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THE UNIDIRECTIONAL INHIBITION OF GLUTAMATE DEHYDROGENASE FROM *BLASTOCLADIELLA EMERSONII*

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SUMMARY

The effect of ATP and EDTA on glutamate dehydrogenase (L-glutamate:NAD^+ oxidoreductase (deaminating), EC 1.4.1.2) from *Blastocladiella emersonii* has been studied by steady-state kinetic methods and by stopped-flow technique. ATP and EDTA inhibit the oxidative deamination of glutamate but have no effect on the reductive amination of α-ketoglutarate under normal assay conditions.

At low NH^{4+} concentration, glutamate is able to stimulate the reductive amination of α-ketoglutarate. This stimulation was reversed by ATP and EDTA. Thus, under certain conditions ATP and EDTA are able to inhibit both the oxidative deamination as well as the reductive amination.

In stopped-flow experiments it was found that the inhibition of the enzyme by ATP and EDTA needs several seconds to be complete. The results indicate that the inhibition may be mediated through conformational changes in the protein.

Evidence is obtained that $(NH_4)_2SO_4$ inhibits the enzyme by acting as an allosteric effector molecule and that glutamate reverses the inhibition. The results suggest that ATP and EDTA act on the enzyme in a similar manner to $(NH_4)_2SO_4$.

It is suggested that the enzyme can occur in three different conformational states, *viz.* the normal state, the AMP-activated state as well as an inhibited state induced by $(NH_4)_2SO_4$ or by chelating agents.

INTRODUCTION

Recently LÉJOHN[1] reported that certain metabolites such as citrate, isocitrate and fructose 1,6-diphosphate and metal-binding agents such as EDTA and 8-hydroxyquinoline elecit a unique form of inhibition of the NAD-specific glutamate dehydrogenase (L-glutamate:NAD^+ oxidoreductase (deaminating), EC 1.4.1.2) from the water mould *Blastocladiella emersonii*. He found that these compounds inhibited the oxidative deamination of glutamate while the reductive amination of α-ketoglutarate was unaffected, a phenomenon which he called unidirectional inhibition[1]. In later

studies LÉJOHN *et al.*[2,3] also found analogous unidirectional inhibition or activation in the case of glutamate dehydrogenases from certain fungi. It was proposed that such unidirectional effects may be of physiological importance[1–3].

In the present work the mechanism underlying the unidirectional inhibition of glutamate dehydrogenase from *Blastocladiella emersonii* was investigated. The inhibition by ATP and EDTA was studied by the stopped-flow technique and steady-state kinetic methods. These two components were chosen since LÉJOHN[1] found that ATP inhibited the enzyme in both directions while the chelating agent EDTA only inhibited the oxidative deamination of glutamate.

MATERIALS AND METHODS

Materials

Glutamate, α-ketoglutarate, AMP, ATP, EDTA, NAD^+, NADH and 5'-adenylic acid deaminase were obtained from Sigma Chemical Co., St. Louis, Mo.

Preparation of enzyme

Glutamate dehydrogenase was prepared from *Blastocladiella emersonii* according to the method of LÉJOHN AND JACKSON[4] with small modifications as previously described[5]. The specific activity was similar to that observed by LÉJOHN *et al.*[1,4].

Protein concentration was determined by the method of LOWRY *et al.*[6]. A molecular weight of 200 000 (ref. 7) was used in the calculations.

Assay of enzyme activity

The activity was measured from the rate of change in absorption at 340 nm upon oxidation or reduction of the coenzyme. The normal kinetic studies were done with a Unicam SP 800 spectrophotometer. Unless otherwise indicated the reaction mixture contained, in addition to enzyme, the following reagents in 0.2 M Tris chloride buffer (total volume 3 ml). Measurements of the oxidative deamination of glutamate: 4 mM NAD^+ and 33 mM glutamate. Measurements of the reductive amination of α-ketoglutarate: 0.33 mM NADH, 2.5 mM α-ketoglutarate and 50 mM $(NH_4)_2SO_4$. The pH was adjusted as indicated in the text. The pre-steady-state kinetic experiments were carried out with a Durrum stopped-flow spectrophotometer (Model D-110). Conditions are given in the text. The change in transmission was displaced on a Tektronix storage oscilloscope. Photographs of the curves were taken with a polaroid camera.

