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PROTEIN FACTOR WHICH INDUCES CONVERSION BETWEEN *PHYSARUM* ORNITHINE DECARBOXYLASE FORMS IN VITRO

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Summary

The rapid activity modulation of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) in *Physarum polycephalum* is closely correlated with a reversible post-translational modification of this enzyme. A factor has now been isolated from homogenates of exponentially-grown microplasmodia which catalyzes the conversion of the active enzyme form, A, into its less active, B state. Partial purification of this A-B converting factor has been achieved using DEAE-Sephacel chromatography and Ultrogel AcA-34 gel filtration. It appears to be a heat labile, acidic protein with a molecular weight of about 35 000 which binds to large macromolecules in crude fractions isolated using low ionic strength buffers. The in vitro converting reaction requires the presence of spermidine or spermine (1 mM) while putrescine is much less effective, and inorganic cations are ineffective at levels up to 5 mM. Enzyme conversion is reduced in elevated ionic strengths and in the presence of polyamine chelators such as ATP, ADP or GTP (1.0 mM). Under current assay conditions the interaction between ornithine decarboxylase and this factor is stoichiometric, yet it is not reversed even by conditions which favor dissociation of protein-protein interactions. This is the first report of the isolation of a protein factor which is involved in the interconversion of ornithine decarboxylase between its alternate enzyme states.

Introduction

In most tissues the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17), the initial enzyme in eukaryotic polyamine biosynthesis, is easily altered by changes in hormonal, nutritive or ionic environment, as well as

other factors which influence cell growth and differentiation [1]. The extreme sensitivity and speed of this response suggests the involvement of post-translational mechanisms in the modulation of this enzyme's activity. Indeed heat-labile, macromolecular cytoplasmic factors, such as ornithine decarboxylase-antizyme [2—4] and ornithine decarboxylase inhibiting factor ('ODIF') [5], have been implicated in the inactivation of ornithine decarboxylase. Other heat labile factors have been discovered, in *Escherichia coli* [6] and sea urchin eggs [7], which serve to directly enhance ornithine decarboxylase activity.

Alternately post-translational modulation of ornithine decarboxylase has been suggested to occur through reversible covalent modification of this enzyme protein [8,9]. In several instances [9—12] the mammalian enzyme has been found to exist in such multiple, catalytically distinct forms, yet their involvement in the modulation of this enzyme's activity has not been demonstrated. To the contrary, variations in the activity of ornithine decarboxylase in the lower eukaryote *Physarum polycephalum* have been shown to be closely correlated with a reversible post-translational modification of this enzyme [8,9, 13,14]. In this organism ornithine decarboxylase exists in either an active (A-form) state, which has a high affinity (K_m = 0.13 μM) for the coenzyme pyridoxal 5'-phosphate, or a less active (B-form) state which is fully activated only by unphysiologically high levels of this coenzyme (K_m = 33 μM). Total enzyme activity (that expressed by saturating coenzyme concentrations) changes only slowly when this system is perturbed by alterations in external polyamines [13], ionic strength [14], metabolic inhibitors [8] or nutrient condition, as well as normal progression through the cell cycle [15], yet these changes will stimulate very rapid alterations in the activity of the A form of this enzyme as indicated by assays using low pyridoxal phosphate concentrations. Although 15 min is sufficient for the majority of the enzyme molecules to be converted from the A to the B form, or from B to A form in vivo, these enzyme states have not been found to be altered once the cell has been disrupted. The in vivo enzyme form ratio is generally maintained during purification and eventual separation of the enzyme forms by hydroxyapatite chromatography or isoelectric focusing [9]. These alternate enzyme states, which coelute from gel filtration columns as molecules of about 80 000, appear to differ by a covalent modification which alters their molecular charge [9,16], yet this modification has not as yet been identified. Recently we noted that, although the addition of polyamines to purified ornithine decarboxylase does not alter its catalytic form [13], their addition to crude cell lysates induces a conversion of the active form of this enzyme to the less active state. This discovery is of intense interest in that it represents an in vitro demonstration of a portion of this post-translational modulation of ornithine decarboxylase. In this paper we report the isolation and partial characterization of a protein factor from homogenates of *Physarum*, which is responsible for the polyamine-stimulated modification of this important enzyme.

