately neutral solution. Notably, reactions 3 and 4 are pH dependent. From data on heats of formation,[6] the heat of reaction for process 2(ΔH_2) is found to be +7.29 kcal/mol urea. The heat of reaction of processes 3 and 4 ($\Delta H_{3,4}$) is given by eq 5. In this expression, K_1, K_2, and K_b represent the ionization constants for H_2CO_3, HCO_3^-, and NH_3, respectively. The ΔH values are for the ionization of a single proton from the subscripted species,[7] and it is assumed that there is always a sufficient amount of buffer present so that the pH change is virtually zero. Figure 1 illustrates the variation of the overall theoretical ΔH ($=\Delta H_2 + \Delta H_{3,4}$) with pH in phosphate buffer. This is a composite of three curves where the buffer systems are H_3PO_4–$H_2PO_4^-$, $H_2PO_4^-$–HPO_4^{2-}, and HPO_4^{2-}–PO_4^{3-}, and the appropriate ΔH_{buffer} was used for each. The overall curve represents the joining of these three segments.

$$H_2O(l) + \text{urea(aq)} \longrightarrow CO_2(aq) + 2NH_3(aq) \quad (2)$$

$$H^+(aq) + CO_2(aq) + 2NH_3(aq) + H_2O(l) \longrightarrow 2NH_4^+(aq) + HCO_3^-(aq) \quad (3)$$

$$H^+\text{buffer(aq)} \longrightarrow \text{buffer(aq)} + H^+(aq) \quad (4)$$

$$\Delta H_{3,4}(\text{kcal/mol urea}) = \frac{[H^+]K_1(\Delta H_{H_2CO_3} - \Delta H_{buffer}) + K_1K_2(\Delta H_{H_2CO_3} + \Delta H_{HCO_3^-} - 2\Delta H_{buffer})}{[H^+]^2 + [H^+]K_1 + K_1K_2} + \frac{2[H^+](\Delta H_{buffer} - \Delta H_{NH_4^+})}{[H^+] + K_w/K_b} \quad (5)$$

The above discussion and results are valid as long as all of the carbon dioxide produced is capable of being aquated. If the solution is saturated with CO_2 and its hydrolysis products before the reaction is initiated, then CO_2(aq) is replaced by CO_2(g) in eq 2. The primary effect of this is to change ΔH_2 to a value of +11.98 kcal/mol urea, while the pH-dependent expression 5 loses the first term involving CO_2 and its hydrolysis products. The dashed curve in Figure 1 illustrates the results expected in this situation. If the solution *becomes saturated* with CO_2 during the course of the hydrolysis, the ΔH observed will lie somewhere between these two extremes.

In addition to the classical products, it has been shown that ammonium carbamate is a product of the hydrolysis of urea in alkaline, unbuffered solutions.[8,9] Reaction 6 sum-

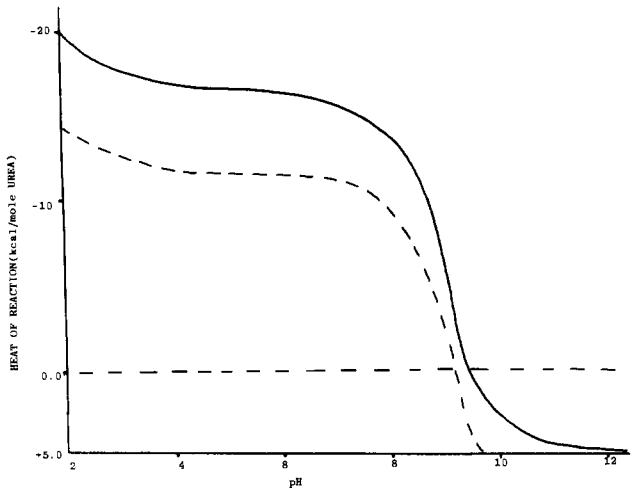

Figure 1. pH dependence of the theoretical ΔH, calculated for phosphate buffer and products related to NH_3 and CO_2. The dashed curve is obtained if the system is saturated with CO_2. Other buffers have similar type curves.