Treatment of data

Determination of kinetic parameters was made from LINEWEAVER–BURK[8] plots or HILL[9] plots. From the data the best line was determined by the least-square method by the use of a Hewlett–Packard calculator combined with an X–Y plotter. The changes in transmission in the stopped-flow experiments were measured and converted to absorption. The results were fitted to second order curves by the least square method and the reaction rate determined from the derivative of the curves.

RESULTS

Glutamate dehydrogenase from *Blastocladiella emersonii* uses NAD as coenzyme. The enzyme is activated by AMP and ADP[4]. On the basis of measurements of the rate constant for the activation of the enzyme by AMP it has been suggested that this activation involves a conformational change in the protein[5]. As pointed out above, LÉJOHN[1] has found that EDTA inhibits only the oxidative deamination of glutamate while ATP inhibits both the oxidative deamination of glutamate and the reductive amination of α-ketoglutarate.

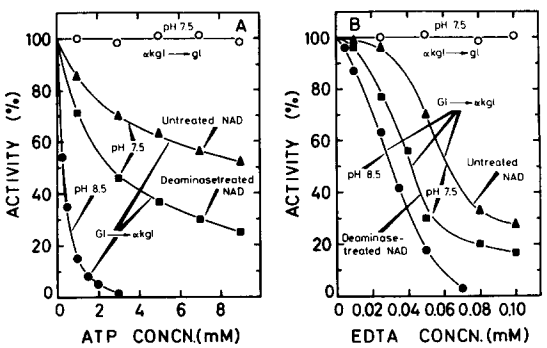


Fig. 1. Inhibition of glutamate dehydrogenase by ATP (A) and EDTA (B). The oxidative deamination of glutamate (gl) (closed symbols) and the reductive amination of α-ketoglutarate (αkgl) (open symbols) were determined at the pH values indicated, in the presence of different amounts of ATP and EDTA. The inhibition of the oxidative deamination at pH 7.5 was measured both with untreated NAD as coenzyme (▲—▲) and with NAD, pretreated with 5′-adenylic acid deaminase (2 μg protein per ml) in 0.01 M citrate buffer (pH 6.5) for 30 min at 30° (■—■). The concentration of glutamate dehydrogenase was $2.8 \cdot 10^{-9}$ M for measurement of the oxidative deamination and $1.4 \cdot 10^{-9}$ M for measurement of the reductive amination.

Unidirectional inhibition by ATP and EDTA

In Fig. 1A is shown the effect of ATP on the enzyme activity. It is apparent that the inhibition of the oxidative deamination of glutamate by ATP depends strongly on the pH during the assay. Thus at pH 8.5 a 50% inhibition was obtained with an ATP concentration of less than 0.5 mM ATP, while at pH 7.5, a 5–20 times higher ATP concentration was needed to give the same inhibition.

LÉJOHN AND JACKSON[4] have previously shown that the ATP inhibition is counteracted by AMP. As we have recently obtained evidence that commercial NAD preparations may contain small amounts of an AMP-like impurity[10], the inhibitory effect of ATP was measured both with untreated NAD and with NAD pretreated with 5′-adenylic acid deaminase. It is apparent that the sensitivity of the enzyme to inhibition by ATP increased when deaminase-treated NAD was used. This finding supports the view that the NAD was contaminated with AMP. The fact that inhibition was obtained with deaminase-treated NAD indicates that the inhibition of the enzyme by ATP cannot be accounted for simply by a competition with AMP.

In contrast to the results of LÉJOHN[1] we did not observe any inhibition of the reductive amination of α-ketoglutarate by ATP (Fig. 1A). The possibility should be considered that this discrepancy could be due to the fact that the Tris buffer used has

a small capacity at pH 7.5. If the pH of the ATP is not accurately adjusted prior to addition to the assay mixture an apparent inhibition will be found due to change in the pH.

The kinetics of the inhibition of the oxidative deamination by EDTA differ from those observed with ATP. The curves for the activity as a function of the EDTA concentration show an initial shoulder which increases with decreasing pH (Fig. 1B). The slope of the curves seem, however, to be nearly independent of pH. When NAD treated with 5'-adenylic acid deaminase was used as coenzyme, a reduction in the shoulder was observed, indicating that the shoulder may in part be due to AMP contamination. In agreement with LÉJOHN[1] we were unable to find any inhibition of EDTA of the reductive amination of α-ketoglutarate (Fig. 1B).