Materials and Methods

Chemicals. Pyridoxal 5'-phosphate, L-ornithine, Blue Dextran, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (Hepps), dithiothreitol, EDTA, soybean

trypsin inhibitor, trypsin and the chloride salts of putrescine, spermidine and spermine were purchased from Sigma Chemical Co; Sephadex G-100 and DEAE-Sephacel from Pharmacia Fine Chemicals, Inc; Ultrogel AcA-34 from LKB; and L-[1-^{14}C]ornithine · HCl (50 Ci/mol) from Amersham/Searle Corp.

Culture techniques. Cultures of *P. polycephalum* were maintained and sampled as described previously [17]. Only exponentially-growing shake-flask cultures of asynchronous microplasmodia were used in these experiments.

Ornithine decarboxylase assay. The details of the assay procedure have been previously reported [14]. A-form enzyme activity was determined using 0.01 M Hepps buffer (pH 8.0)/0.5 mM dithiothreitol/0.5 mM EDTA/0.5 μM pyridoxal 5′-phosphate/0.1 mM L-[1-^{14}C]ornithine (0.02 μCi). Total assayable enzyme was determined using pH 8.4 Hepps and 100 μM pyridoxal 5′-phosphate. The difference between these assays was attributed to the B enzyme form. 1 unit of activity was designated to be 1 nmol CO_2 released/h.

Ornithine decarboxylase purification. Partially purified preparations (250—500-fold) or ornithine decarboxylase used in the detection and characterization of the conversion factor were prepared as described by Mitchell et al. [13].

Assay of A to B conversion factor. Converting activity was detected by measuring the loss of A-form activity induced by mixing a 0.05—0.5 ml sample of converting factor in 0.02 M Hepps (pH 8.0) buffer (0.5 mM EDTA/2.0 mM $MgCl_2$/1.0 mM spermidine/2.5 mM dithiothreitol/0.01—0.05 ml partially purified ornithine decarboxylase in the same buffer. Samples (0.05 ml) extracted immediately upon mixing and after 60 min incubation at 30°C were assayed for A-form and total ornithine decarboxylase activity. During this time period total activity remained constant, while A-form enzyme decreased in proportion to the amount of conversion factor present.

Trypsin inactivation of conversion factor. Trypsin and $CaCl_2$ were added to purified conversion factor to final concentrations of 0.5 μg/ml and 10 mM, respectively. After incubation for 10 min at 30°C, soybean trypsin inhibitor was added to a concentration of 1.0 μg/ml. Conversion factor activity was assayed in this fraction as well as a control which had been similarly treated with calcium and trypsin inhibitor but without initial trypsin exposure.

Results

Form conversion in crude homogenates

Sonicates of exponentially growing *P. polycephalum* contain both active (A) and less active (B) forms of ornithine decarboxylase. Although these alternate enzyme states rapidly interconvert in vivo, previous reports suggest a lack of such enzyme modification in vitro [9,16]. As shown in Fig. 1, however, incubation of such crude homogenates at 30°C in the presence of 1 mM spermidine induced a progressive decrease in the A-form of ornithine decarboxylase while the total enzyme activity remained relatively constant. Consistent with previous in vivo studies, such a decrease in A-form enzyme without loss of total assayable activity is interpreted to indicate a conversion of the A-form enzyme into B. Thus, these crude homogenates were shown to contain a factor which catalyzes this change in ornithine decarboxylase from form A to B. Of interest, ATP (1 mM) was found to prevent this conversion reaction (Fig. 1), yet ATP

