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MAGNESIUM SENSITIVITY OF L-ASPARTATE:2-OXOGLUTARATE AMINOTRANSFERASE IN *ESCHERICHIA COLI*

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SUMMARY

Strains 619 and 961, derived from *Escherichia coli* K12, show growth inhibition by 0.4 M Mg^{2+}. The inhibition is overcome by L-aspartate. The related strain 977 exhibits Mg^{2+} resistance, which arose concurrently with a mutation to *argR*$^-$. Transduction experiments indicated that the genetic determinant for Mg^{2+} sensitivity is closely linked to, but not identical with, *argR*.

Since aspartate reverses the inhibition, aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), an enzyme of aspartate synthesis, was examined. Incubation of 961 in a growth medium containing Mg^{2+} leads to a sharp decrease in the specific activity of aspartate aminotransferase in 2 h, followed by a gradual increase. In similar experiments with 977, a greatly reduced drop and a much sharper rise occur. Extracted aspartate aminotransferase is virtually insensitive to Mg^{2+}. Extracts of Mg^{2+}-treated 961, on incubation at 37 °C, show nearly full restoration of aspartate aminotransferase activity within several hours. The reversible inactivation appears to affect the finished enzyme, in the absence of protein synthesis, as demonstrated in cell suspension experiments. Under these conditions, the inactivating effect of Mg^{2+} is enhanced by aspartate. Tests with growing cells suggest that aspartate may repress aspartate aminotransferase formation.

Growth of 961 in the presence of 0.4 M Mg^{2+} *plus* aspartate leads to relatively elevated levels of aspartate aminotransferase activity. In this case, on extraction, no appreciable restoration occurs, and in cell suspension experiments, the aspartate aminotransferase shows no Mg^{2+} sensitivity. The aminotransferase from Mg^{2+} *plus* aspartate-grown cells and the normal enzyme are similar in affinity for aspartate, sedimentation behavior, and inactivation at 60 °C, but differ in their response to heat treatment at 50 °C. It is thought that the two enzymes may be specified by the same gene, but may differ structurally, as a function of availability of Mg^{2+} during their formation.

INTRODUCTION

Magnesium ions at relatively high concentration (0.4 M) have two effects on strain 961, a derivative of *Escherichia coli* K12: (a) a pronounced lowering of growth rate and (b) a reduction in the activities of the arginine biosynthetic enzymes, acetylornithine aminotransferase (α-*N*-acetyl-L-ornithine:2-oxoglutarate aminotransferase, EC 2.6.1.11) and argininosuccinase (L-argininosuccinate arginine-lyase, EC 4.3.2.1)[1]. Strain 961 is an arginine-requiring mutant carrying a wild-type regulatory gene (*argR*$^+$) for the arginine system. The effects of Mg^{2+} are not observed in the *argR*$^-$ strain 977, which produces the enzymes of arginine biosynthesis at derepressed levels[1]. The Mg^{2+} effect on the acetylornithine aminotransferase activity represents a reversible alteration of the aminotransferase thought to arise while the enzyme, arginine (or an arginine derivative), and the protein product of the *argR*$^+$ gene are in contact[2]. The present communication reports an analysis of the Mg^{2+} effect on growth rate. Although the Mg^{2+} resistance of strain 977 is associated with the introduction of the *argR*$^-$ mutation[3], the effect on the growth rate of the sensitive strain 961 is not directly related to arginine metabolism but rather to aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), an enzyme of aspartate synthesis.

MATERIALS AND METHODS

Organisms

The organisms used were the F$^-$ strain 619, *argR*$^+$, *his*$^-$, *ile*$^-$, *met*$^-$ (ref. 4); strain 961, an arginine auxotroph derived from 619 (refs 5 and 6); and strain 977 (ref. 6), a *his*$^+$ recombinant of 961, which has received the *argR*$^-$ gene from the Hfr strain 3134 (ref. 4). Phage Plkc (ref. 7) was kindly furnished by Dr V. Bryson.