marizes this process. The heat of reaction calculated from

$$H_2O(l) + \text{urea}(aq) \longrightarrow H_2NCOO^-NH_4^+(aq) \quad (6)$$

available data[6] is −5.8 kcal/mol urea. Over the pH range of interest in this work (6.7 to 7.5), there is little protonation of the carbamate or ionization of the ammonium ion which would cause the observed ΔH to vary much from −5.8 kcal/mol. This lack of ionization means that there is virtually no contribution to the ΔH from ionization processes of the buffer. Therefore, the heat of reaction observed will be *independent of the buffer used.*

The large differences in the calculated ΔH values for the two possible processes allows for unambiguous interpretation of the results in most cases. In one particular case, this is not possible and it was found that results in mixed buffers must be utilized for the interpretation of the data. Plots of ΔH observed versus buffer fraction were found to be the most useful form for evaluating and presenting data. Buffer fraction (f) is defined as the fraction of the total buffer concentration which is made up of the buffer with the lowest pK_a (buffer 1 in eq 7). The concentrations refer to the total

$$\text{buffer fraction} = f = \frac{(\text{buffer 1})}{(\text{buffer 1} + \text{buffer 2})} \quad (7)$$

analytical concentrations of the buffers. For a reaction which involves the ionization or protonation of a buffer, a linear change in ΔH as a function of buffer fraction will be observed provided that the pH of analysis is $\frac{1}{2}pK_1 + \frac{1}{2}pK_2$. Since reaction 6 does not involve the buffer while 2-4 do, they may be readily distinguished from each other (vide infra). Plots of ΔH versus buffer fraction will therefore allow for the elucidation of products when the ΔH values are inconclusive by themselves.

Experimental Section

Chemicals. Urease, equivalent in purity to the first ammonium sulfate precipitation after dialysis in the purification method of Hanabusa,[10] was used for the majority of this work. Additionally, to be certain that large quantities of extraneous protein did not interfere, a rather highly purified form of urease corresponding to the first dissolution of crystals with mercapto ethanol as given by ref 10 was used. As expected, there was no difference, within experimental error, between the two preparations. Starting materials were obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Urease solutions were prepared by suspending an arbitrary amount of solid material (approximately 1 g) in 40 ml of buffer, allowing the heavier particles to settle, and then using the cloudy supernatant. It is estimated that each experiment involved approximately 10-20 Sumner units of urease.

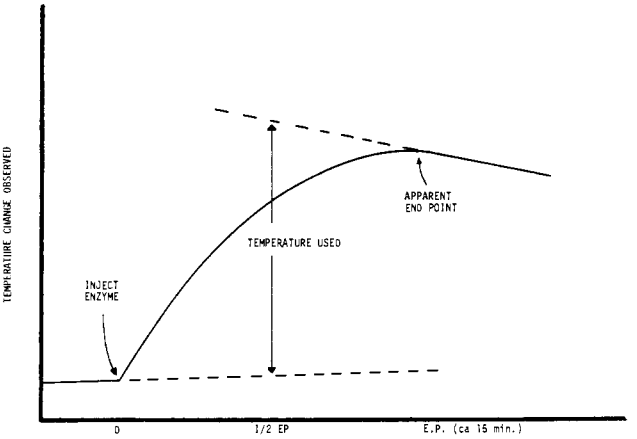

Figure 2. Extrapolations performed to obtain overall temperature changes (ΔT).

The urea used was the "absolute" grade from Research Plus Labs. Denville, N.J. Solutions for study were prepared by weighing 0.3 g to the nearest 0.1 mg and dissolving it to 100 ml with the appropriate buffer mixture. More dilute solutions were prepared from quantitative dilutions of this stock solution. The pH was rechecked after preparation and never needed adjusting. These solutions, as expected, were stable over time spans of up to 2 weeks as evidenced by the consistency of the results.

Buffer solutions were prepared by dissolving 0.50 mol of the relevant acid or base in distilled water. They were then neutralized to the desired pH with HCl or NaOH, and diluted to 1 l. All materials were reagent grade except the maleic acid which was prepared from the purest maleic anhydride available by hydrolysis. In that case, 0.25 mol of maleic anhydride was used. Mixed buffers were prepared by mixing the above buffer solutions in the desired ratio. The pH was checked after mixing and needed no adjusting.

Ammonium carbamate (obtained from Pfaltz and Bauer Co., Flushing, N.Y.) solutions were prepared by dissolving a weighed amount in the desired buffer or distilled water. The pH necessarily had to be readjusted after this process indicating some decomposition occurred upon dissolution. Due to the instability and the imprecisely known concentration of ammonium carbamate, these solutions were used only qualitatively.