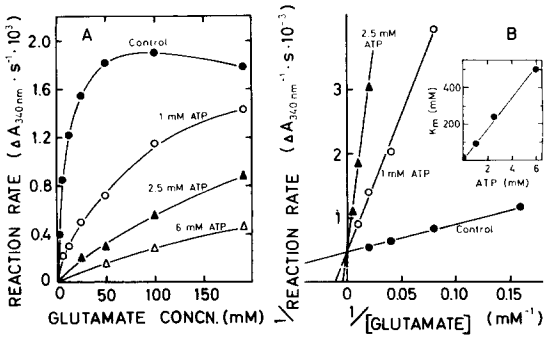


Fig. 2. Effect of glutamate concentration on the inhibition of the oxidative deamination by ATP. (A) Reaction rate in the presence of different amounts of ATP as a function of the glutamate concentration. (B) LINEWEAVER–BURK plots[8] of the data in Part A. The inserted figure shows K_m for glutamate as a function of the ATP concentration. The experiments were carried out at pH 8.0 with an enzyme concentration of $2.8 \cdot 10^{-9}$ M.

Inhibition of the oxidative deamination

The extent of inhibition by ATP decreases strongly with increasing glutamate concentrations (Fig. 2A). By plotting the results according to the method of LINEWEAVER AND BURK[8], it was found that ATP acts as a linear competitive inhibitor with regard to glutamate. Thus, v_{max} was independent of the presence of ATP, while K_m for glutamate increased linearly with the ATP concentration (Fig. 2B). The fact that K_m for glutamate is independent of the pH[5] while the inhibition by ATP decreases with decreasing pH (Fig. 1A) suggests that a particular ionic species of ATP (*e.g.* ATP^{4-}) is involved in the inhibition.

In Fig. 3A is shown the effect of the glutamate concentration measured in the presence of different concentrations of EDTA. It is apparent that ATP and EDTA affect the glutamate concentration curves differently (*cf.* Figs. 2A and 3A). In the presence of EDTA the curve for the activity as a function of the glutamate concentration seemed sigmoidal. However, the curves cannot be fitted to a straight line in a HILL[9] plot and furthermore the activity is close to zero until a limiting concentration of glutamate was reached. Thus, EDTA acts by displacing the activity curve to higher glutamate concentrations. The curve was displaced to approximately 70 mM higher glutamate concentration per 0.1 mM EDTA present in the assay mixture.

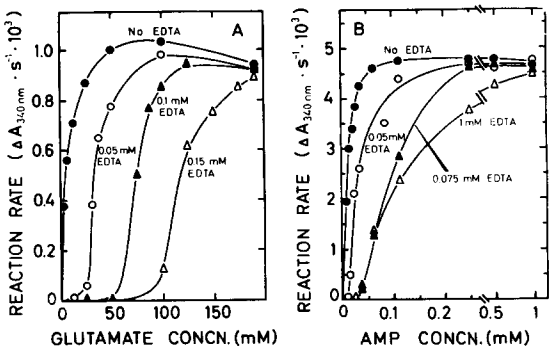


Fig. 3. Effect of glutamate concentration (A) and of AMP concentration (B) on the inhibition of the oxidative deamination by EDTA. The experiments were carried out at pH 8.0 with an enzyme concentration of $1.4 \cdot 10^{-9}$ M in the presence of different amounts of EDTA. In the results in Part B, NAD pretreated with 5'-adenylic acid deaminase, as described in legend to Fig. 1, was used.

As pointed out above, LÉJOHN AND JACKSON[4] found that AMP reverses the ATP inhibition. In order to study whether EDTA acts by competing with AMP, the rate of the oxidative deamination was measured for increasing concentrations of AMP in the presence of EDTA (Fig. 3B). It is apparent that at low EDTA and AMP concentrations EDTA competes with AMP. Unexpectedly it was found, however, that the inhibitory effect of EDTA did not increase significantly when the EDTA concentration was increased from 0.075 to 1 mM. The possible mechanism underlying this observation will be discussed further below.