Media and cultivation

Unless otherwise indicated, the organisms were grown, at 37 °C with aeration, in glucose-salts medium supplemented with L-arginine hydrochloride, L-histidine hydrochloride, L-isoleucine, and L-methionine, each at 0.1 mg per ml (ref. 2). The magnesium treatment medium contained 0.03 M potassium phosphate (pH 7.0), 0.004 M $(NH_4)_2SO_4$, 0.001 M trisodium citrate, 0.5% glucose, and 0.4 M $MgSO_4$. For autoclaving, the glucose and $MgSO_4$ were omitted; glucose was autoclaved separately, and solid $MgSO_4 \cdot 7H_2O$ (unsterilized) was added immediately before use. When the magnesium treatment medium was used as a growth medium, it was supplemented with arginine, histidine, isoleucine, and methionine, as given above. For transduction experiments, the media of Lennox[7] and Dempsey[8] were used. Arginine-free Difco arginine assay medium was prepared from the solid commercial mixture, at a concentration of 25 g per l.

Chemicals and reagents

L-Aspartic acid, α-ketoglutaric acid, and pyridoxal 5-phosphate were obtained from Calbiochem; malate dehydrogenase, from Worthington Biochemical Corporation, and NADH, from C. F. Boehringer & Soehne G.m.b.H.

Preparation of extracts for enzyme assays

For the preparation of extracts, cells were cultivated, washed, and collected, as indicated, and suspended in 0.1 M phosphate (pH 7.0), and were disrupted, with chilling, in a MSE 100 W Ultrasonic Disintegrator. The resulting extracts were clarified at 3 °C by centrifugation at 20 000 × *g* for 30 min.

Aspartate aminotransferase assay and unit

The enzyme mediates the pyridoxal 5-phosphate-dependent conversion of L-glutamate and oxaloacetate to α-ketoglutarate and L-aspartate. The assay of Amador and Wacker[9] depends on the transamination (in the reverse of the aspartate-yielding direction) of aspartate to oxaloacetate, which gives rise to malate in the presence of malate dehydrogenase and NADH, which in turn is monitored by following the decrease in absorbance at 340 nm.

The assay is carried out at 25 °C with the aid of 1-ml quartz cuvettes (light path, 1 cm). The following reagents are used: 0.15 M L-aspartic acid in 0.04 M K_2HPO_4, adjusted to pH 7.4 with NaOH; malate dehydrogenase in 0.04 M potassium phosphate, pH 7.4 (1.0 Worthington unit per ml); 6.6 mM α-ketoglutaric acid in water, adjusted to pH 7.4 with NaOH; 1.0 mM pyridoxal 5-phosphate in water; and 0.22 mM NADH in 5 mM Tris–HCl, pH 7.4. The reaction mixture contains 0.5 ml of the aspartic acid reagent and 0.1 ml of each of the remaining reagents *plus* 0.1 ml of aminotransferase preparation (total volume 1.0 ml). The reactions are started by the addition of NADH and rapid mixing. The absorbance at 340 nm is measured repeatedly over a 5-min interval, and the decrease in absorbance per min is computed. For blank determinations, α-ketoglutarate is omitted from the reaction mixture. One unit of aspartate aminotransferase is defined as the amount of enzyme that will bring about a decrease in absorbance of 1.00 per min, under the conditions described.

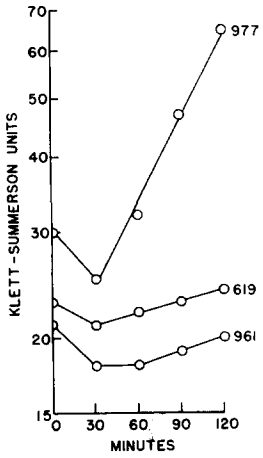
Protein determination

Protein was determined by the method of Lowry *et al.*[10]. Bovine albumin powder (Fraction V) was used as the protein standard.