Apparatus and Methods. The experimental approach was simple. Isothermal solutions of urease and urea were mixed in a quasiadiabatic cell and the temperature change was monitored until the reaction was complete. Instrumentation for these experiments has been previously described,[11] the only alteration being the use of a syringe (rather than a syringe pump) for the rapid addition of enzyme suspension to the substrate.

Methodologically, all experiments involved the following steps. The two solutions were thermostated so that their temperature difference was less than 1% of the temperature change due to the hydrolysis reaction. After thermostating, 0.5 ml of the buffered urease solution was rapidly (ca. 0.5 sec.) injected into 5.00 ml of the buffered urea solution. (The reverse approach of injecting urea into urease failed to give good results due to the imprecision of the injected volumes). After injection, the reaction was then allowed to go to completion, the criteria for determining that equilibrium had been reached being the attainment of a linear temperature versus time region. After this, the sensitivity of the detection system was determined by classical electrical calibration methods.

To ascertain the magnitude of the heats of dilution of the reagents, buffered urease was injected into the buffer solution (without urea) and buffer (without urease) was injected into the urea solutions. The effects were found to be negligible.

Data were evaluated in the following manner. The post reaction region and the initial base line were usually not identical in slope except for very small temperature changes. This indicated the existence of some heat leakage from the cell. All curves were corrected for this heat leak by extrapolating the base line and the post reaction line as shown in Figure 2. The temperature change midway between the start and the apparent end point (Figure 2) is taken as the overall temperature change. Due to the typically small slopes,

Table I. Heats of Reaction Determined in Tris and Phosphate Buffers[a] at 25° and pH 7.5

Urea concn, m*M*	ΔH_{Tris}, kcal/mol urea[b]	$\Delta H_{phosphate}$, kcal/mol urea[b]
100	−4.27	−14.60
50	−4.70	−14.83
25	−4.40	−14.76
5	−4.42	−14.54
Experimental mean[c]	−4.47 ± 0.15	−14.65 ± 0.19
Theoretical[d]	−4.03	−14.66
Theoretical[e]	−5.8	−5.8

[a] Buffer concentration = 0.50 *M*. [b] 1 cal = 4.184 J. [c] Differing numbers of runs at each concentration gives a mean slightly different from the above values. [d] Assuming products of NH_4^+ and HCO_3^-. [e] Assuming a product of $H_2NCOONH_4$.

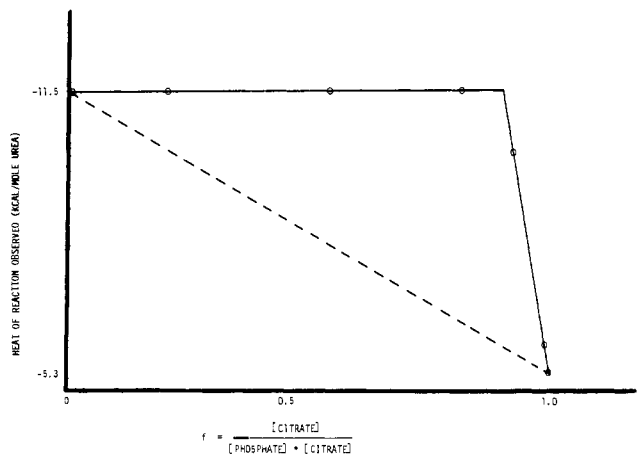

Figure 3. Plot of ΔH observed versus buffer fraction for the mixed citrate, phosphate buffers at pH 5.7. The solid line connects the experimental points while the dashed line would be obtained if the products in both buffers were the same.

an error in the placement of this point usually results in an insignificant change in the calculated ΔH. (The slopes in the figure are magnified for clarity.) This treatment tacitly assumes that the heat leaks are zero for the first half of the curve and that they are the maximum for the second half. Reproducibility of the data indicates that this is a fair approximation of the integral heat leak over the entire time course of the experiments. Once the overall temperature change is known, along with the urea concentration of the heat capacity of the cell (k), the heat of reaction in terms of moles of urea hydrolyzed is readily calculated from the equation

$$\Delta H(\text{kcal/mol urea}) = -\Delta T(k)/[\text{urea}](5.00) \quad (8)$$

In the present methodology, ΔT is expressed in recorder chart divisions and the heat capacity in calories/chart division, 5.00 in eq 8 represents the initial volume of the urea solution in milliliters.