In previous studies LÉJOHN[1] found that the inhibition of the oxidative deamination by ATP and EDTA can be counteracted by addition of divalent metal ions such as Mg^{2+} and Ca^{2+}. Attempts were therefore made to study whether the inhibition by EDTA could be accounted for by removal of divalent cations from the enzyme or the substrate. It was found (not shown) that pretreatment of the enzyme with 3 mM EDTA and subsequent dialysis of the enzyme against 0.25 mM EDTA did not influence its susceptibility to EDTA inhibition. Recrystallization of the glutamate from an EDTA solution likewise had no effect on the inhibition. Thus, the inhibition by EDTA cannot be accounted for simply by removal of metal ions from the enzyme or substrate solutions.

Stimulation of the reductive amination by glutamate

In the results presented in Fig. 4A the effect of glutamate was measured for different concentrations of $(NH_4)_2SO_4$. Since glutamate is a product in the enzymatic reaction measured, it might be expected that glutamate would inhibit the activity. Surprisingly, it was found that glutamate stimulated the activity to a considerable extent, particularly at low $(NH_4)_2SO_4$ concentrations. In the presence of glutamate the K_m for $(NH_4)_2SO_4$ was reduced to less than 20 mM, while in the absence of glutamate the activity increased nearly linearly, at least to 400 mM $(NH_4)_2SO_4$. A similar decrease in K_m for $(NH_4)_2SO_4$ has previously been found in the presence of AMP and metal ions.

The results in Fig. 4B indicate that the stimulation of the reductive amination of α-ketoglutarate by glutamate occurs according to a sigmoid curve. The curve gives

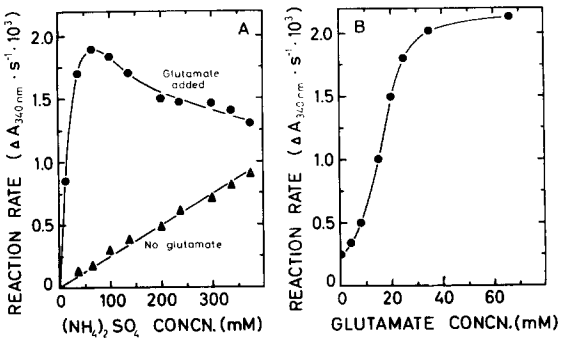


Fig. 4. Stimulation of the reductive amination of α-ketoglutarate by glutamate. (A) Reaction rate as a function of the $(NH_4)_2SO_4$ concentration in the presence and absence of 33 mM glutamate. (B) Reaction rate as a function of the glutamate concentration. The enzyme was assayed at pH 7.5 in the presence of 50 mM $(NH_4)_2SO_4$. The enzyme concentration was $1.4 \cdot 10^{-9}$ M.

a straight line in a HILL[9] plot with $n = 2$ (see Fig. 7B). It was found that the stimulation was not affected by recrystallization of the glutamate from an EDTA solution, or by treatment of glutamate with 5'-adenylic acid deaminase (not shown). Thus, it seems unlikely that the stimulation by glutamate is caused by an impurity in the glutamate preparations used.

Further information on the mechanism of stimulation by glutamate was sought in stopped-flow experiments. When $(NH_4)_2SO_4$ was premixed with the enzyme prior to mixing with NADH and α-ketoglutarate, the absorption at 340 nm decreased linearly with the reaction time (Fig. 5A). On the other hand, when $(NH_4)_2SO_4$ was premixed with the other substrates prior to mixing with the enzyme, the absorption decreased initially rapidly and subsequently the curve levelled off. When glutamate was

