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Carbon Isotope Effects on the Enzymatic Decarboxylation of Glutamic Acid[1,2]

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Abstract: Steady-state kinetic parameters and carbon isotope effects have been measured for the enzymatic decarboxylation of glutamic acid from pH 3.6 to 5.5. Over this range the maximum velocity changes by less than 50%, reaching a maximum at pH 4.4. The carbon isotope effect varies from a minimum of $k^{12}/k^{13} = 1.014$ at pH 4.0 to a maximum of 1.022 at pH 5.5. These data indicate that the energy barriers for decarboxylation and for formation of the enzyme–substrate complex are very similar. Decomposition of the enzyme–substrate complex to reform enzyme and substrate occurs with general base or general acid–specific base catalysis.

The enzymatic decarboxylation of amino acids usually requires the assistance of pyridoxal 5′-phosphate and proceeds according to the general mechanism of Scheme I.[4,5] Although this mechanism is qualitatively correct, it does not specify many important details of this sequence. Except for the binding of the coenzyme to an ε-amino group of a lysine residue of the enzyme,[6] little is known about the detailed role of the enzyme. Other catalytic groups are presumably involved, but this has not been demonstrated. As a start toward elucidation of this mechanism we have used isotope effects and steady-state kinetics to investigate the pH dependences and relative rates of the individual steps in the reaction sequence.

Carbon isotope effects have been used extensively in studies of organic reaction mechanisms.[7-11] Large isotope effects are observed only in cases where a bond to the isotopic atom is formed or broken in or before a slow step. Thus the decarboxylations of azulene-1-carboxylic acid[10] and of 4-methoxyanthranilic acid[11] show no carbon isotope effects at low acidity (protonation of the starting material is rate determining) and a "full" isotope effect ($k^{12}/k^{13} = 1.04$) at high acidity (decarboxylation is rate determining). The carboxyl carbon isotope effect on an enzymatic decarboxylation should provide information about the relative rate of the decarboxylation step in the overall reaction sequence. If decarboxylation is rate determining the reaction should exhibit a substantial carbon isotope effect.

Few carbon isotope effects on enzymatic reactions have been reported. Seltzer, Hamilton, and Westheimer[12] reported that there is no isotope effect on the enzymatic decarboxylation of oxalacetic acid, whereas the metal-catalyzed reaction shows an isotope effect (k^{12}/k^{13}) of 1.06. Studies of carbon isotope effects on the urease reaction[13] are not easily interpretable because of unexplained variations in the observed isotope effect with various enzyme preparations. Other studies[14] are deficient because of insufficient precision in the measurement of isotopic abundances.

Bacterial L-glutamate decarboxylase is a particularly suitable object for a detailed kinetic and isotope effect

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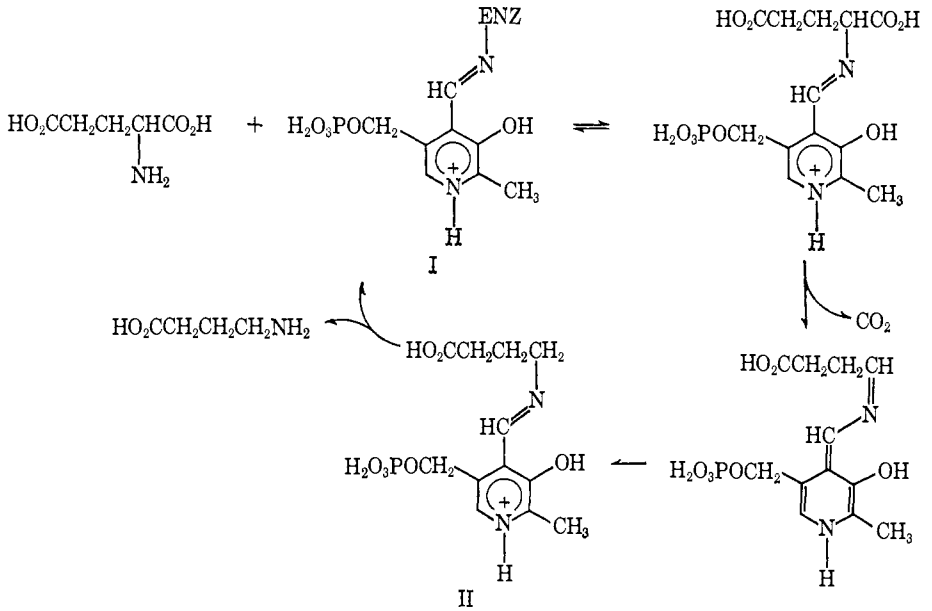
Scheme I