Results

Table I lists the results obtained at pH 7.5 for the hydrolysis of urea in phosphate and Tris buffers. Table II gives the results obtained from the maleate, citrate, and phosphate buffers at pH 6.7. The pH change was necessitated in order that the buffers have sufficient capacity to react with the hydroxide ions produced at the highest concentrations of urea. All results in these tables are the average of between two and five determinations, while the relative average deviation for all results is consistently around 1%. As will be seen later, this small imprecision has no bearing at all on the interpretation of the results.

Results from the mixed buffer experiments are more conveniently presented in graphical form. These are plots of ΔH observed versus the buffer fraction (f). For the two systems used (i.e., Tris and phosphate; phosphate and citrate) f is given by eq 9 and 10. In the above expressions, the denominator is always 0.50 *M*. Figure 3 gives the results for the citrate–phosphate system at pH 6.7 while Figure 4 gives the results for Tris–phosphate at pH 7.5. The pH values used are dictated by the pK_a values of the buffer pairs as discussed earlier.

Table II. Heats of Reaction Determined in Phosphate, Maleate, and Citrate Buffers at 25° and pH 6.7

Buffer (0.50 *M*)	ΔH observed, kcal/mol urea	ΔH,[a,b] kcal/mol urea	ΔH,[c] kcal/mol urea
Phosphate	−11.53 ± 0.21	−14.97	−5.8
Maleate	−13.54 ± 0.18	−17.29	−5.8
Citrate	−5.24 ± 0.14	−17.26	−5.8

[a] Assumed products are NH_4^+ and HCO_3^-. [b] Add 3.99 kcal/mol urea to this column to obtain the ΔH if CO_2(g) is formed. [c] Assumed product is ammonium carbamate.

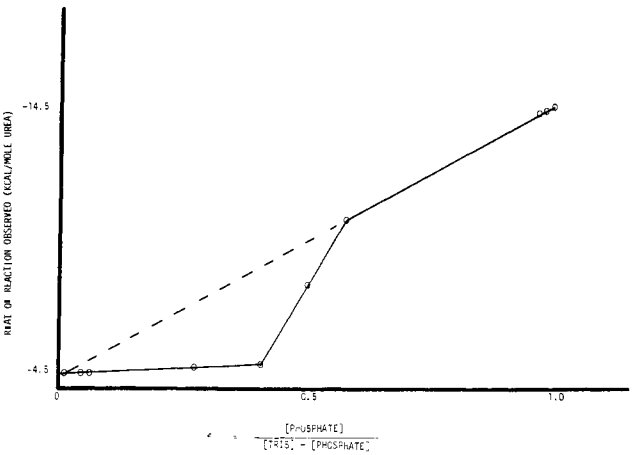

Figure 4. Plot of ΔH observed for the mixed phosphate, Tris buffers at pH 7.5. The solid line connects the experimental points while the dashed line would be obtained if the products were the same. Note the constant nature of the ΔH below F = 0.50.

$$f = C_{phosphate}/(C_{phosphate} + C_{Tris}) \quad (9)$$

$$f = C_{citrate}/(C_{citrate} + C_{phosphate}) \quad (10)$$

Additional qualitative results using ammonium carbamate are given with the discussion in support of the above quantitative results.

Interpretation of Results

Position of Equilibrium. Results in Table I confirm the well-established fact that the hydrolysis of urea catalyzed by urease is virtually complete. Indeed, over a 20-fold concentration range, there is essentially no change in the ΔH measured for both Tris and phosphate. The other buffers exhibit similar behavior although a smaller concentration range was used. These results are important from the standpoint that they show that the reaction proceeds with no evidence of *complete* inhibition by-products. Therefore the different ΔH values calculated are not due to an incomplete reaction. Additionally, it must be emphasized that due to the high ionic strength of the medium, these heats of reaction are *not* standard heats of reaction ($\Delta H°$).