EXPERIMENTAL

Growth tests on Mg^{2+}-sensitive and Mg^{2+}-resistant strains and reversal of Mg^{2+} inhibition by aspartate

The inhibition of the growth of strain 961 by 0.4 M Mg^{2+} is shown as follows. The organism is cultivated in a 250-ml sidearm flask in 40 ml of supplemented glucose-salts medium (see *Media and cultivation*). This culture (approx. 2 ml) is used to inoculate a fresh portion of the same medium, and growth is allowed to proceed in the same general manner, to a turbidity corresponding to a reading of approx. 40 in the Klett-Summerson colorimeter (No. 66 filter). The resulting culture is divided into two equal portions, which are centrifuged. One pellet, for control purposes, is suspended in the same growth medium as before (1 ml, Inoculum A). The other pellet is suspended in magnesium treatment medium (1 ml, Inoculum B). For the control culture, Inoculum A is added to supplemented glucose-salts medium (40 ml), contained in a 250-ml sidearm flask. For the experimental culture, Inoculum B is introduced into supplemented magnesium treatment medium, also contained in a 250-ml



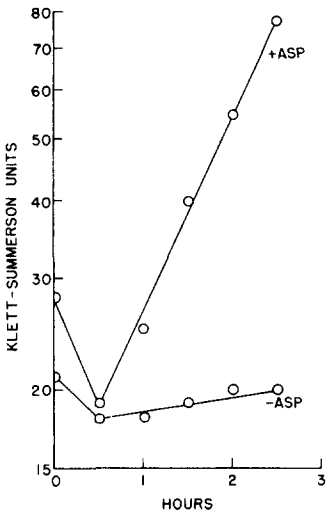


Fig. 1. Growth behavior of Mg^{2+}-sensitive (619, 961) and Mg^{2+}-resistant (977) strains in the presence of 0.4 M Mg^{2+}.

Fig. 2. Effect of aspartate on the growth inhibition of strain 961 by Mg^{2+}. Asp: L-aspartate.

sidearm flask. Incubation is carried out at 37 °C with shaking, and the turbidity of the cultures is followed.

The Mg^{2+} inhibition of the growth of strain 961 is illustrated in Fig. 1. Also shown is a similar effect of Mg^{2+} on strain 619, the arginine-independent parent of strain 961. The effect thus did not arise with the introduction of the mutation leading to the arginine requirement. Fig. 1 furthermore shows the growth behavior of the Mg^{2+}-resistant strain 977, which is an *argR*$^-$ derivative of strain 961. It was soon found, however, that the *argR*$^-$ character itself does not confer resistance to Mg^{2+} since, for example, independently isolated *argR*$^-$ derivatives proved Mg^{2+}-sensitive. As described below, the Mg^{2+} sensitivity seems to be determined by a genetic factor that is closely linked to the *argR* locus.

Experiments were then performed in an effort to pinpoint the biochemical lesion that seems to occur in strains 961 and 619 but not in strain 977, when these organisms are treated with Mg^{2+}. In growth tests, first with amino acid mixtures and then with individual amino acids, it was found that L-aspartate (0.1 mg per ml, as monopotassium salt) is able to reverse the Mg^{2+} effect, as shown in Fig. 2. L-Glutamate (0.1 mg per ml, as monosodium salt) counteracted the Mg^{2+} inhibition to an extent. These results suggest that Mg^{2+} affects a step in the synthesis of aspartate.