study. This enzyme has been extensively purified[15,16] and a number of its properties have been examined in detail.[5,6,16,17] We have previously reported[1] that the decarboxylation of glutamic acid by an enzyme purified from *E. coli* is accompanied by a carbon isotope effect of 1.0173 at pH 4.7, suggesting that decarboxylation is at least partially rate limiting. We have now extended our studies of this isotope effect to other pH's and have measured steady-state kinetic parameters for this reaction. The availability of a variety of kinetic information enables us to make conclusions about the reaction mechanism without recourse to elaborate theoretical calculations of isotope effects.

Table I. Kinetic Parameters for the Decarboxylation of Glutamic Acid at 37.0°

pH	Buffer	V_{max}[a]	K_m, mM
3.60	Isonicotinamide–HCl	14.3 ± 0.6	0.44 ± 0.06
3.80	TMAO[b]–HCl	14.2 ± 1.1	0.62 ± 0.18
4.00	Isonicotinamide–HCl	10.4 ± 0.2	0.21 ± 0.02
4.00	TMAO–HCl	13.5 ± 0.4	0.27 ± 0.05
4.40	Pyridine–HCl	23.4 ± 1.4	0.95 ± 0.18
4.90	Pyridine–HCl	21.8 ± 0.9	0.89 ± 0.11
5.20	Pyridine–HCl	19.9 ± 1.5	1.17 ± 0.22
5.40	Pyridine–HCl	17.8 ± 0.6	2.01 ± 0.17

[a] In microliters of CO_2/100 sec/20 units of enzyme. A unit of enzyme is the amount of enzyme needed to form 1 μl of CO_2/100 sec under standard assay conditions. [b] Trimethylamine N-oxide.

Results

Kinetics. Steady-state kinetic parameters for the enzymatic decarboxylation of glutamic acid at 7 pH values from pH 3.6 to 5.4 were determined by a modified manometric method. All measurements were made at 37° in 0.1 M buffer in the presence of 10^{-5} M pyridoxal 5′-phosphate. Lineweaver–Burke plots were computer calculated[18] from triplicate determinations of the decarboxylation rate at each of four substrate concentrations. Values of the Michaelis constant, K_m, and the maximum velocity, V_{max}, corrected to the same enzyme activity are given in Table I.

Isotope Effects. Carbon isotope effects on the enzymatic decarboxylation of glutamic acid were obtained by comparison of the isotopic composition of the carbon dioxide produced during the first few per cent reaction with that of the α-carboxyl group of the glutamic acid used (obtained by carrying the decarboxylation to completion and measuring the composition of the product carbon dioxide). All experiments were conducted with glutamic acid containing only the natural abundance of carbon-13. For each isotope-effect experiment two aliquots of the same substrate solution were degassed and decarboxylated with the same enzyme preparation in degassed buffer. Reactions were stopped by addition of concentrated sulfuric acid, and the product carbon dioxide was separated and purified by standard methods.

The "isotope ratios" obtained from the mass spectrometer are uncorrected decade settings for the ratio m/e 45:44 and are not themselves absolute isotope ratios. Thus, a standard sample of CO_2 gives a ratio of about 0.014150. These decade settings are directly proportional to isotope ratios and can be converted to the same scale for comparison. All samples are measured relative to a tank CO_2 standard and are converted to the constant tank standard value of 0.014150 by multiplication of the observed ratio by the factor $0.014150/R_t$, where R_t is the observed 45:44 ratio for the tank standard in that particular determination.

The observed ratios corrected to a constant reference standard must be corrected for the presence of oxygen-17 in the CO_2. The observed isotope ratio m/e 45:44 is proportional to the relative abundance ($^{13}C^{16}O_2$ + $^{12}C^{17}O^{16}O$)/$^{12}C^{16}O_2$. The oxygen-17 correction is made

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by subtracting 0.000800 from the observed ratio. The isotope effects obtained are quite insensitive to the exact value of this correction factor.