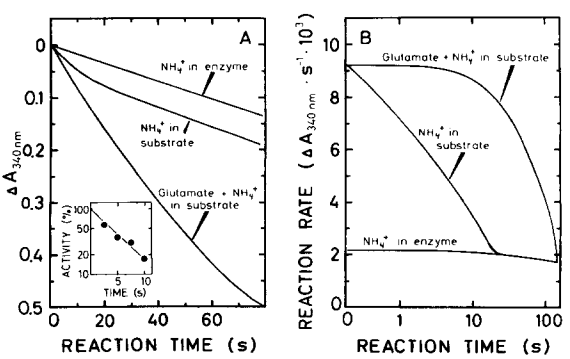


Fig. 5. Effect of $(NH_4)_2SO_4$ and of glutamate on the reductive amination of α-ketoglutarate. (A) Decrease in absorption at 340 nm as a function of the reaction time. The different curves represent, from the top: $(NH_4)_2SO_4$ preincubated with enzyme prior to mixing with the substrates; $(NH_4)_2SO_4$ premixed with substrate prior to mixing with enzyme and glutamate and $(NH_4)_2SO_4$ premixed with substrate prior to mixing with enzyme. (B) Reaction rate as a function of time after mixing. The experiments were carried out with stopped-flow spectroscopy. The final concentrations were: 2.5 mM α-ketoglutarate, 0.09 mM NADH, 50 mM $(NH_4)_2SO_4$ and $6 \cdot 10^{-9}$ M enzyme in 0.2 M Tris chloride buffer, pH 7.5. Glutamate (50 mM) as indicated. The inserted figure in Part A shows the decrease in activity when $(NH_4)_2SO_4$ was premixed with substrate. The calculations were carried out as described in the text.

present with the other substrates a significant increase in the reaction rate was observed.

The results are more clearly demonstrated in Fig. 5B, where the instantaneous reaction rates are plotted *versus* the time in a semilogarithmic scale. It is seen that when $(NH_4)_2SO_4$ was premixed with the other substrates, the initial rate of reaction was 4–5 times higher than when $(NH_4)_2SO_4$ was preincubated with the enzyme. However the reaction rate decreases rapidly during the first 20 sec, and eventually reached the same level as when $(NH_4)_2SO_4$ was preincubated with the enzyme. From the decrease in reaction rate with time the rate constant for the conversion of the enzyme to the less active state (the state present when $(NH_4)_2SO_4$ is preincubated with enzyme) can be calculated. The inserted figure (Fig. 5A) shows the relative activity determined from the reaction rate observed when $(NH_4)_2SO_4$ was premixed with the substrate *versus* time in a semilogarithmic scale. From the straight line it follows that $(NH_4)_2SO_4$ converts the enzyme to the less active state in a first order reaction with a rate constant of 0.2 sec^{-1}.

When glutamate was present together with the other substrates the reaction rate remained for almost 20 sec at the same high level as that initially observed when $(NH_4)_2SO_4$ was premixed with the other substrates. Then the rate decreased rapidly due to a decrease in the NADH concentration. On the basis of the results obtained the possibility should be considered that $(NH_4)_2SO_4$, as well as glutamate, acts as an allosteric effector molecule for the enzyme.

Inhibition of the reductive amination

It was shown above (Fig. 1) that under normal assay conditions ATP and EDTA are unable to inhibit the reductive amination of α-ketoglutarate. In subsequent experiments (not shown) it was likewise found that even when small amounts of AMP were present to stimulate the activity, ATP and EDTA had no significant inhibiting effect. The results in Fig. 6 demonstrate, however, that when the reductive amination was stimulated by the presence of glutamate both ATP and EDTA inhi-

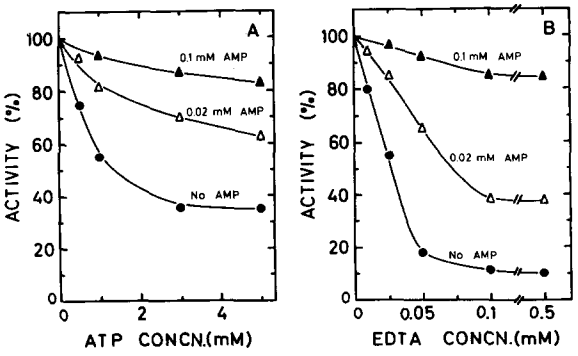


Fig. 6. Effect of AMP on the inhibition of the reductive amination of α-ketoglutarate by ATP (A) and EDTA (B) in enzymes stimulated by glutamate. The assay was carried out in the presence of 33 mM glutamate and different concentrations of AMP as indicated, at pH 7.5. Since the activity is increased in the presence of the activator AMP, the results have been normalized and the activity in the absence of ATP and EDTA is set equal to 100%. The enzyme concentration was $1.4 \cdot 10^{-9}$ M.

