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EFFECT OF MANGANESE ON THE QUATERNARY STRUCTURE OF HUMAN LIVER ARGINASE

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SUMMARY

1 The molecular weight of human liver arginase (L-arginine amidinohydrolase, EC 3 5 3 1) as determined by gel filtration was found to be 118 000

2 Incubation of the enzyme with EDTA followed by dialysis resulted in an inactivated enzyme with a molecular weight of about 30 000 It is suggested that the native enzyme is composed of four subunits

3 Addition of Mn^{2+} to the inactive subunits resulted in the regeneration of the enzymatic activity The molecular weight of the regenerated enzyme was found to be that of the native enzyme

4 The native enzyme and the subunits differ in ion exchange chromatography and electrofocusing behaviour

INTRODUCTION

The activation of arginase (L-arginine amidinohydrolase, EC 3 5 3 1) by divalent metal ions, especially Mn^{2+}, is a well documented fact[1] However, the exact role of the metal ion in the catalytic activity of the enzyme has not been clearly established The work presented in this report describes the effect of Mn^{2+} on the quaternary structure of human liver arginase and relates this effect to the activity of the enzyme Conditions are described for the reversible dissociation of the enzyme into subunits

MATERIALS AND METHODS

Arginase activity was determined, after activation with 1 mM $MnCl_2$, by incubation in arginine solutions for 10 min at 25°, and measuring the urea produced with isonitrosopropiophenone[2] Protein concentrations were determined by 280 nm absorption or by the method of LOWRY *et al.*[3], using bovine serum albumin as a standard

Human liver arginase was purified as described by BASCUR *et al.*[4] According to this procedure, the chromatography of partially purified preparations on a CM-

cellulose column equilibrated with 5 mM Tris–HCl (pH 7 4) resolves the enzymatic activity into two protein fractions The experiments reported in the present paper were performed with the fraction containing the highest enzymatic activity, that eluting from the column at 0 10–0 14 M KCl The enzyme preparation obtained under these conditions is active even in the absence of added Mn^{2+}, but to be sure that the enzyme was fully activated it was incubated with 1 mM $MnCl_2$ in 5 mM Tris–HCl (pH 7 5) for 30 min at 37° After this incubation the enzymatic activity increases less than 10% and no further increase is observed after longer incubation even with higher concentration of the metal ion

Inactivation of the enzyme was performed by incubation with 30 mM EDTA in 50 mM Tris–HCl (pH 7 5) for 60 min at 37°, followed by an exhaustive dialysis against glass-bidistilled water Under these conditions, an enzyme preparation is obtained with no enzymatic activity in the absence of added Mn^{2+} Throughout this work this enzyme preparation will be referred to as "EDTA-treated enzyme" In the gel filtration, ion exchange chromatography and electrofocusing experiments performed with this enzyme preparation, the fractions from the columns were analyzed for arginase activity after incubation with 1 mM $MnCl_2$ for 30 min at 37°

Molecular weights were determined by gel filtration on Sephadex G-100 and G-200 columns, as described by ANDREWS[5,6] Elution was performed with 10 mM Tris–HCl (pH 7 5 or 8 0) containing 20 mM KCl, and 2-ml fractions were collected The column was standardized with blue dextran, determined by its absorption at 625 nm, and some proteins of known molecular weight Rat liver arginase (mol wt 118 000)[7] was determined as described for the human liver enzyme Lactate dehydrogenase (L-lactate NAD^+ oxidoreductase, EC 1 1 1 27) (mol wt 140 000)[6] was assayed by the method of KORNBERG[8] Bovine serum albumin (mol wt 69 000)[9] was determined by its absorption at 280 nm Rennin (EC 3 4 4 3) (mol wt 34 400)[5] was determined by its absorption at 230 nm Horse heart cytochrome *c* (mol wt 12 400)[6] was determined by its absorption at 425 nm

Isoelectric focusing was performed essentially as described by VESTERBERG AND SVENSSON[10], with the use of carrier ampholytes of LKB-Produkter, Stockholm, to establish a pH gradient between 7 and 10 The amount of protein applied to the column was 4 mg, and the voltage applied was increased stepwise from 300 to 500 V, and then kept constant at 500 V The column temperature was 8° For each fraction of 2 ml, enzyme activity after Mn^{2+} activation, and pH was determined

L-Arginine and all the calibrating proteins used except rat liver arginase were purchased from Sigma Chemical Rat liver arginase was purified according to the procedure of SCHIMKE[11] Sephadex G-100, G-200, and Blue Dextran 2000 were purchased from Pharmacia, Uppsala All other chemicals were of analytical grade or of the highest purity available