The isotope effect is calculated from the corrected isotope ratios by eq 1, where R is the corrected m/e 45:44 ratio. This isotope effect is then corrected for per cent reaction by the method of Bigeleisen and

$$k^{12}/k^{13} = R(100\%\text{ reaction})/R(5\%\text{ reaction}) \quad (1)$$

Wolfsberg.[7] This last correction is small, being about +0.0010 in the isotope effect for a reaction carried to 10% completion.

The results of 27 determinations of the carbon isotope effect are summarized in Table II. All experiments were conducted at 37° with 0.010 M glutamic acid in 0.1 M pyridine hydrochloride or isonicotinamide hydrochloride buffer. The isotope ratios in Table II have been corrected to a constant standard value, but have not been corrected for the presence of ^{17}O.

All experiments were conducted with the same batch of L-glutamic acid, so the isotope ratios for all of the 100% reaction samples should be the same. The data in Table II fit this prediction quite well—the mean of 27 determinations of the isotopic composition of the carboxyl group is 0.014307 ± 0.000003. This degree of reproducibility indicates the absence of gross errors or contamination in the samples. Unfortunately the reproducibility of the measurements at 5% reaction at a given pH is not as good. The most extensive data were obtained at pH 4.7. In that case the mean of the low conversion samples is 0.014084 ± 0.000014. The larger error in this case than in the 100% samples arises from the fact that this sample is four times smaller and has to be removed from five times as much solution. In spite of this error, the precision of our isotope-effect measurements is similar to the precision of most reported measurements.

The most extensive study of this isotope effect was conducted at pH 4.7. This isotope effect is independent of per cent reaction, enzyme concentration, and the presence or absence of 10^{-5} M pyridoxal 5′-phosphate. The same isotope effect was obtained over a period of several months by two independent investigators. The same isotope effect was found with very pure enzyme (specific activity 140 μmol/mg of enzyme-(min)) and with impure enzyme (17 μmol/mg of enzyme-(min)). Most experiments were conducted with enzyme having a specific activity of 100–120 μmol/mg of enzyme-(min).

Although less extensive experiments were carried out at other pH's the isotope effects were established with sufficient precision to be sure that the variation in isotope effect with pH in Table II is real. The isotope effects measured at pH 4.4 appear to depend on the nature of the buffer, but it is possible that the variation might simply be due to experimental error.

Table II. Carbon Isotope Effects on the Enzymatic Decarboxylation of Glutamic Acid at 37°

Per cent reaction	Isotope ratios[a] × 10^6 Low conversion	100% conversion	k^{12}/k^{13}
	pH 3.6, Isonicotinamide–HCl		
10	14113	14310	1.0157
10	14119	14309	1.0152
10	14101	14310	1.0166
5	14110	14309	1.0150
		Mean	1.0156 ±0.0007
	pH 4.0, Isonicotinamide–HCl		
6	14128	14314	1.0141
10	14124	14309	1.0148
10	14130	14304	1.0140
10	14125	14310	1.0148
		Mean	1.0144 ±0.0004
	pH 4.4, Isonicotinamide–HCl		
5	14134	14309	1.0131
5	14129	14310	1.0146
	Pyridine–HCl		
10	14104	14305	1.0160
10	14105	14303	1.0158
		Mean	1.0146 ±0.0015
	pH 4.7, Pyridine–HCl		
2.5	14070	14303[b]	1.0176
5	14071	14303[b]	1.0175
5	14089	14305	1.0163
5[c]	14083	14304	1.0166
6	14100	14303[d]	1.0153
6[c]	14104	14303[d]	1.0150
5	14087	14304	1.0163
2.5	14068	14310	1.0182
		Mean	1.0166 ±0.0011
	pH 5.2, Pyridine–HCl		
5	14065	14308	1.0183
5	14055	14308	1.0190
5	14059	14308	1.0188
10	14069	14306	1.0189
		Mean	1.0188 ±0.0003
	pH 5.5, Pyridine–HCl		
5	14008	14309	1.0228
5	14021	14312	1.0220
5	14019	14307	1.0218
		Mean	1.0222 ±0.0005

[a] m/e 45:44 corrected to tank standard 14150. Not corrected for ^{17}O. [b] Same sample. [c] 0.01 mM pyridoxal 5′-phosphate added. [d] Same sample.

