BROMIDE ION PROBE N.M.R. STUDIES OF GLUTAMATE DECARBOXYLASE (E. Coli)

J. T. Gerig and L. Kwock

Department of Chemistry, University of California
Santa Barbara, California 93106

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Summary. Rate and bromine-81 nmr spectroscopic investigations of the binding of bromide and chloride ions to bacterial L-glutamate decarboxylase have been carried out. A mild acceleration of the decarboxylation reaction by these anions is observed and the nmr results suggest that bromide and chloride bind competitively to the enzyme. Bromide ion binding appears to have the same pH dependence as the rate of the enzymatic reaction.

A number of glutamate decarboxylase enzymes have been isolated and studied including those from E. Coli (1-6), mouse brain (7,8) and squash (9). An intriguing aspect of the reactions catalyzed by these proteins is the effect of halogen anions; chloride ion enhances the rate of decarboxylation by the bacterial enzyme (1,10) while the mouse brain enzyme is inhibited by this anion (7). Halide ion probe nuclear magnetic resonance experiments have been revealingly applied to the study of metal-containing proteins and to the examination of nonspecific ion-protein binding (11-15). The present exploratory work was undertaken to define the applicability of this technique to the investigation of an enzyme system that does not contain metal ions but which apparently rather specifically binds chloride or bromide in a way that influences the catalyzed reaction.

Materials and Methods

L-(+)-glutamic acid was obtained from Matheson, Coleman and Bell. The inorganic salts were reagent grade (Baker and Adamson); doubly distilled water was used for all solutions.

Glutamate decarboxylase (E. Coli, ATCC 11246) was isolated from the crude acetone powder by a modified procedure based on that of Homola and Dekker (16). Protein concentration (17) and pyridoxal-5'-phosphate content determinations (18) showed that between 50 and 60% of the total protein in the sample was the desired decarboxylase. The kinetics of decarboxylation were followed with a constant volume Warburg manometer system at 37°.

Transverse relaxation times were determined with a Bruker 321S spectrometer operating at 15-16 MHz. A Fabritek 1074 computer was used to enhance signal-to-noise ratios. The relaxation times were estimated from the off-resonance decay patterns according to the equation

$M_t = A \cos(\omega-\omega_0)e^{-t/T_2}$ where M_t is proportional to the signal intensity at time t after a 90° pulse. At least three separate determinations were made for each datum; reported T_2 values are the average of these. Field homogeneity was checked with the sodium-23 resonance of a saturated NaCl solution; this correction to T_2 was negligible. The sample temperature was 31°.

Results

The effect of the sodium, potassium, lithium and ammonium halides on the activity of the enzyme was surveyed at a salt concentration of 0.1 M. These experiments showed that activation of the enzyme is dependent only upon the nature of the anion with the order of effectiveness being $Cl^- \geq Br^- > I^- > F^-$. The influence of chloride and bromide was explored more fully (Figure 1). These results indicate that the rate effect is maximum at about 0.2 M salt and is not primarily an ionic strength effect.

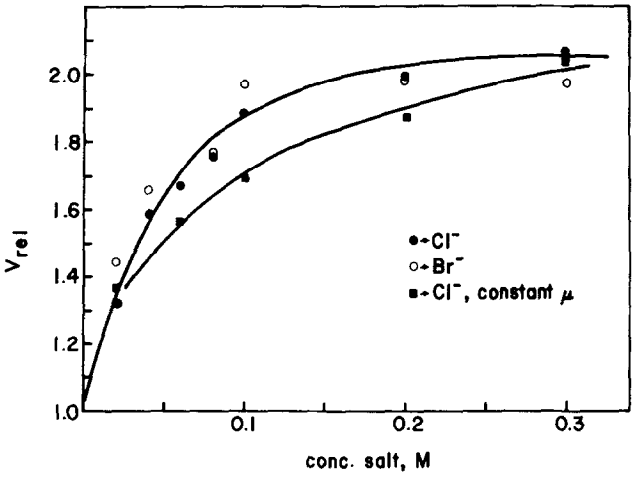

Figure 1. The effect of halide ions on the activity of glutamate decarboxylase. Protein concentrations were approximately 0.08 mg/ml; solutions were made up in 0.1 M pyridine-pyridine sulfate buffer at pH 4.6, 37°. The same enzyme preparation was used for all runs. V_{rel} is the reaction velocity relative to the value found in the absence of added electrolyte. For the lower curve, the total added anion concentration was held constant at 0.3 M using KNO_3.

The transverse relaxation rate of ^{81}Br in 0.25 M KBr solution was determined in the presence and absence of the enzyme at various solution acidities. It was found that inclusion of the enzyme in the sample led to a substantial increase in relaxation rate and that this effect was maximal at approximately the same pH where the enzyme is optimally active (Figure 2).