Reaction Products. Results given in Tables I and II may be compared with the theoretical heats of reaction which were calculated for the two possible sets of reaction products. It is observed that phosphate and maleate buffer yield the classical products of bicarbonate and ammonium ions. It is also apparent that, at the lower pH, the phosphate solution used was essentially saturated with CO_2 before starting while the one at pH 7.5 was not. The ΔH observed for citrate buffer corresponds nicely to the formation of ammo-

nium carbamate, while the products of Tris buffer are not distinguishable from the ΔH values alone. The product in Tris buffer may be deduced from the shape of the curve in Figure 4. Here it is seen that there is a nonlinear relationship between ΔH and buffer fraction. Indeed, while Tris is in excess, there is virtually no change in the ΔH observed. This clearly indicates that the reaction does not involve the buffer, as would be expected in the formation of ammonium carbamate.

Effect of Phosphate. Figure 5 is a composite of two qualitative experiments involving ammonium carbamate and phosphate. The major portion depicts the curve obtained for the enzymatic reaction in citrate buffer; after that reaction was complete, 0.5 ml of 0.5 *M* phosphate buffer at pH 6.7 was injected into the mixture. The sharp rise in temperature is consistent with the decomposition of ammonium carbamate but not with the heat of dilution or ionization of the phosphate, both of which are endothermic. Indeed, the heat liberated is almost quantitatively the same as would be expected if the products in citrate buffer were bicarbonate and ammonium ions. The inset to Figure 5 represents the injection of a similar aliquot of phosphate into an ammonium carbamate mixture, without the enzyme or buffer, at a pH of approximately 7. The similarity between the two injections is obvious. Injection of a similar aliquot of phosphate into the products in Tris buffer yields no temperature change of any significance as the results in Figure 4 predict.

The above observations along with the data in Figures 3 and 4 elucidate a possible mode of action of the phosphate. It is seen in Figures 3 and 4 that there are regions where the ΔH observed is apparently due to the formation of a mixture of the two possible sets of products. This may be due to a required stoichiometric reaction between phosphate and ammonium carbamate (as opposed to a catalytic effect). However, while this explains the data in Figure 3 adequately, it does not hold true for Figure 4. The most plausable explanation encompassing both figures is that the ammonium ion, or the Tris buffer (an ammonium ion analog), deactivate the phosphate in its capacity to decompose ammonium carbamate. Indeed, it seems to be necessary to have at least 1 mol of phosphate for every mole of ammonium-type ions produced, and already present, or the result is a mixed product in terms of the heat evolved. In light of this behavior, a reasonable explanation (although not the only one) of the role of phosphate is that a fairly strong interaction between it and the ammonium ion, to form an ammonium phosphate anion, removed the NH_4^+ from the ammonium carbamate, allowing the carbamate ion to decompose rapidly, in the presence of 10^{-7} *M* H^+.

Qualitative Determination of Products. A relatively easy test to determine if the product of the hydrolysis of urea will result in the classical products or ammonium carbamate has evolved from this work. It involves the simple addition of solid ammonium carbamate to the buffer solution of interest. In phosphate and maleate, solid ammonium carbamate decomposes on contact with the buffer, yielding carbon dioxide and ammonia in a violent frothing reaction. In Tris, citrate, and pure water, solid ammonium carbamate seems to dissolve quite readily with no obvious signs of violent decomposition. Since it is the buffer which governs the *final* product, via a nonenzymatic process, this test dramatically shows which set of products to expect.

Discussion

Perhaps the most important feature of this study is the demonstration of the virtually quantitative formation of ammonium carbamate in approximately neutral solutions in the presence of some buffers. This has never been shown

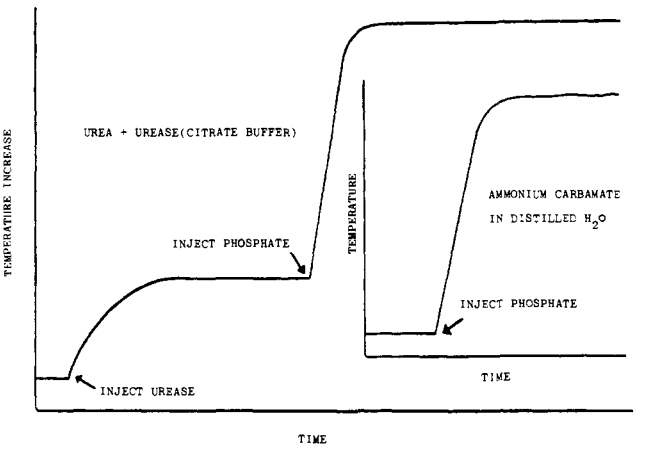

Figure 5. Qualitative results from the reaction of ammonium carbamate with phosphate. In the major curve, ammonium carbamate is produced enzymatically in citrate buffer. The inset shows the reaction of phosphate with ammonium carbamate which was prepared by dissolving the solid compound in distilled water (no enzyme present). Time scale for the enzymatic portion of the figure is in minutes while it is in seconds for the curves produced by the injection of phosphate.