Discussion

The enzymatic decarboxylation of glutamic acid is accompanied by a substantial carbon isotope effect at all pH's examined. Although this decarboxylation occurs by a multistep mechanism, several of the steps can be neglected because: (1) the isotope effects on all steps except decarboxylation are negligible compared to the isotope effect on that step; (2) only the steps in the reaction sequence up to and including the decarboxylation step can influence the isotope effect because the decarboxylation step is essentially irreversible. If steps other than decarboxylation are kinetically important, then the observed isotope effect will be smaller than the isotope effect on the decarboxylation step. Although the isotope effects observed in this case (k^{12}/k^{13} = 1.014 – 1.022) are somewhat smaller than those usually observed in organic reactions (1.03 – 1.06)[9-12] there is no doubt that the decarboxylation step must be rate determining or nearly so.

Carbon isotope effects and steady-state maximum velocities are plotted against pH in Figures 1 and 2. The velocity of the enzymatic decarboxylation under

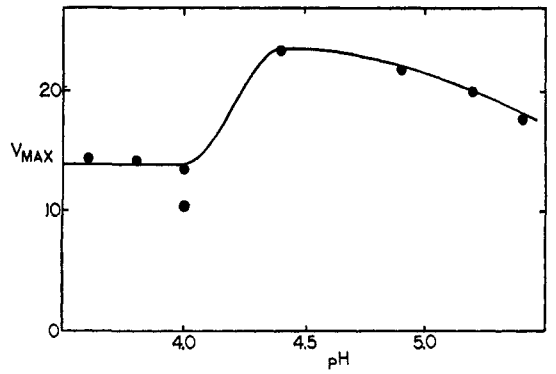

Figure 1. Steady-state maximum velocities *vs.* pH for the enzymatic decarboxylation of glutamic acid at 37.0°.

saturating substrate conditions does not change much with pH between pH 3.6 and 5.5. However, over the same range the carbon isotope effect varies from a minimum of 1.014 to a maximum of 1.022. Taken together these facts indicate that both decarboxylation and some step prior to it are kinetically significant.

Our detailed consideration of the mechanism of the decarboxylation is based on Scheme I and eq 2. Scheme I is not correct in detail because it does not consider the (presumed) tetrahedral intermediates which

$$E + Glu \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}Glu \xrightarrow{k_2} E + CO_2 + \text{product} \qquad (2)$$

occur between the starting materials and intermediate I and in the decomposition of the enzyme–product Schiff base.[19] However, the steady-state concentrations of these intermediates are probably quite small compared to the concentrations of the Schiff base intermediates, and the data available can be accommodated within the framework of eq 2, so a more complex scheme will not be used. The inclusion of another intermediate prior to decarboxylation makes no qualitative difference to the arguments presented here.

The dependence of the observed isotope effect on the rate constants of eq 2 has been derived previously[12] and is given in eq 3, where $P = k_2^{12}/k_{-1}$. It is assumed in the derivation of eq 3 that there is no isotope

$$k^{12}/k^{13} = (k_2^{12}/k_2^{13} + P)/(1 + P) \qquad (3)$$

effect on k_1 or k_{-1}. Thus the observed isotope effect is a function of two variables: the isotope effect on the decarboxylation step, and the partitioning of intermediate I between reversion to starting materials and decarboxylation. Unfortunately, neither of these parameters is known. It is possible to make a reasonable guess of the ratio k_2^{12}/k_2^{13} based on carbon isotope effects on nonenzymatic decarboxylation reactions. If we assume that the ratio is in the range usually observed for such isotope effects (1.03–1.06),[9–12] we can estimate that P is between 0.81 and 2.6 at pH 4.7. Electronic interactions involving pyridoxal 5′-phosphate probably make the transition state more stable and more reactant-like in this case than in the nonenzymatic decarboxylations which have been studied. For this reason k_2^{12}/k_2^{13} is probably at the low end of the expected range. In any case, it is clear that k_2 and k_{-1} differ by no more than a factor of three.

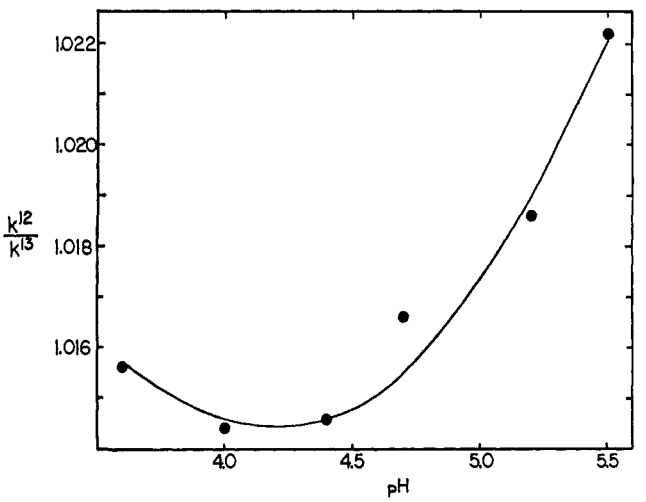

Figure 2. Carbon isotope effects on the enzymatic decarboxylation of glutamic acid *vs.* pH at 37.0°.