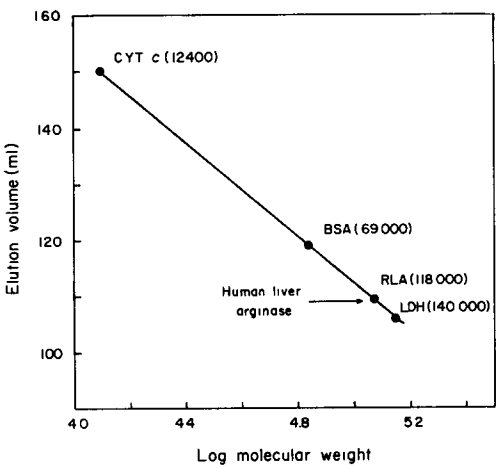
RESULTS

Molecular weight of activated and EDTA-treated enzyme

Purified human liver arginase was fully activated and examined by gel filtration in order to obtain an estimation of its molecular weight The results obtained on a Sephadex G-200 column calibrated with a high molecular weight dextran and 4 proteins of known molecular weight, are shown in Fig 1 It is seen that the elution

volume of the human liver arginase corresponds exactly to that of the rat liver arginase, for which a molecular weight of 118 000 has been reported[7] The coincident elution volumes then indicate that the enzymes obtained from both mammalian sources have a very similar molecular weight

When the EDTA-treated enzyme was analyzed by gel filtration on Sephadex G-100, a molecular weight of about 30 000 was obtained (Fig 2) The activity of the fractions was assayed after activation with Mn^{2+} as described in MATERIALS AND METHODS The molecular weight determined for the EDTA-treated enzyme is again in agreement with the molecular weight of the subunits of arginase obtained from other sources[7,12] and suggests that the human liver enzyme is a tetramer



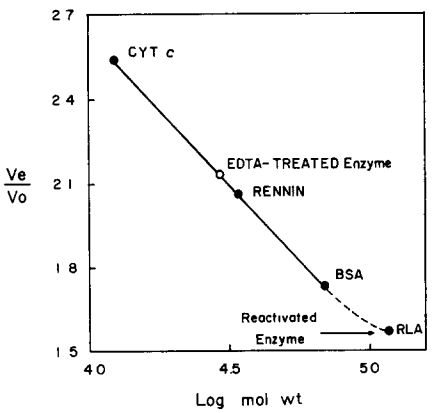


Fig 1 Estimation of the molecular weight of fully activated human liver arginase on Sephadex G-200 Column size 2 cm × 50 cm, buffer 10 mM Tris–HCl (pH 7 5) containing 20 mM KCl The proteins used as standards were cytochrome *c* (CYT *c*), bovine serum albumin (BSA), rat liver arginase (RLA) and lactate dehydrogenase (LDH)

Fig 2 Molecular weight of EDTA-treated enzyme The column of Sephadex G-100 (1 7 cm × 50 cm) was calibrated with cytochrome *c* (CYT *c*), rennin, bovine serum albumin (BSA) and rat liver arginase (RLA) The results are presented as a plot of the ratio of the protein elution volume (V_e) to the column void volume (V_0) against log mol wt of the proteins The arrow indicates the result obtained after reactivation of the low molecular weight species of the human liver arginase

The reversibility of the dissociation process of the native human liver arginase was also investigated For this purpose the fractions obtained in the gel filtration experiments of the EDTA-treated enzyme were concentrated by lyophylization and activated by incubation with 1 mM $MnCl_2$ in Tris–HCl (pH 7 5) for 30 min at 37°, followed by dialysis for 6 h against 5 mM Tris–HCl (pH 7 5) containing 1 mM $MnCl_2$ After this treatment, the enzyme was filtered into the same Sephadex G-100 column The elution volume of the protein activated under these conditions was identical with that of the activated native enzyme, and the activity measured in the absence or presence of added Mn^{2+} was found to be essentially the same This indicates that the reactivation was accompanied by the reassociation of the subunits

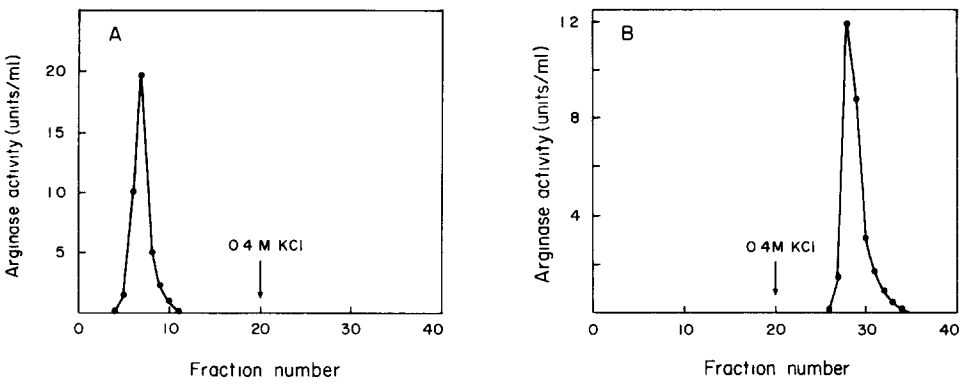


Fig 3 Chromatography of activated and EDTA-treated enzyme on DEAE-cellulose Column size 1 7 cm × 19 cm Other details are given in MATERIALS AND METHODS A Activated enzyme B EDTA-treated enzyme

Behaviour of activated and EDTA-treated enzyme on ion-exchange chromatography and electrofocusing