Genetic determinant of Mg^{2+} sensitivity

Since it was found that strain 977, in contrast to strain 961, is both *argR*$^-$ and Mg^{2+}-resistant, it seemed possible that the genetic determinant for Mg^{2+} sensitivity is closely linked to *argR*. Evidence that this is indeed the case was provided by transduction experiments performed according to the general method of Lennox[7], as modified by Dempsey[8]. The transducing phage P1kc was grown on the Mg^{2+}-resistant *argR*$^-$ arginine auxotroph 977, and the lysate obtained was used for the

transduction of the Mg^{2+}-sensitive *argR*$^+$ strain 619. Selection for *argR*$^-$ transductants was made on arginine-free Difco assay medium containing L-canavanine sulfate (0.05 mg per ml) and these transductants were tested for Mg^{2+} sensitivity. Some were found to be Mg^{2+}-resistant, and some were Mg^{2+}-sensitive, which is indicative of a fairly close linkage between the genetic determinant of Mg^{2+} sensitivity and *argR*.

Implication of aspartate aminotransferase in Mg^{2+} sensitivity

In view of the indication that Mg^{2+} affects a step in the synthesis of aspartate, aspartate aminotransferase, which mediates aspartate production, was examined in this connection. For this purpose, the Mg^{2+}-sensitive strain 961 and the Mg^{2+}-resistant strain 977 were cultivated, in the presence of 0.4 M magnesium, in supplemented magnesium treatment medium, and the activity levels of aspartate aminotransferase were followed (see Materials and Methods). The results obtained are listed in Table I. It can be seen that, in the case of the sensitive strain, the relative specific

TABLE I

ASPARTATE AMINOTRANSFERASE LEVELS OF STRAINS 961 AND 977 ON INCUBATION IN MAGNESIUM TREATMENT MEDIUM (AS RELATIVE SPECIFIC ACTIVITY)*

Strain	*Response to Mg^{2+}*	*Incubation (h)*	*Relative specific activity*
961	Sensitive	1	43
961		2	13
961		3	32
961		4	57
977	Resistant	1	58
977		2	40
977		3	102
977		4	105

* See the text for details. The relative specific activity of the inocula is taken as 100.

activity drops to a level of 13% within 2 h. Thereafter, the relative activity gradually increases. In the case of the resistant strain, the minimum relative specific activity is higher by a factor of three and the initial activity level is exceeded within 3 h. These results, therefore, support the inference that the Mg^{2+} sensitivity of the normal aspartate aminotransferase is responsible for the inhibition of the growth of the sensitive strain by Mg^{2+}. The lesser sensitivity of the aminotransferase of the resistant strain is in harmony with this inference.

Insensitivity of extracted aspartate aminotransferase to Mg^{2+}

To test whether the Mg^{2+}-dependent reduction in aminotransferase activity, which was observed in growing cells, is demonstrable under cell-free conditions, the following experiment was carried out. The sensitive strain 961 was cultivated in glucose-salts medium supplemented with the required amino acids. The organisms were collected by centrifugation and suspended in magnesium treatment medium *minus* $MgSO_4$ and glucose. A cell-free extract was prepared (see Materials and Methods) and was divided into four portions. To one portion, no Mg^{2+} was added, and

the remaining three portions were made 0.02 M, 0.1 M and 0.4 M in Mg^{2+}. The four portions were incubated at 37 °C for 2 h and were individually dialyzed at 3 °C, first against Mg^{2+}-free and glucose-free magnesium treatment medium for 2 h, and subsequently against 0.1 M phosphate buffer (pH 7.0) for 16 h. Aminotransferase assays of the four dialyzed preparations showed that Mg^{2+} up to a concentration of 0.4 M does not produce a lowering of the activity of the extracted enzyme. It therefore appears that the intracellular environment of the aminotransferase offers structural features that are required for the diminution of enzyme activity in the presence of Mg^{2+}.