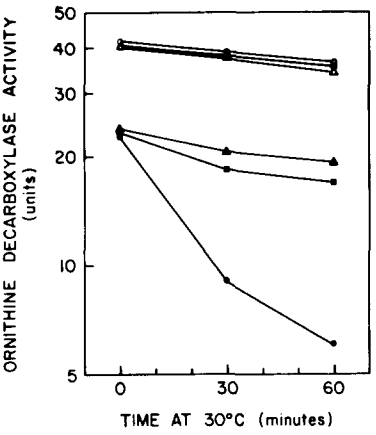


Fig. 1. Spermidine-induced enzyme form conversion in crude homogenates. Exponentially-grown microplasmodia were disrupted by sonication in 10-times their volume of 0.01 M Hepps (pH 8.1) buffer/1 mM EDTA/1 mM dithiothreitol. Aliquots of this crude homogenate were incubated at 30°C with no additions (△,▲), 1 mM spermidine (○,●) or 1 mM spermidine plus 1 mM ATP (□,■). At time 0, 30 and 60 min, 0.1 ml samples were withdrawn and assayed for A form (closed data points) or total (open units) ornithine decarboxylase activity as detailed in the text.

concentrations up to 10 mM, even in the absence of spermidine, did not promote ornithine decarboxylase B to A conversion (data not shown). The partial inhibition of A-form to B-form conversion appeared common to nucleotides as GTP, UTP and ADP also were effective, while equimolar Mg^{2+} or polyamines overcame this effect.

Isolation of form-converting activity

Extensive dialysis of these crude homogenates did not diminish their ability to alter the form of ornithine decarboxylase, yet heating these fractions to 90°C for 10 min completely inhibited this response. In the latter experiment purified *Physarum* ornithine decarboxylase was added to the homogenate to replace the heat-killed enzyme. Such purified enzyme had been previously shown not to be converted between alternate enzyme states by the addition of polyamines [13]. Since this A-B converting activity was observed to be present in crude homogenates and not in purified ornithine decarboxylase it appeared reasonable to assume that this reaction was induced by a heat labile, nondialyzable factor which could be isolated away from this enzyme. Such a separation was attempted using the conversion of added, purified ornithine decarboxylase as a bioassay for this factor. For convenience, 1 unit of A-B conversion activity was established to be responsible for changing 1 unit of A-form enzyme to B in 60 min when using 0.02 M Hepps (pH 8.0) buffer/0.5 mM EDTA/2 mM $MgCl_2$/1 mM spermidine/2.5 mM dithiothreitol at 30°C.

Columns of a weak anion exchange resin, DEAE-Sephacel, readily bound both ornithine decarboxylase and the A-B converting activity from crude homogenates of *Physarum*. When eluted with a 0.1—0.3 N NaCl gradient, ornithine decarboxylase emerged as a sharp peak at about 0.15 N salt (Fig. 2). The A to B converting activity emerged as a much broader peak which overlapped the decarboxylase. Our initial attempts to recover conversion activity from this