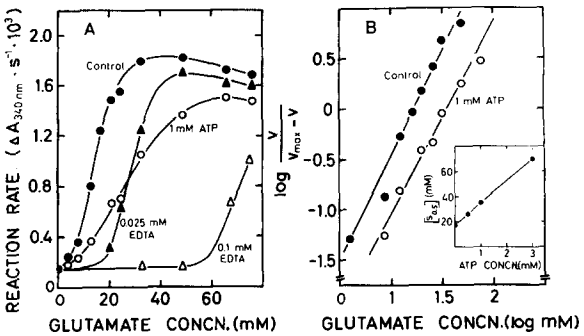


Fig. 7. Effect of glutamate concentration on the inhibition of the reductive amination of α-ketoglutarate by ATP and EDTA. (A) Reaction rate in the presence of ATP and EDTA as a function of the glutamate concentration. (B) HILL plot[9] of the glutamate stimulation in the absence and presence of 1 mM ATP. The inserted figure shows the effect of different ATP concentrations on $[S]_{0.5}$. The experiments were carried out at pH 7.5. The enzyme concentration was $1.2 \cdot 10^{-9}$ M.

bited the reaction. In agreement with the results for the oxidative deamination, the inhibition was counteracted by AMP.

If we compare the inhibition by ATP and EDTA on the reductive amination in the presence of glutamate with that obtained with the oxidative deamination at the same pH, it is apparent that ATP and EDTA inhibit the reductive amination as effectively as the oxidative deamination.

Experiments were then made to study whether glutamate counteracts the inhibitory effect of ATP and EDTA in a similar way as that found for the oxidative deamination. In Fig. 7A the stimulatory effect of glutamate on the reductive amination was studied in the presence of different amounts of ATP and EDTA in the assay mixture. In the presence of 1 mM ATP a sigmoidal curve for the activity as a function of the glutamate concentration was obtained, as in the control. When the data were plotted according to the method of HILL[9] (Fig. 7B) a straight line was obtained which is parallel to that obtained in the absence of ATP. The fact that the slope of the line was unaffected while $[S]_{0.5}$ increased linearly with the ATP concentration (inserted figure) demonstrates that ATP acts in a competitive manner with regard to glutamate.

When EDTA was added, the curve for the glutamate stimulation was displaced to higher glutamate concentrations in a similar way as found for the oxidative deamination of glutamate. Addition of 0.1 mM EDTA resulted in a displacement of the curve, corresponding to 50–60 mM glutamate. This is in good agreement with that for the oxidative deamination (see Fig. 3A).

Time course of inhibition

In order to investigate the mechanism of the inhibition by ATP and EDTA in further detail, the initial rate of inhibition was studied in stopped-flow experiments.

In Fig. 8A is shown the initial increase in absorption at 340 nm, when the oxidative deamination of glutamate was measured in the presence and absence of ATP and EDTA. When the activity was assayed with ATP or EDTA premixed with the substrates, prior to mixing with the enzyme, the absorption increased initially nearly at the same rate as in the absence of inhibitory substances. However, after a few

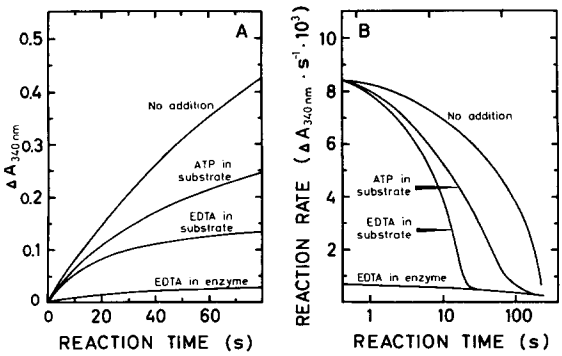


Fig. 8. Effect of EDTA and ATP on the initial rate of the oxidative deamination of glutamate. (A) Increase in absorption at 340 nm as a function of the reaction time. The different traces represent from the top: no additions; 10 mM ATP, premixed with substrate; 0.1 mM EDTA premixed with substrate; 0.1 mM EDTA premixed with enzyme. (B) Reaction rate calculated at different time after mixing. The experiments were carried out with stopped-flow spectroscopy. NAD was in all cases premixed with enzyme. The final concentrations were: 33 mM glutamate, 4 mM NAD, and $1.2 \cdot 10^{-8}$ M enzyme in 0.2 M Tris chloride buffer, pH 8.5, and EDTA and ATP as indicated.

seconds the curves levelled off. On the other hand, if EDTA was preincubated with the enzyme prior to the mixing with substrates, the inhibition was complete when the reaction started.