Restoration of aspartate aminotransferase activity

Since it seemed possible that the loss of activity of the aminotransferase reflects a structural alteration of the enzyme in response to Mg^{2+}, in the context of the appropriate intracellular environment, it was endeavored to obtain a restoration of aminotransferase activity in cell extracts. Strain 961 was subjected to Mg^{2+} treatment for 2 h, in 40-ml batches, as described above. The organisms from four such batches were harvested, washed with distilled water by centrifugation, suspended in 4 ml of 0.1 M phosphate (pH 7.0), and disrupted sonically. The extract obtained was tested for *in vitro* restoration of enzyme activity, as follows. The extract was maintained at 37 °C, without any other treatment, and samples for assay of the aminotransferase activity were taken at intervals from 0 to 6 h. As can be seen in Fig. 3, over a period of 4 to 6 h, a recovery of enzyme activity from an initial 12% level to a 97% level was noted. The aminotransferase activity of control extracts from organisms not subjected to Mg^{2+} treatment remains virtually constant during maintenance of the extracts at 37 °C for 6 h. It is inferred that, as a result of the Mg^{2+} treatment, the aminotransferase undergoes a structural alteration that appears to be reversible on keeping the enzyme in cell-free extract.

Cell suspension experiments

Since the inferred alteration of aspartate aminotransferase in response to Mg^{2+}

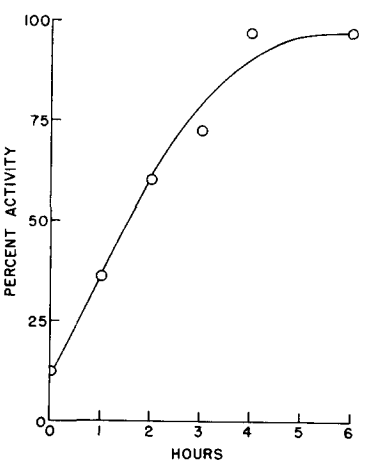


Fig. 3. Restoration of aspartate aminotransferase activity at 37 °C. The source of the enzyme was strain 961 cultivated in the presence of 0.4 M Mg^{2+}.

was indicated to occur in cells present in a growth medium, but not in cell extracts, cell suspension experiments were performed under conditions where protein synthesis does not occur as a result of amino acid deprivation. Washed cells of strain 961 were suspended in magnesium treatment medium from which the Mg^{2+} had been omitted, so as to give a Klett-Summerson reading of approximately 100. The amino acids required for the growth of the strain were withheld. Six 40-ml portions of the suspension, with additions as indicated below, were incubated at 37 °C with shaking. The additions were (a) potassium L-aspartate, (b) sodium L-glutamate, (c) $MgSO_4$, (d) $MgSO_4$ and potassium L-aspartate, and (e) $MgSO_4$ and sodium L-glutamate; a flask with no addition was included. Aspartate and glutamate were provided at 1 mg/ml, and Mg^{2+} at 0.4 M. Incubation was carried out for 2 h, and 10-ml samples of the suspension were taken for assays. The cells from each sample were harvested by centrifugation, washed with distilled water, suspended in 2 ml of 0.1 M phosphate (pH 7.0), and disrupted sonically. The extracts were clarified by centrifugation at 3 °C, and were assayed for aspartate aminotransferase activity and protein content. The results are given in Table II. The treatment with Mg^{2+} is seen to lower the relative

TABLE II

EFFECT OF MAGNESIUM, ASPARTATE, AND GLUTAMATE ON THE ASPARTATE AMINOTRANSFERASE ACTIVITY OF CELL SUSPENSIONS OF STRAIN 961

The cells were incubated in the absence of the amino acids required for growth, with additions as indicated. The results are expressed relative to the specific activity of the inoculum (0.250 unit/mg protein) taken as 100. See the text for details.

Addition to medium	*Relative specific activity*
None	100
L-Aspartate	97
L-Glutamate	96
Mg^{2+}	46
Mg^{2+} + L-aspartate	18
Mg^{2+} + L-glutamate	25

specific activity to 46%, and this lowering is considerably enhanced when aspartate is present in addition to Mg^{2+}. Glutamate in the place of aspartate gives a similar, although less pronounced, effect. Incubation with aspartate or glutamate as sole additions has virtually no effect on the activity. The table also shows that the activity is completely stable when the cells are incubated without any additions. These results with cell suspensions, taken together with the results on the restoration on the activity of extracts, support the view that the Mg^{2+} effect reflects a reversible alteration of finished enzyme.