The interpretation of the pH dependence of the observed isotope effects depends to some extent on the assumed kinetic scheme. The simple scheme of eq 2 is adequate to interpret the data between pH 4.4 and 5.5. Decomposition of the enzyme–product complex II can be neglected because it would be expected to be more rapid than decomposition of the enzyme–substrate complex I as a result of the greater basicity of the Schiff base nitrogen in II. The reactivity of a Schiff base increases with increasing basicity of the Schiff base nitrogen.[20] The small pH dependence of V_{max} (which must reflect primarily k_2) requires that there be no pH dependence of k_2^{12}/k_2^{13}.

Assuming that the mechanism of the decarboxylation is adequately expressed by eq 2 in the range from pH 4.4 to 5.5, quantitative information about the variation in k_{-1} with pH can be obtained from the pH variation of the isotope effect. A value for the isotope effect on k_2 can be assumed and the corresponding values of P at pH 4.4 and 5.5 can be calculated. Since k_2 does not vary much with pH, the ratio of the two values of P gives the approximate ratio of the values of k_{-1} at the two pH's. As the assumed value of k_2^{12}/k_2^{13} varies from 1.03 to 1.06 the calculated ratio of k at the two pH's varies from 3.0 to 1.8. Thus, independent of the exact value assumed for k_2^{12}/k_2^{13}, k_{-1} increases threefold or less when the pH is increased from 4.4 to 5.5. This increase in k_{-1} with increasing pH indicates that this step is subject to general base catalysis or the kinetically equivalent general acid-specific base catalysis. Although it is not possible to identify the catalytic group involved, the magnitude of the pH variation of k_{-1} indicates that if the catalysis is general base the catalytic group must have a pK_a of 4.0 or below; if the catalysis is general acid-specific base, the pK_a must be 6.0 or above.

The change in V_{max} which occurs just above pH 4 can probably be attributed to the influence of the γ-carboxyl group of glutamic acid. If the pK_a of

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this group in Schiff base I is about the same as it is free in solution (4.25),[21] then the decrease in V_{max} at low pH may indicate that the enzyme catalyzes the decarboxylation of glutamic acid slightly less efficiently when the γ-carboxyl group is protonated than when it is not protonated. This difference would not be expected to affect k_2^{12}/k_2^{13}, but would decrease P, thereby making the observed isotope effect larger than would be predicted from data at higher pH. This consequence is most notable at pH 3.6, where the isotope effect is actually somewhat larger than at pH 4.0 or 4.4. Alternatively, the observed variation in V_{max} might be due to a conformational change of the enzyme.

Conclusions

Subject to the assumption that the enzymatic mechanism is adequately represented by eq 2 and that the isotope effect on this decarboxylation is similar to that on other decarboxylations, we conclude that the enzyme–glutamic acid Schiff base undergoes decarboxylation and decomposition to glutamic acid and free enzyme at very similar rates. The latter reaction is subject to general base catalysis or to the kinetically equivalent specific base–general acid catalysis.

The principal problems in the present study are the lack of a good model for the isotope effect on the decarboxylation step and our lack of knowledge of the importance of tetrahedral intermediates in the reaction sequence. Both of these matters are under further investigation. Clearly carbon isotope effects in systems such as this one are capable of providing information which is not easily accessible by any other method.

Experimental Section

Measurements of pH were made with a Radiometer Model 26 pH meter at 22°. Standard buffers (E. H. Sargent and Co.), pH 4 and pH 7, were used to calibrate the meter by the two-buffer method. Optical rotations were measured using a Perkin-Elmer Model 141 polarimeter. Amounts less than 10 mg were weighed on a Cahn Model M-10 microbalance.