In Fig. 8B the reaction rates are plotted *versus* time in a semilogarithmic scale. The decrease in the reaction rate in the control experiment is probably due to product inhibition[5]. It is seen that when EDTA was preincubated with the enzyme prior to mixing with the substrate, the reaction rate was only one tenth of the initial rate observed in the control experiment. Furthermore, the rate was nearly constant during the first 100 sec. On the other hand, when EDTA and ATP were premixed with substrates, prior to mixing with the enzyme, the reaction rate was initially the same as in the control experiment. However, the rate levelled off considerably faster and reached that observed when EDTA was preincubated with the enzyme after 20–30 sec, in the case of the EDTA and 50–100 sec, in the case of ATP.

On the basis of the initial reaction rate observed immediately after mixing and

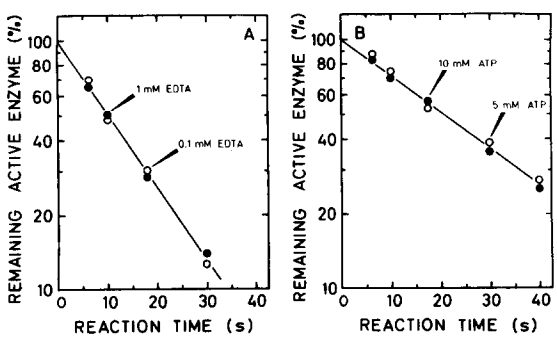


Fig. 9. Rate of inhibition of the oxidative deamination by EDTA (A) and ATP (B). Remaining active enzyme in percent was calculated as described in the text. The inhibitory substances were in all cases premixed with the substrates. Experimental conditions as in Fig. 8.

the final reaction rate observed when the rate approached a constant value, the inhibition was calculated as a function of the reaction time. In Fig. 9 the remaining active enzyme is plotted in a semilogarithmic scale *versus* the reaction time. In the calculation corrections were made for the decrease in the reaction rate occurring in the absence of ATP or EDTA. The fact that straight lines were obtained shows that the conversion of the enzyme to the inhibited state follows first-order reaction kinetics. It is apparent (Fig. 9A) that the rate of conversion is independent of the EDTA concentration in the range 0.1–1 mM EDTA. The rate constant for inhibition of the enzyme by EDTA corresponds to 0.07 sec^{-1}. The rate constant was somewhat lower (0.03 sec^{-1}) when the enzyme was inhibited by ATP (Fig. 9B). In this case the same reaction rate was observed with 5 and 10 mM ATP.

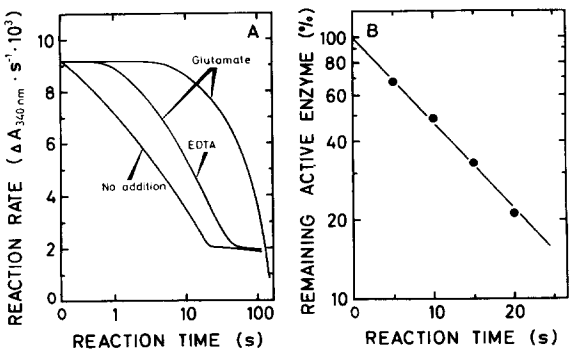


Fig. 10. Effect of EDTA on the initial rate of the reductive amination of α-ketoglutarate. (A) Reaction rate as a function of time. The different curves represent from the top: 50 mM glutamate premixed with the substrates; 0.25 mM EDTA and 50 mM glutamate premixed with the substrates; no additions. (B) Rate of inhibition of the enzyme by EDTA. Ammonium sulphate was in all cases premixed with the other substrates. Otherwise experimental conditions as described in Fig. 5.