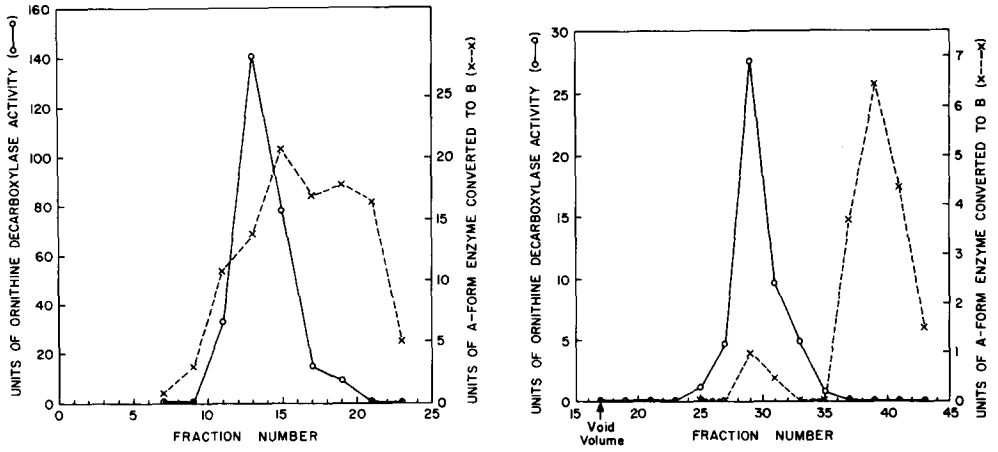


Fig. 2. Elution of A-B conversion factor from a DEAE-Sephacel column. Acetone precipitable material from exponentially-grown microplasmodia was suspended by sonication in 0.02 M Hepps (pH 8.0) buffer/0.5 mM EDTA/2 mM $MgCl_2$/0.1 M NaCl/2.5 mM dithiothreitol and applied to a 35 ml column of DEAE-Sephacel equilibrated with the same buffer. The column was eluted with a 200 ml gradient of NaCl from 0.1 to 0.3 N, and 5 ml fractions collected. 1-ml samples of the indicated fractions were dialyzed overnight against the isolation buffer without NaCl, and subsequently assayed for total ornithine decarboxylase (o———o) and conversion factor (X———X) activity as indicated in Materials and Methods.

Fig. 3. Separation of ornithine decarboxylase and converting factor on an Ultrogel AcA-34 gel filtration column. Fractions 13 through 20 of Fig. 2 were concentrated on a Amicon ultrafiltration membrane PM-10, and 2.5-ml fractions eluted through a 170 ml column of Ultrogel AcA-34 equilibrated with the initial extraction buffer. The indicated 4-ml fractions were assayed, as in Fig. 2, for total ornithine decarboxylase (o———o) and conversion factor (X———X) activity.

column were thwarted by the fact that NaCl concentrations as low as 0.1 N strongly inhibit conversion activity. We therefore found it essential to dialyze portions of the effluent samples in preparation for this assay.

Additional purification and greater separation between the decarboxylase and the converting activity were achieved when the peak of conversion activity from the ion exchange column was concentrated on an Amicon membrane filter (PM-10) and eluted through an Ultrogel AcA-34 filtration column (Fig. 3). As with the ion exchange column, however, some of the A-B converting activity remained associated with the ornithine decarboxylase. The delayed elution of the A-B converting activity from the gel filtration column indicated that this factor was significantly smaller than ornithine decarboxylase. Similar elutions of this converting factor from calibrated Sephadex G-100 columns in buffer containing 0.3 N NaCl suggested that this factor has a molecular weight of about 35 000. Gel filtration column chromatography of crude homogenates in low ionic strength buffer resulted in A-B conversion activity emerging with the column void volume and spreading over several of the early eluted fractions. Thus, it appears that this converting factor either polymerizes extensively or readily aggregates with other macromolecules in the crude homogenate.

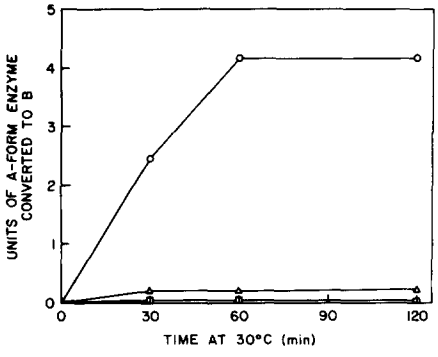
Partially purified A-B conversion factor was prepared by concentrating, using Amicon PM-30 membrane filtration, the most active fractions of columns prepared and eluted as described in Fig. 3. Such conversion factor preparations,

which were found to be stable for 2—4 days at 4°C, were used for subsequent characterization of this reaction.