Materials. Pyridoxal 5′-phosphate (Sigma), dithiothreitol (Cal-Biochem), isonicotinamide (Aldrich), hydrochloric acid (Baker), sodium acetate (Baker), and glacial acetic acid (Baker) were reagent grade materials and were used as received. Pyridine (Allied Chemical) was distilled from barium oxide through a 20-cm Vigreux column and only the constant boiling middle fraction was used. Trimethylamine N-oxide was prepared as described by Meisenheimer and Bratring,[22] and melted at 94–96°. Deionized doubly distilled water was used for all solutions. Glutamic acid (Free acid, Sigma) had α_D (6 N HCl) +30.2° (lit. +31.2°).[23]

Glutamate decarboxylate was isolated from *E. coli* (ATCC 11246) as previously described,[15] except that some of the preparations were chromatographed twice on DEAE cellulose. Bacteria were grown in the preparations laboratory of the University of Wisconsin Biochemistry Department under the supervision of Professor John Garver. The purified enzyme was stored in the precipitated state until needed. Before use it was centrifuged, dissolved in a small amount of buffer, pH 4.9, and desalted on a small column of Sephadex G-25.

All buffers were 0.1 M in total buffering material and were adjusted to the proper pH at 22°. The pH of solutions containing glutamic acid was adjusted after the acid was added. All buffers for the kinetic measurements contained 0.1 mM dithiothreitol and 0.01 mM pyridoxal 5′-phosphate.

Kinetics. All kinetic measurements were made on a Gilson respirometer at 37.0°. The general assay procedure has been described.[15] For determination of kinetic parameters 36-ml Warburg flasks were used. Each flask contained 25 ml of the appropriate glutamic acid solution in the main chamber and an appropriate amount (20–100 μl) of enzyme (measured with an Eppendorf pipet) in the side arm. The quantity of evolved gas was recorded each 100 sec for at least 1200 sec. The amount of enzyme used was such that the maximum decarboxylation rate never exceeded 25 μl per 100 sec.

Rates obtained in a particular series of determinations were adjusted to a constant standard activity for comparison with other rates by duplicate determination of the activity of the enzyme at pH 4.90 with 25 mM substrate.

In an individual kinetic run the plot of milliliters of gas evolved *vs.* time in the decarboxylation was very nearly linear from 200 or 300 sec to at least 1000 sec. Accurate determination of the initial rate could easily be accomplished by laying a straight edge along the early portion of the curve. Under a given set of conditions these rates were reproducible to about ±5% except for very slow rates. Observed volumes of CO_2 evolved were corrected to actual volumes of CO_2 evolved at standard temperature and pressure by multiplication by a factor calculated by method D of Gregory and Winter.[24] Since all of our determinations were made at the same temperature in the same apparatus this correction is dependent only on the barometric pressure. The magnitude of the correction factor was always about 0.91. Above pH 5 it is necessary to correct for the presence of carbonate and bicarbonate in the solution.[25] Under the conditions of our experiments this correction is a function only of pH.

For the computation of the Michaelis constant (K_m) and maximum velocity (V_{max}) a series of rate measurements was made at constant pH with varying concentrations of substrate. Substrate concentrations were chosen to give equally spaced points on the double-reciprocal plot as suggested by Cleland.[18] Triplicate determinations of the rate at each of four substrate concentrations were made. Exact values of the kinetic constants were calculated using a computer program supplied by Cleland[18] which gives appropriate weighting to all points and discards points which deviate from the computed concentration–velocity curve by more than 2.6 standard deviations.

Isotope Effects. Solutions used for isotope effects did not contain dithiothreitol or pyridoxal 5′-phosphate. Each isotope effect experiment was done with a freshly prepared solution of 0.01 M glutamic acid. For each experiment a 100-ml sample of the glutamic acid solution was put into a 250-ml flask equipped with a side arm with a stopcock and a serum cap, and a 20-ml sample of the same solution was put into another such flask. The solutions were then degassed by bubbling CO_2 free nitrogen through them for 30 min. The top of the flask, equipped with a joint for connection to the vacuum system and a stopcock, was then put on and nitrogen was swept in through the top and out through a hypodermic needle in the serum cap for 30 min. The flasks were then closed and equilibrated at 37.0° for 30 min.

The enzyme used for each experiment was incubated for at least 15 min with 0.1 mM pyridoxal 5′-phosphate and then desalted on a small column of Sephadex G-25 with pyridine hydrochloride buffer, pH 4.9, which had been degassed by bubbling CO_2 free nitrogen through it for at least 30 min. The time required to reach 5% reaction was determined by assay of 20 μl of the enzyme using 5 ml of the same glutamic acid solution used above.