previously, although the data of Wang and Tarr[12] and Hanss and Rey[13] may be interpreted in a manner which supports these findings. Ammonium carbamate has been demonstrated by others[12,9] to be an "intermediate" in the classical reaction; the above results add another piece of solid evidence to this interpretation. Indeed, considering the pH used in this work, and the quantitative yield of ammonium carbamate, no other conclusion is possible.

Results from the effects of phosphate on ammonium carbamate indicate that the term "intermediate" is incorrect and that it should be changed to "product." Decomposition of this product to ammonia and carbon dioxide has been shown to be independent of the presence of the enzyme, but dependent upon the buffer present, particularly in the case of phosphate.

The two sets of products found have interesting characteristics which may have had significant effects on kinetic studies of this reaction. In phosphate and maleate buffers, the "classical" products are evolved, particularly the ammonium ion. In citrate and Tris buffers, ammonium carbamate is formed where the *maximum* amount of ammonium ions produced is half that in the other two buffers. Indeed, it is quite possible that the free ammonium ion concentration in the citrate and Tris buffers is even lower due to the apparent necessity of a rather strong interaction of NH_4^+ with the carbamate anion in order to achieve stability. The importance of ammonium ion concentration is seen in the results of Hoare and Laidler[14] where it was found that 0.2 m*M* concentrations will inhibit crystalline urease by approximately 10%. Many buffer effects may be shown to arise in situations where the concentration of ammonium ions greatly exceeds this value.

Perhaps the clearest evidence for the effect of products (and therefore buffer) on reaction kinetics is found in the studies of Howell and Sumner.[1] They show that the maximum activity of urease in citrate buffer is consistently 20–30% greater than it is in the other buffers studied. Fortunately, they give sufficient information on their experimental methods to calculate that the concentration of ammonium ions in their reaction mixtures was on the order of 1 m*M* or more. Thus, their results are a reflection of a much greater inhibition of urease in buffers other than citrate, where greater amounts of NH_4^+ are expected. The maximum activity of urease in Tris may be compared to that in phosphate[2,4] and a similar increase is found. Although it

Table III. Relationship between Buffer pK_a and pH of Maximum Urease Activity

Buffer	pK_a	pH_{max}
Phosphate	7.21	6.7–7.6[a]
Citrate	6.40	6.5–6.7[a]
Acetate	4.74	6.4–6.7[a]
Tris	8.08	~8.0[b]
Maleate	6.59	~6.5[c]

[a] See ref 1. [b] See ref 2. [c] Estimated from G. B. Kistiakowsky, et al., *J. Am. Chem. Soc.*, 74, 5015 (1952).

may be argued that the differences in relative reaction rates are due to inhibiting or activating properties of the buffers themselves, there is no corresponding evidence reported which would show this to be the case. Indeed, small differences in maximum activities do exist. However, two groups of buffers are readily recognized, and differentiated, on the basis of the products of the reaction.

It is not possible to rationalize, on the basis of ammonium ion inhibition, the change in the pH of maximum activity with the buffer which was also observed by Howell and Sumner.[1] It is, however, of interest to note that this maximum usually occurs at a pH which is close to the pK_a of the buffer. Since the product of the concentration of the acid and basic forms of the buffer is at a maximum at this pH, it is possible that this may be a unique case of a *simultaneous* general acid, general base catalysis. Acetate "buffer" is one anomality in this consideration; however, using its pK_a and the pH at which the maximum is observed, it is obvious that the solution used is by no means a buffer at pH 7, therefore, proton transfer must depend on the solvent rather than the buffer. Table III shows this correspondence clearly.