Time course of A to B enzyme form conversion

Polyamine-stimulated conversion of A-form ornithine decarboxylase to B had frequently been observed to occur for only 45—60 min in crude homogenates. We examined the time course of this reaction using purified enzyme and partially purified A-B conversion factor prepared as above. As shown in Fig. 4 this conversion reaction also stops within 60 min even though there remains an excess of A-form ornithine decarboxylase. In the particular experiment shown, the added purified enzyme contained about 22 units of A and 6 units of B, and conversion was completed when about 18 units of A and 10 units of B remained. This reaction did not stop due to lack of spermidine as the addition of another 1 mM of the polyamine failed to elicit additional conversion (data not shown).

One possible explanation of such abrupt cessation of conversion activity after 45—60 min is that this converting factor was in some way depleted by its reaction with the decarboxylase. To test this, conversion experiments were performed where the level of purified ornithine decarboxylase was held constant, while the amount of conversion factor added to the reaction mixture was varied. As shown in Fig. 5 there appears to be a linear relationship between maximal conversion and amount of conversion factor added, up to the greatest concentration of conversion factor tested, which changed approx. 50% of the available A-form enzyme to B. Complementary experiments were performed where the concentration of conversion factor was held constant and the amount of purified ornithine decarboxylase varied. Consistent with the above



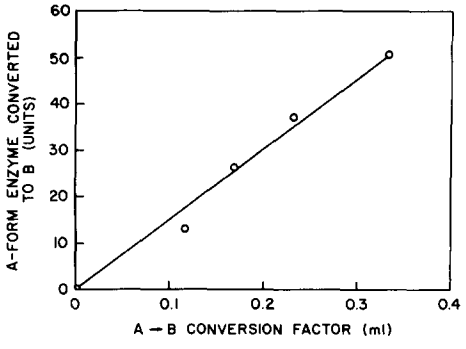


Fig. 4. Time dependence of A to B conversion reaction. Purified conversion factor was prepared by concentrating the peak activity fractions of gel filtration columns such as described in Fig. 3. A sample of this factor was added to 28 units (22 units A and 6 units B) of purified ornithine decarboxylase in the presence (○———○) or absence (△———△) of 1.0 mM spermidine. A control flask contained an equivalent amount of enzyme in buffer with 1.0 mM spermidine but without any conversion factor (□———□). At the indicated times, samples were withdrawn and assayed for A-form and total ornithine decarboxylase enzyme.

Fig. 5. Relationship between amount of conversion factor and the amount of A-form enzyme altered. Purified ornithine decarboxylase containing 115 units of A-form enzyme was mixed with varying amounts of conversion factor prepared as in Fig. 4. After 60 min, when all reactions were complete, the amount of A-form enzyme converted to B was estimated as in the text.

result, the total amount of A-form enzyme converted to B was not altered as long as the decarboxylase was in excess. These observations strongly suggest that this converting factor acts stoichiometrically rather than catalytically under these in vitro conditions.

Characterization of the A-B conversion reaction

In characterization of this form conversion reaction attempts were made to distinguish between factors which altered the final stoichiometry of this reaction and those parameters which served to alter the time required to attain this stoichiometric equilibrium. Varying the reaction temperature, for example, did not affect the amount of A-form enzyme which reacted with a given quantity of conversion factor, but it did inversely alter the time required for completion of this reaction. Similarly, reduction of spermidine levels in the reaction to 0.1 mM, which reduced normal 60 min conversion by 90%, did not inhibit the eventual attainment of the same conversion level as the 1 mM spermidine control. Where significant conversion was not evident within the first 2 h, as in the absence of spermidine (Fig. 4), it was assumed that the conversion reaction had been effectively prevented.