Effect of aspartate on the aspartate aminotransferase activity of growing cells

In view of the enhancement of the Mg^{2+} effect by aspartate (and to a smaller degree, by glutamate) in cell suspension, the effect of aspartate and glutamate on the aspartate aminotransferase activity of growing cells was examined. For this purpose, strain 961 was cultivated in the usual manner, at 37 °C, with shaking, in glucose-salts medium, supplemented with L-arginine hydrochloride, L-histidine hydrochloride, L-

TABLE III

EFFECT OF ASPARTATE AND GLUTAMATE ON THE ASPARTATE AMINOTRANSFERASE ACTIVITY OF GROWING CELLS OF STRAIN 961

See the text for details. The results are expressed relative to the aminotransferase activity of the inoculum (0.300 unit/mg protein) taken as 100.

Addition to medium	*Relative specific activity*
None	100
L-Aspartate	25
L-Glutamate	45

isoleucine, and L-methionine (0.1 mg/ml, each). Additionally, potassium L-aspartate or sodium L-glutamate (1.5 mg/ml) was added to the medium as indicated. The cells were allowed to grow from a reading of 8 to a reading of 40 in the Klett-Summerson colorimeter and were harvested by centrifugation. Extracts were then prepared and were assayed for aminotransferase activity and protein (see Materials and Methods). The results obtained are listed in Table III. The data show that cultivation in the presence of aspartate leads to a sharp decrease in specific activity. Glutamate, possibly after conversion to aspartate, also gives a decrease which, however, is smaller than that obtained with aspartate. The action of aspartate may include a repression phenomenon, although continued cultivation in the presence of aspartate does not lead to a commensurate drop in specific activity. It is noteworthy that, in cell suspension experiments described above, aspartate had no appreciable effect on the activity of the aminotransferase.

Effect of aspartate and Mg^{2+} on the aspartate aminotransferase activity of growing cells

The combined effect of aspartate and Mg^{2+} on the aminotransferase activity of growing cells was examined as follows. Cells of strain 961 were grown as usual in magnesium treatment medium, supplemented with arginine, histidine, isoleucine, and methionine, either in the absence or the presence of potassium L-aspartate (1 mg/ml). Incubation was carried out for the period indicated, and samples of the cells were collected, disrupted sonically, and assayed for aminotransferase activity and protein content. As shown in Table IV, when aspartate is omitted, the low relative specific activity characteristic of the magnesium effect is obtained. On

TABLE IV

EFFECT OF ASPARTATE *Plus* MAGNESIUM ON THE ASPARTATE AMINOTRANSFERASE ACTIVITY OF GROWING CELLS OF STRAIN 961

See the text for details. The results are expressed relative to the specific activity of the inoculum (0.300 unit/mg protein) taken as 100.

L-*Aspartate*	*Incubation (h)*	*Relative specific activity*
Omitted	2	11
Added	2	78
Added	4	214

addition of aspartate much higher activities are noted, which can exceed the specific activity of the inoculum by a considerable margin. These findings are in sharp contrast to the results obtained with cell suspensions, in which case it was observed that aspartate further lowers the specific activity compared to the value obtained with Mg^{2+} alone.

Attempted restoration of aminotransferase activity from aspartate plus Mg^{2+}-grown cells and lack of Mg^{2+} sensitivity of the enzyme from such cells

In restoration experiments like those described above, no appreciable restoration of aminotransferase activity was demonstrable in extracts of cells of strain 961 grown in the presence of potassium L-aspartate (1 mg/ml) *plus* 0.4 M Mg^{2+}. In cell suspension experiments, the aminotransferase from such cells of strain 961 grown in this manner is not susceptible to the Mg^{2+} effect, whether aspartate is added to the suspension or not. It would thus appear that cells grown in the presence of Mg^{2+} and aspartate, under the conditions described, produce a species of aspartate aminotransferase that is Mg^{2+}-insensitive.