In Fig. 10A is shown the time course of inhibition of the reductive amination of α-ketoglutarate by EDTA. When we compare the curve obtained in the presence of EDTA with that in the absence of EDTA and glutamate, it is seen that the enzyme retained its initial rate for a longer time. The rate decreased, however, more rapidly than in the control experiment with glutamate. The final rate was the same as that observed in the absence of glutamate. When the rate of inhibition was calculated in the same manner as for the oxidative deamination a straight line was observed when the activity was plotted *versus* time in a semilogarithmic scale. (Fig. 10B). From this line the rate constant for inhibition was calculated to be 0.08 sec^{-1}, which corresponds closely to that found for the oxidative deamination of glutamate. The results thus support the view that the mechanism of the enzyme inhibition by EDTA is similar for the reductive amination and for the oxidative deamination. Furthermore, the results suggest that $(NH_4)_2SO_4$ inhibits the reductive amination of α-ketoglutarate by a similar mechanism as does EDTA, although the reaction rate for the inhibition is 2–3 times faster (see Figs. 5 and 10).

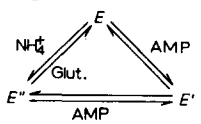
DISCUSSION

In the present paper evidence is obtained that glutamate and $(NH_4)_2SO_4$, in addition to acting as substrates for the enzyme, also act as allosteric effector molecules. At low ammonium sulphate concentration, glutamate stimulates the reductive amination of α-ketoglutarate. This stimulation is counteracted by ATP and EDTA. Thus, under certain conditions ATP and EDTA are able to inhibit both the oxidative deamination of glutamate, and the reductive amination of α-ketoglutarate.

ATP and the compounds which previously have been found to cause unidirectional inhibition of the enzyme[1] are all able to bind divalent cations. The simplest explanation of the inhibition would be that the compounds act by removal of metal ions from the media or the enzyme. However, we have been unable to demonstrate any requirements for metal ions for the reaction. The possibility cannot be excluded, however, that the enzyme contains metal ions, which are so strongly bound that they are not removed by the chelating agent, and that the main purpose of the metal ion is to participate in the binding of the inhibitory compound. The fact that the inhibition by EDTA is more efficient than is ATP, supports this view.

It has been claimed that ATP reverses the activating effect of AMP in a competitive manner[4]. The fact that ATP and EDTA do not affect the AMP activation of the reductive amination of α-ketoglutarate unless glutamate is present and that the two compounds seems to act in a linear competitive manner with AMP only at very low concentrations indicate that ATP and EDTA do not bind to the same allosteric site as AMP. Since the inhibition takes a considerable time to manifest itself, it seems likely that the inhibition involves a conformational change in the enzyme. Such conformational changes do, in general, need considerable time to be complete[11,12]. The fact that the rate of inhibition is independent of the EDTA and ATP concentration, indicates that the rate limiting step is not the interaction of the inhibitor with the enzyme but rather the structural changes induced by the binding.

On the basis of the results obtained here, as well as in previous experiments on the mechanism of activation of the enzyme by AMP[5], it is proposed that the enzyme may be present in three different conformational states as indicated below.



E represents the normal state of the enzyme. In the presence of AMP the enzyme is converted to E' in a bimolecular reaction[5]. The rate of this reaction has been shown to depend on the pH, but is independent of the presence of glutamate and NAD[5]. In the E' state the enzyme is able to catalyze the oxidative deamination as well as the reductive deamination at a much faster rate than for the E state.

In the presence of $(NH_4)_2SO_4$ the E state of the enzyme is converted to the E'' state in a time-dependent reaction. The fact that glutamate stimulates the enzyme at low ammonium sulphate concentration can be explained if it is assumed that glutamate is able to convert E'' back to the E state. In the E'' state the enzyme has considerably less activity than in the E state. It seems likely that E'' can be converted directly to E' as this reaction seems to occur in the absence of glutamate, when the reductive amination of α-ketoglutarate is measured in the presence of AMP[5]. From

earlier experiments[5] on the mechanism of activation of the enzyme by AMP, we can conclude that the apparent K_m for the substrates are different in the three states of the enzyme.

Several similarities exist between the inhibitory effect of ATP and EDTA and of $(NH_4)_2SO_4$. The finding that ATP and EDTA reverse the stimulating effect of glutamate on the reductive amination of α-ketoglutarate indicates that they act in a similar manner to $(NH_4)_2SO_4$. Furthermore, it is shown that they compete with glutamate in the oxidative deamination. It has previously been found[7] that $(NH_4)_2SO_4$ acts as a competitive inhibitor for glutamate. The rate constant for converting the enzyme from E to E'' is, however, greater for $(NH_4)_2SO_4$ than for ATP and EDTA.

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