As shown in Table I the conversion reaction appears to specifically require spermidine or spermine, while putrescine is considerably less effective and inorganic divalent cations elicit essentially no response. This summary table also shows that elevating the ionic strength of the reaction buffer with NaCl, or adding ATP, each diminish the conversion reaction. Similarly, pretreatment of

TABLE I

FACTORS INFLUENCING THE A-B CONVERSION REACTION

This table summarizes the results of nine overlapping experiments on the factors necessary for optimal enzyme form conversion. Since 1.0 mM spermidine was found to stimulate complete reaction between the conversion factor and ornithine decarboxylase within 60 min, this reaction was valued at 100% (control) and used as the basis for comparison of the various experiments. The basic conversion reaction (no addition) contained partially purified conversion factor and excess partially purified ornithine decarboxylase in 0.02 M Hepps buffer (pH 8.0)/0.5 mM EDTA/2 mM $MgCl_2$ (except when ATP was tested)/2.5 mM dithiothreitol. Treatment of conversion factor with trypsin was described in Materials and Methods. Conversion responses less than 5% of the spermidine control were essentially undetectable.

Addition to conversion reaction	Fraction of control response after 60 min (%)
No addition	0
1.0 mM spermidine (control)	100
0.1 mM spermidine	10.3
1.0 mM spermine	88.7
1.0 mM putrescine	6.7
5.0 mM Mn^{2+}	<5
5.0 mM Ca^{2+}	<5
1.0 mM spermidine + 0.2 N NaCl	<5
1.0 mM spermidine + 1.0 mM ATP	40
1.0 mM spermidine + 5.0 mM ATP	<5
1.0 mM spermidine + trypsin inhibitor	82
1.0 mM spermidine + trypsin inhibitor + trypsin-treated conversion factor	<5

the partially purified conversion factor preparation with trypsin essentially eliminates all A- to B-form conversion.

Characterization of B-form enzyme produced by the partially purified conversion factor

Since the amount of conversion factor protein added directly determined the units of A-form enzyme converted to B, it was reasoned that the conversion factor functioned by binding to the A-form, thereby altering its affinity for coenzyme and mimicking the catalytic properties of ornithine decarboxylase B.