There is one last buffer effect which must be commented upon in light of its apparent significance in the urease literature over the past 20 years. This involves the observations that the measured activation energies in phosphate[4] and Tris[2] buffers differ significantly. Not only are the reported magnitudes of the activation energies different, but in Tris buffer, the activation energy increases with urea concentration while in phosphate buffer it decreases. Table IV summarizes these data. This along with the characteristic rate versus urea concentration plots for the two buffers has been used to suggest a direct interaction of one or both of the buffers with urease.[15]

Two points are of interest in explaining the above data. First, the present results indicate that reactions run in phosphate buffer will produce at least twice as much NH_4^+ as the Tris buffer, due to the difference products. Second, the experimental methodology[3] used in obtaining the data for Table IV reveals that inhibiting levels of ammonium ions were almost certainly present. Using the information presented,[3] it is possible to estimate that the inhibiting concentrations of NH_4^+ (0.2 m*M*)[14] are reached after 60 sec in the most dilute urea solutions and after only 2 sec in the most concentrated solutions. Another calculation using the molar absorptivity of the Nessler's complex (approximately 3500 l. mol^{-1} cm^{-1}) and an assumed 1-cm cell leads to the conclusion that the reacting systems had inhibiting levels of ammonium ions present whenever the absorbance was above 0.07 units. If the absorbances determined were always below 0.07, then a very large instrumental error exists in the analyses.[16] Data obtained under conditions where inhibition is present will necessarily lead to inaccurate results if extrapolations to zero time are made as suggested by these authors.

As a consequence of the above, it may be deduced that the rate versus urea concentration curves in phosphate buffer should show the effects of this inhibition. Indeed, this is the case; severe curvature of these plots is one indication.

Table IV. Activation Energies for the Hydrolysis of Urea in Tris and Phosphate Buffers

Urea concn, mol/l.	Phosphate buffer[a]		Tris buffer[b]	
	pH 6.2	pH 6.8	pH 7.1	pH 8.0
0.005	12.4	12.5	6.2	7.9
0.250			9.1	10.5
0.299	6.7	6.1		
1.496	5.9			

[a] See ref 4. [b] See ref 3.

Since the concentration of ammonium ions present will be somewhat proportional to the urea concentration, at least for the ascending portion of the curve, it is not at all surprising that these effects will be masked by the well-known urea inhibition of the process. However, the effect of ammonium ions is evident in a small region of the curve presented which does not fit the postulated equations. This deviation has been ascribed to the "failure of the Langmuir isotherm owing to the strong electrostatic forces involved." [3] A more consistent interpretation in this case would be that the concentration of ammonium ions was so great at this particular portion of the curve that the reaction period had to be decreased in order to avoid precipitation of the Nessler complex. This reduction in time would reduce the ammonium ion concentration and concomitantly increase the apparent rate measured, giving the distorted curve.

It is apparent that inhibition by NH_4^+ has played a large part in the shape of the rate versus urea concentration curves in phosphate. It also would have a correspondingly large effect on the activation energies measured. In particular, it will raise the activation energy. The upper limit of this increase in activation energy is equal to $-\Delta H$ for reaction 11. There are several ways to obtain an estimate for

$$\text{urease} + NH_4^+ \longrightarrow \text{urease-}NH_4^+ \qquad (11)$$

this ΔH assuming that the results in Tris buffer are for the noninhibited reaction. The first estimate is obtained from the difference between the phosphate and Tris buffers at low urea concentrations and a value of −5.4 kcal/mol is obtained (see Table IV). A second possibility is that the difference between the results in high and low urea solutions should be the same. In that case, a heat of reaction of −8.2 kcal/mol is obtained. It is of interest that this latter value approaches the heat of reaction expected between ammonia (NH_3) and a carboxylic acid group to yield ammonium and carboxylate ions.

The above treatment completely ignored the effect of ammonium ion inhibition on the activation energy of urease in phosphate buffer at high urea concentrations. The reason for doing so is embodied in the observations that crystalline urease seems to be more susceptible to ammonium ion inhibition at low urea concentrations.[17] This would suggest that systems high in urea concentration would be inhibited by urea and not NH_4^+, both species utilizing the same site for inhibition.