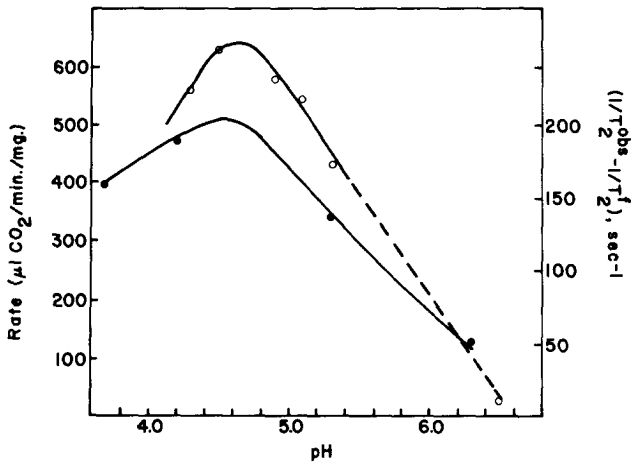

Figure 2. The dependence of reaction rate and bromine-81 relaxation times on pH. For the rate studies, the protein concentration was approximately 2 mg/ml; reactions were done in 0.1 M pyridine-pyridine sulfate buffers at 37° with substrate at 13 mM. For the relaxation data (lower curve, right axis) all samples contained 0.25 M KBr with the protein concentration at 1.5 mg/ml in the same buffers.

In interpreting the data, we assume that there are two possible environments for the bromide ions, either a protein-bound state or the free-solution state. Under these conditions the observed relaxation rate is given by

$$\frac{1}{T_2^{obs}} = \frac{\chi_f}{T_2^f} + \frac{\chi_b}{T_2^b} \qquad (1)$$

where χ_f and χ_b are the mole fractions of free and protein-bound anions respectively, and T_2^f and T_2^b are the transverse relaxation times which characterize each state. Equation (1) is valid only when exchange between the free and bound states is rapid. For our experiments $\chi_f \approx 1$ and T_2^f can be measured in the absence of protein. Since other bromide-binding materials may be present in the enzyme preparations, rigorous quantitation of these data is not yet possible. However the pH-relaxation rate results would obtain most simply if the mole fraction of bound bromide ions reached a maximum at a pH near 4.2. This effect is likely not due to viscosity changes since the protein concentration was constant throughout the experiments. The relaxation rate for ^{79}Br was also determined at several pH values. If the fast-exchange assumption made in writing equation 1 is valid, the ratio of the ^{81}Br and ^{79}Br relaxation rates should be 1.48 (12,19). At pH 4.7 and 5.6 the observed ratios were 1.45 and 1.35 respectively, close to the value expected for the fast-exchange situation. At pH 7.1, the $^{81}Br/^{79}Br$ ratio dropped to 1.18, indicating a slowing of the exchange rate at this pH.

In another series of determinations (Table I) it was noted that the addition of increasing amounts of chloride decreased the bromide relaxation rate. This result indicates that chloride competes with bromide for the same protein binding sites since the effect of protein on the relaxation rate of bromide becomes less and less pronounced as the amount of chloride increases. cis-Aconitate and D,L-α-methylglutamate are good competitive inhibitors of glutamate decarboxylase (6,20) but when present at concentrations in excess of their respective binding constants, have little effect on the bromine-81 relaxation processes. The principal sites of halogen ion binding, therefore, may not be the same as those used by these inhibitors of the enzyme.

Table I

Bromine-81 Relaxation Rates in the Presence of Added Anions[a]

Anion	Concentration	$\frac{1}{T_2^{obs}} - \frac{1}{T_2^{f}}$, sec^{-1}
None	0	107
Chloride	0.05	70
Chloride	0.10	37
Chloride	0.15	32
Chloride	0.20	37
D,L-α-Methylglutamate	0.03	92
cis-Aconitate	0.04	85

[a] All samples contained 0.25 M KBr; a pyridine-pyridine sulfate buffer at pH 5.0 was used. In the absence of protein $\frac{1}{T_2^{obs}} - \frac{1}{T_2^{f}}$ The concentration of protein in each sample was 1 mg/ml.

It thus appears that the halide ion probe nmr technique can be of value in supplying details of the halide-induced rate effects in glutamate decarboxylases. The potential of halogen nmr spectroscopy to reveal the nature of the halide ion binding site(s) has not been fully realized in the present study primarily because the low inherent sensitivity to detection of bromine made it necessary to work at high concentrations of Br^- relative to enzyme. There is also the possibility that extraneous proteins not removed by the purification steps contributed to the observed effects. We hope to overcome these limitations and to expand this study using crystallized, high-purity protein.

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