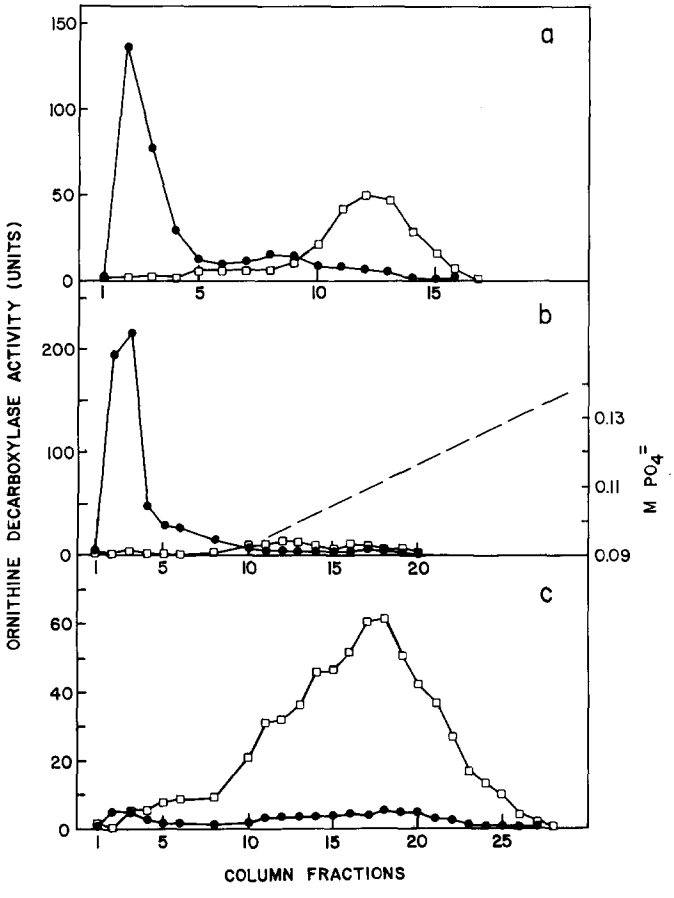


Fig. 6. Differential elution of alternate ornithine decarboxylase forms from hydroxyapatite columns. All samples were dialyzed against 0.09 M phosphate buffer (pH 7.2; 2 mM dithiothreitol/0.5 mM EDTA/4 μM pyridoxal 5'-phosphate) and applied to 2 ml hydroxyapatite columns. Material not adsorbing to the column at this ionic strength was assayed in fractions number 1—9. After this application and wash, the columns were eluted with a 100 ml phosphate gradient from 0.09 to 0.2 M. 2.8-ml fractions were collected in panel a and 2.0 ml in panels b and c. Fractions up to number 30 were assayed for the A (●———●) and B (□———□) forms. In all cases approx. 1000 units of enzyme activity were applied, and generally 60—70% was recovered. Panel a: purified ornithine decarboxylase containing approximately equal amounts of each enzyme form. Panel b: purified ornithine decarboxylase containing 89% A-form activity. Panel c: another aliquot of the same sample shown in panel b was treated for 2 h with 1100 units of conversion factor in 0.02 M Hepps buffer (pH 8.0; 2 mM dithiothreitol/0.5 mM EDTA), then dialyzed into the phosphate buffer and chromatographed as in panel b.

Yet, when 90% A-form enzyme was treated with conversion factor to yield 90% B-form there was no corresponding alteration in the effective size of the enzyme as determined by elution from Sephadex G-200 in 0.05 M phosphate buffer (pH 7.2; 2 mM dithiothreitol). Thus, any association between this converting factor and ornithine decarboxylase must be reversible, while the alteration in the enzyme is stable, under these column conditions.

To demonstrate that the in vitro enzyme conversion produced B-form enzyme similar to that isolated directly from cultures, we separated the enzyme forms chromatographically before and after treatment with partially purified conversion factor. Fig. 6a shows the separation of these two enzyme states on a hydroxyapatite column when the applied purified sample contained approximately equal amounts of each form. When a purified fraction containing about 90% A-form enzyme (Fig. 6b) was treated with partially purified conversion factor, the product eluted at the same location as the B-form enzyme produced in vivo (Fig. 6c).

Discussion

The capacity for rapid in vivo interconversion between active and less active forms of ornithine decarboxylase has been repeatedly demonstrated in *Physarum*. It is, therefore, not surprising to find that a factor can be isolated from crude lysates of *Physarum* which has the ability to change the A form of ornithine decarboxylase into the B form. This discovery, however, is of major significance in that it opens the way to further investigation into the regulatory elements involved in this very sensitive post-translational modulation of ornithine decarboxylase.

The heat and trypsin sensitivity of this A to B converting factor, and its pattern of elution from ion exchange and gel filtration columns suggest that this is an acidic protein with a molecular weight of about 35 000. In low ionic strength buffers this factor appears to bind readily to larger proteins and perhaps to ornithine decarboxylase itself. Further characterization of the chemical nature of this factor will require preparation of more highly purified fractions.

The apparently absolute requirement for either spermidine or spermine in this A to B enzyme form conversion is of considerable interest. Their role in modulating the activity of this enzyme is appropriate since the decarboxylation of ornithine is considered to be the initial and rate-limiting reaction in the biosynthesis of polyamines. However, the role of the polyamines in the mechanism of this enzyme form conversion is not yet known. They could serve as effector ligands which alter the conformation of either the conversion factor or the decarboxylase and thereby promote this form conversion. This is consistent with the observation that these levels of spermidine and spermine, but not putrescine, alter the physical conformation of ornithine decarboxylase as noted by its elution from gel filtration columns [13]. Alternatively the polyamines may be covalently linked to ornithine decarboxylase in this conversion reaction. In this view the conversion factor could be a transglutaminase which uses the added polyamines to produce a less active polyamine-ornithine decarboxylase conjugate [18,19]. It has not yet been feasible to test this attractive hypothesis due to the stoichiometric nature of this reaction, as it is currently

catalyzed, and our limited ability to produce purified conversion factor.