The above evaluation was based solely upon previously reported data[2-4] and the results reported here. Additional evidence does exist, to support this interpretation, in the data presented by Kistiakowsky and Rosenberg.[18] Unfortunately, these authors placed little emphasis upon these results, and as a consequence the apparent activation energy differences between Tris and phosphate buffers in the urease system have become an established fact by default. Briefly, their data may be used to calculate the activation energies in the phosphate system over a range of urea concentrations from 0.3 m*M* to 1.0 *M*. Their analytical methods avoid the problems of ammonium ion inhibition while the admitted impurity of their urease also has the effect of

reducing ammonium ion concentrations. The calculated activation energies are 7.5 kcal at 1.3 mM urea and 9.5 kcal at 1.3 M urea. These correspond very well with the data obtained in Tris buffer, both in terms of magnitude and the direction in which they change.

Summary

The products of the hydrolysis of urea catalyzed by urease have been determined calorimetrically in four buffer systems. Two of these (phosphate and maleate) yield the classical products NH_4^+ and HCO_3^-, while the other two (citrate and Tris) give an almost quantitative yield of ammonium carbamate.

Additional, qualitative studies show that the formation of the classical products from ammonium carbamate is a nonenzymatic process dependent solely upon the nature of the buffer. This reinforces the ammonium carbamate concept (especially considering the low pH at which the results were obtained) and also suggests that rather than being an intermediate, ammonium carbamate is the true product of this enzymatic process.

Several buffer effects are discussed. Two are found to be readily explained on the basis of ammonium ion inhibition. The third, involving the change in optimum pH with buffer, is perhaps the only true buffer effect, involving a unique example of simultaneous general acid and general base catalysis.

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Luminescence and Simplified Photophysics of Methylglyoxal

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Abstract: Steady state spectra and time-resolved luminescence decays have been observed for methylglyoxal. Absorption spectra ascribed to the $^1A''(^1A_u^1) \leftarrow {}^1A'(^1A_g^1)$ transition have been described and the 0-0 band assigned to be at either 22,260 or 22,090 cm^{-1}. Several vibrational progressions in ~270–280 cm^{-1} have also been observed. The emission spectra indicate the presence of the $^3A''(^3A_u^1)$ state approximately 2400 cm^{-1} lower in energy from the $^1A''(^1A_u^1)$ level. A simple kinetic scheme is presented which explains the behavior of the relative intensities of fluorescence and phosphorescence as well as the luminescence decay behavior for methylglyoxal pressures above ~2.0 Torr. Below 2.0 Torr more complicated behavior may occur due to the reversible nature of the S_1–T_1 coupling.

Extensive examinations of photophysical and photochemical behavior for low-lying excited electronic states of the simple dicarbonyl molecules glyoxal[1-4] (HCOCHO) and biacetyl[5-9] [$CH_3COC(CH_3)O$] have been recently carried out. We report here absorption spectra, emission spectra, and time-resolved luminescence decays for methylglyoxal (pyruvaldehyde, CH_3COCHO) which is structurally intermediate to glyoxal and biacetyl. We also present a simplified kinetic scheme which describes the observed luminescence behavior for total pressures above ~2 Torr. Such an investigation is of considerable importance for several reasons.

(1) Only two ($^1A_u^1$, commonly denoted S_1, and $^3A_u^1$, commonly denoted T_1) of the eight low-energy n,π^* excited electronic states expected for *trans*-dicarbonyl systems have been observed and definitively assigned in gaseous glyoxal and biacetyl. The reduction of C_{2h} to C_s symmetry formally allows transitions between the ground state and the other n,π^* excited singlet states, opening the possibility for the direct spectroscopic observation of these states and thus leading to a better definition of the photophysical possibilities of the $^1A_u^1$ state.

(2) Finlayson, Pitts and Atkinson[10] have recently observed chemiluminescence from reactions of isobutene, 2-methyl-2-butene, and 2,3-dimethyl-2-butene with 2% O_3 in O_2. Part of the chemiluminescence spectrum closely corresponds to that observed in this work. A characterization of the luminescence features is necessary for the reliable interpretation of such photochemical data and for reliable product identification.

(3) Recent photophysical experiments on biacetyl and glyoxal have revealed a number of striking differences in behavior.[1,2,6,7] Examination of corresponding behavior in methylglyoxal may provide insight concerning the changing photophysical behavior in these systems.

Experimental Section

Chemicals. Methylglyoxal (40% aqueous solution) was obtained from Aldrich Chemical Co. In order to obtain pure methylglyoxal the sample was first vacuum distilled to increase the methylglyoxal

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