Some properties of aminotransferase from cells grown with or without aspartate plus Mg^{2+}

For a comparison of some properties of aminotransferase corresponding to different growth conditions, batches of cells of strain 961 were cultivated (a) in the presence of and (b) in the absence of potassium L-aspartate (1 mg/ml) *plus* 0.4 M $MgSO_4$. The cells from each of these batches were collected, and extracts were prepared, clarified by centrifugation at 10 000 $\times$ *g* for 20 min, and dialyzed against 0.1 M potassium phosphate buffer (pH 7.0) at 3 °C for 18 h.

Centrifugation[11] in linear gradients of 5–25% sucrose did not show any detectable difference in sedimentation behavior for aminotransferase from the two sources.

For additional experiments, the dialyzed aminotransferase preparations (8 ml each, approx. 10 mg protein per ml) were partially purified at 0–3 °C, as follows. Each preparation received 1.73 g powdered $(NH_4)_2SO_4$, and the precipates obtained were removed by centrifugation. To each supernatant liquid, 1.73 g $(NH_4)_2SO_4$ were added. The resulting precipitates were separately collected, dissolved in 0.1 M phosphate (pH 7.0), and dialyzed against the same buffer to give solutions (5 ml each) containing approximately 3.5 mg protein per ml and having a specific aminotransferase activity of approx. 2 units per mg protein.

In experiments on the effect of substrate concentration on reaction velocity, the partially purified aminotransferase preparations from the two sources behaved fairly similarly; half-maximal velocity occurred at approx. 1.5 or 2 μmoles of L-aspartate per ml for the preparation from organisms without or with aspartate *plus* $MgSO_4$, respectively.

Heat treatment experiments were performed with the two partially purified enzyme preparations as follows. The preparations (in pH 7.0 phosphate buffer, at 3.5 mg protein per ml) were heated in test tubes, in water baths at the desired temperature, and samples were taken at the intervals indicated, chilled, and assayed for aminotransferase activity. For treatment at 60 and 50 °C, samples were taken at 2-min and 20-min intervals, respectively. The results obtained are illustrated in Figs 4A and 4B. At 60 °C, the activity of aminotransferase from the two sources decreases by over 80%, within 8 min, in virtually the same manner (A). At 50 °C,

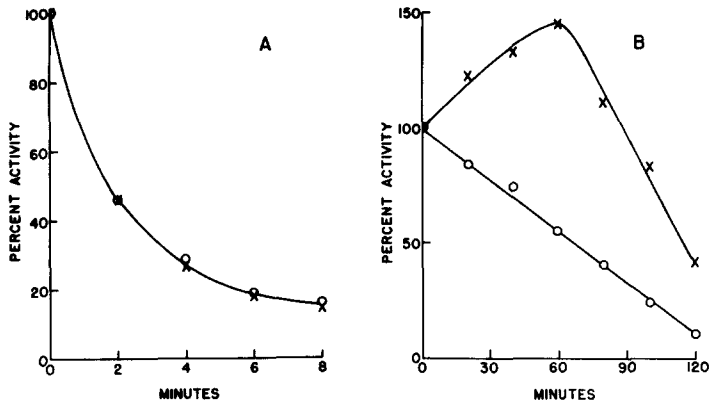


Fig. 4. Activity of aminotransferase from strain 961 grown without (×—×) or with (○—○) an aspartate *plus* Mg^{2+} supplement, as a function of time of heat treatment of the enzyme preparations at 60 °C (A) or 50 °C (B).

the aminotransferase activity from Mg^{2+} *plus* aspartate-grown cells diminishes steadily by nearly 90% within 120 min; in contrast, aminotransferase from cells grown without the Mg^{2+} *plus* aspartate supplement shows a heat activation effect, with maximal activity occurring at approximately 60 min (B).