The reaction was initiated by adding 400 μl of enzyme to the 100-ml solution and 0.5–1.0 ml of enzyme to the 20-ml solution. The reaction in the 100-ml solution was stopped at the time computed above by addition of 5 ml of concentrated H_2SO_4. The other solution was allowed to react for at least 1 hr before addition of 5 ml of H_2SO_4.

For removal and purification of the product CO_2 each flask was attached to the vacuum line, frozen with liquid nitrogen, and the nitrogen in the flask was removed. The flask was then warmed slightly and refrozen with Dry Ice and the CO_2 distilled into another

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flask cooled with liquid nitrogen. This flask was then thawed, refrozen with Dry Ice, and the CO_2 distilled into a third vessel. The thawing and freezing were repeated and the CO_2 was transferred to a sample tube for mass-spectrometric analysis.

Isotopic analyses were conducted on a Nuclide Associates RMS 6-60 isotope-ratio mass spectrometer equipped with a double inlet system. Isotope ratios *m/e* 45:44 were measured alternately for the sample and the tank standard, at least six such cycles being used for calculation of the isotope ratio. All measurements were made at approximately the same pressure. In a particular determination the isotope ratio never varied by more than ±0.000002.

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Proton Magnetic Resonance Studies of the Cation-Binding Properties of Nonactin. II. Comparison of the Sodium Ion, Potassium Ion, and Cesium Ion Complexes[1]

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Abstract: A comparison of the ion-binding properties of the macrocyclic antibiotic nonactin to Na^+, K^+, and Cs^+ has been made. Pmr spectroscopy (220 MHz) has been used to study the complexation of nonactin to these alkali ions in anhydrous acetone-d_6 and acetone-d_6–water mixtures containing as much as 0.5 mol fraction of water. Complex formation constants of 7×10^4, 7×10^4, and 1×10^4 are obtained for the Na^+, K^+, and Cs^+ complexes, respectively, in dry acetone; in wet acetone, the respective binding constants are 210, 2×10^4, and 400. Thus, all three ions bind to nonactin with nearly equal affinity in dry acetone, but the binding constants are drastically reduced when the solvent system is altered by the addition of appreciable amounts of water. It is significant that the reduction is far greater for Na^+ and Cs^+ than for K^+ itself, making the binding of K^+ to nonactin highly favored in the more aqueous medium. It is shown that in wet acetone, the alkali ion must be stripped of its hydration shell prior to its accommodation in the nonactin cavity, and hence we surmise that hydration of the various ions in wet acetone must contribute significantly to the ion selective behavior of nonactin in the more aqueous media. It is felt that these results are pertinent to the selective potassium ion transport induced by nonactin in experimental lipid bilayers. Analysis of the pmr data also indicates that the nonactin ring undergoes sizable conformation changes on incorporation of these alkali ions. The extent of this conformational change is slightly different depending on the ion, but on the whole, the three complexes studied appear to exhibit quite similar structures.

Nonactin, the macrocyclic antibiotic depicted in Figure 1, has been shown to be influential in the regulation of metabolic behavior and is thought to act by selectively enhancing the transport of potassium ion through cell membranes.[4,5] Because of its structural simplicity and its potential importance as a lipid soluble ion carrier, it has become the object of a number of recent biophysical and biochemical studies, all aimed at obtaining an understanding, at the molecular level, of the action of this physiologically active agent.[6,7]

With this same objective in mind, we recently reported a proton magnetic resonance (pmr) study of the potassium ion binding properties of nonactin in dry acetone and in acetone–water mixtures.[8] Pmr spectroscopy has proven useful in the investigation of the conformational properties, and cation-binding properties of macrocyclic antibiotics, not only in this study but in studies of other ionophores as well. Several groups of workers, for example, have applied pmr spectroscopy to the study of valinomycin, a depsipeptide which exhibits an ion selectivity in metabolic behavior and membrane permeability similar to that observed for nonactin.[9-11]

In our recent study of nonactin the most significant among the results obtained were the conformational changes observed for the nonactin ring upon ion complexation and the implication of the solvent (H_2O) as an important factor in the origin of the ion selectivity of this antibiotic. The pmr data for the K^+–nonactin complex were shown to be in general consistent with the molecular structure determined earlier by crystallographic studies,[12] but in addition, comparison of the

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