The observation that ATP and other nucleotides protected ornithine decarboxylase A from conversion to the B form is interesting in view of the similar protection ATP affords to tyrosine aminotransferase, another closely regulated, pyridoxal 5′-phosphate-requiring enzyme [20]. In the latter case ATP, potentiated by cyclic AMP, interacts with membranous fractions from liver to alter the form of tyrosine aminotransferase, and thereby reactivates and stabilizes it. Ornithine decarboxylase form conversion was not found to be reversed by ATP levels as high as 10 mM. Furthermore, we found ATP prevented form conversion even when purified fractions of conversion factor and decarboxylase enzyme were used. Although such nucleotides may interact with the conversion factor or ornithine decarboxylase directly, it is more likely that they act by sequestering the necessary polyamine by chelation as suggested by Tabor and Tabor [21]. A similar conclusion was reached by Jamdar [22] in explaining the observation that spermine-induced aggregation of microsomal membranes was reversed by ATP. Our observation that Mg^{2+} reduces the effect of nucleotides also supports this interpretation.

It is tempting to speculate that the A to B conversion factor reported here could also catalyze the reverse reaction and thereby be in complete control of this post-translational modification. Our attempts to find the appropriate substrate and assay conditions to reverse this reaction have not been successful. Conceivably, entirely different factors, perhaps membrane bound or energy requiring, may be needed to catalyze this B to A-form conversion.

Although the precise chemical difference between the two forms of this enzyme is not known it appears to entail an alteration in molecular charge, as seen by different isoelectric points and binding to hydroxyapatite columns, without a major variation in molecular weight determined by gel filtration. Conversion from A form to B was therefore speculated to be by catalytic action of a regulatory enzyme adding or deleting a small, charged molecule. Since the current assay of this conversion reaction shows it to have a stoichiometric nature the following reaction models must be further investigated: (1) in catalyzing the conversion of form A to B the conversion factor itself may be inactivated, requiring the presence of another factor or enzyme before it can be regenerated to catalyze additional conversion reactions; (2) the conversion factor may bind to the A form, catalyze the A to B conversion and then remain attached to the B form until some further reactions or buffer additions release it so that it may be free to bind to additional A-form enzyme.

What is the possible relationship between the ornithine decarboxylase-antizyme demonstrated in several mammalian systems and the conversion factor described in this report? Both are low molecular weight proteins which alter ornithine decarboxylase activity. The latter interacts with, and perhaps binds to, ornithine decarboxylase in the presence of spermidine or spermine and induces a change in the structure and activity of this enzyme which remains even after the removal of the conversion factor. Antizyme binds to ornithine decarboxylase producing an inactive enzyme-antizyme complex. However, any further modification of this enzyme has not as yet been demonstrated. The elevation of the ionic strength of the buffer with 0.3 N NaCl or 10% $(NH_4)_2SO_4$ separates the antizyme-enzyme complex [2—4]. Similarly, 0.2 N NaCl will

prevent the conversion factor reaction, yet once the B form of the enzyme is produced then even 0.5 N NaCl or up to 20% $(NH_4)_2SO_4$ (data not shown) will not reverse this change. Antizyme is a normal component of uninduced H-35 and rat liver cells [23] and is released from subcellular particles by low levels of polyamines or elevated ionic strengths. Similarly, the *Physarum* conversion factor is associated high molecular weight cell components in crude homogenates and requires high ionic strength buffers for its release as a separate protein.

Despite the obvious differences between these two ornithine decarboxylase-regulating factors there exist enough similarities to suggest that further experimentation may actually prove that we have been looking at different aspects of a common regulatory mechanism. Certainly further investigation of the A-B converting reaction will greatly aid our attempts to elucidate the biochemical difference between these forms, the reaction catalyzing B to A conversion, and eventually the complete mechanism and control of the post-translational modulation of this critical enzyme in polyamine synthesis.

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