DISCUSSION

Experiments with strain K12 have shown that aspartate aminotransferase, an enzyme of aspartate synthesis, is Mg^{2+}-sensitive in that the finished enzyme molecules seem to undergo a reversible (presumably conformational) alteration on exposure of the intact cells to Mg^{2+} at relatively elevated concentration (0.4 M). This case exhibits some interesting parallels to that of acetylornithine aminotransferase[1,2]. Both aspartate aminotransferase and acetylornithine aminotransferase are pyridoxal 5-phosphate-dependent aminotransferases. Both enzymes are Mg^{2+}-sensitive in whole cells but not in extracts. For acetylornithine aminotransferase, a particularly pronounced effect is observed in cell suspension experiments under repressive conditions, in the presence of arginine. Analogously, for aspartate aminotransferase, the Mg^{2+} effect in cell suspension is enhanced by aspartate and, although repression of this aminotransferase by aspartate has not been definitely established, the data on enzyme levels of cells grown with added aspartate are consistent with repressibility of the enzyme. The present experiments on cell suspensions, carried out in the absence of certain amino acids required for protein synthesis, seem to show convincingly that it is indeed the finished enzyme that is effected. The results of the restoration experiments with enzyme extracts indicate that the modification of the aspartate aminotransferase, like that of the other aminotransferase is reversible. Thus, an intriguing situation emerges where two functionally related enzymes, under very comparable physiological conditions, seem to undergo very similar changes in response to elevated Mg^{2+} concentrations. In both cases, the enzyme level is lowered without being completely abolished, and the original activity is virtually completely restored by maintaining the extracted enzymes at 37 °C for several h. It is tempting

to speculate that the affected regions of the two enzymes represent functionally (and perhaps evolutionarily) related parts or subunits, such as those involved in the binding of pyridoxal 5-phosphate or glutamate. For acetylornithine aminotransferase, it was inferred that the Mg^{2+} exerts its effect while the finished enzyme is in contact with functional repressor[2].

Support for the idea that the Mg^{2+} sensitivity of the normal aspartate aminotransferase underlies the growth inhibition of such strains as 619 and 961 by Mg^{2+} comes from the finding that aspartate reverses the growth inhibition and from the observation that the aspartate aminotransferase activity of strain 977 (which is resistant to the growth inhibition) shows a much less pronounced drop and a faster rise in response to Mg^{2+}, compared to the aminotransferase activity of the sensitive strains.

The transduction experiments with strain 977 as the donor indicate that the gene responsible for Mg^{2+} sensitivity is closely linked to *argR*. In view of the results with aspartate aminotransferase, it might tentatively be inferred that the genetic determinant for Mg^{2+} sensitivity is the structural gene for this enzyme; however, no direct evidence is available. Deficiency mutants for the aminotransferase apparently are unknown.

The experiments on the aspartate aminotransferase from strain 961 grown in the presence of relatively high concentrations of Mg^{2+} and aspartate seem of particular interest. The findings are that (a) the aminotransferase activity of cells grown in this manner can be considerably greater than usual, (b) incubation of the extracted aminotransferase does not give a restoration effect, (c) in cell suspension experiments, the enzyme appears to be insensitive to 0.4 M Mg^{2+}, even in the presence of aspartate, (d) compared to the normal aminotransferase, the Mg^{2+}-insensitive enzyme shows similar substrate affinity, sedimentation, and 60 °C heat inactivation behavior, and (e) there is a differential response to heat treatment at 50 °C. As for the origin of the Mg^{2+}-insensitive aminotransferase, we suggest the hypothesis that the normal and the insensitive enzymes are specified by the same gene and that, in cells grown with 0.4 M Mg^{2+} *plus* aspartate, the nascent enzyme undergoes a modification that preserves the aminotransferase activity but renders the enzyme insensitive to subsequent modification by Mg^{2+} (such as occurs in the normal case). Thus, two species of aminotransferase are postulated which could account for the similarities as well as the differences between the sensitive and insensitive enzymes.

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