Upon chromatography of the fully activated enzyme on DEAE-cellulose equilibrated with 5 mM Tris–HCl (pH 7 4), all the enzymatic activity emerges from the column at the buffer front If after elution of the enzyme, the column is washed with the eluting buffer and then with 5 mM Tris–HCl (pH 7 4) containing 0 4 M KCl no additional activity is detected, confirming the previously reported findings of BASCUR *et al* [4] (Fig 3A) However, when the EDTA-treated enzyme was chromatographed under the same conditions as that employed for the fully activated enzyme, no enzymatic activity could be detected at the buffer front, in this case, the enzyme elutes with the buffer containing 0 4 M KCl (Fig 3B) The activity of the fractions was again measured after reactivation with manganese

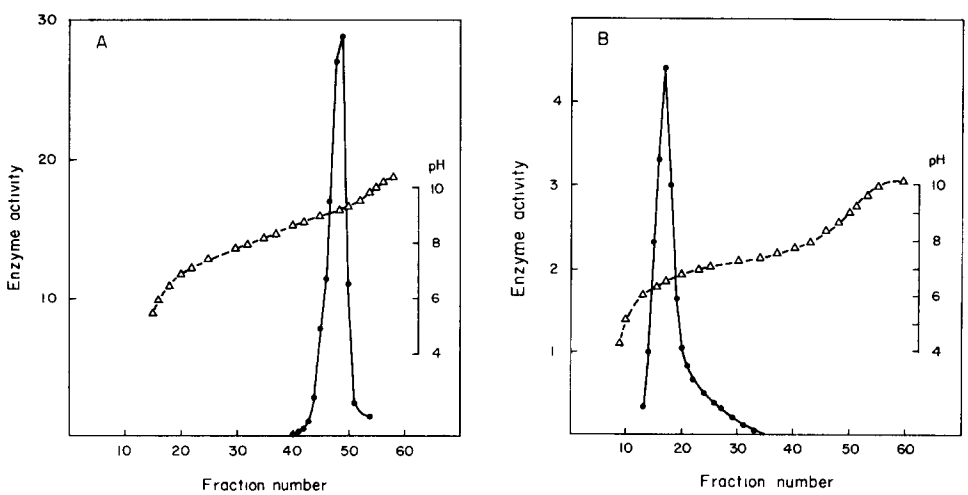


Fig 4 Isoelectric focusing of activated and EDTA-treated enzyme Electrofocusing experiments were carried out as described in MATERIALS AND METHODS A Activated enzyme B EDTA-treated enzyme

The results obtained by chromatography on DEAE-cellulose were confirmed by electrofocusing of the activated and EDTA-treated enzymes as described in MATERIALS AND METHODS The results presented in Fig 4A show that in the case of the activated enzyme, the maximal arginase activity is found in the fraction corresponding to pH 9 2 On the other hand, when the EDTA-treated enzyme is submitted to isoelectric focusing, the maximal activity corresponds to the fraction with pH 6 6 (Fig 4B) The results obtained with the fully activated enzyme are in good agreement with those reported by HIRSH-KOLB *et al* [13] for rat liver arginase

DISCUSSION

The experiments presented above demonstrate the effect of Mn^{2+} on the quaternary structure of human liver arginase The presence of this metal ion is apparently required to maintain the active conformation of the enzyme, which has a molecular weight of 118 000 The molecular weight of the human liver enzyme is similar to that previously described for the rat liver tetramer[7]

Withdrawal of the metal ions apparently results in dissociation of arginase into subunits of molecular weight of about 30 000 These subunits are inactive when assayed in the absence of manganese but can be reactivated by preincubation with Mn^{2+} under the conditions described The reactivation treatment causes the enzyme to reassociate to yield again the active tetramer

Rat liver arginase has been dissociated with 8 M urea into subunits having a molecular weight of 30 800 by HIRSH-KOLB *et al* [7] These authors do not report the reassociation of the subunits More recently, these same researchers[13] have described experiments in which they have withdrawn manganese by extensive dialysis and found that this treatment causes the metal ion to be reduced from 4 to 2 atoms per molecule of tetrameric enzyme Under these conditions the enzyme retains 50% of its original activity Further removal of metal caused irreversible inactivation of their enzyme preparation This apparent discrepancy with our results may be explained by the different method used in the removal of manganese We have found that it is essential to incubate the enzyme at 37° with 30 mM EDTA prior to dialysis in order to remove quickly and efficiently the manganese ions That this discrepancy is not due to an intrinsic difference between the enzyme from both sources is suggested by the findings of SOROF AND KISH[14] These researchers have found that the soluble proteins of rat liver obtained in the absence of added Mn^{2+} can be resolved by gel filtration into two or more molecular species of arginase, one of these species, detected after activation with Mn^{2+}, was presumed to be an enzyme subunit No reassociation experiments were, however, presented in this case In the light of the results reported in the present paper, the findings of SOROF AND KISH[14] would be then explained by the removal of manganese followed by dissociation of the enzyme Very recently, HIRSH-KOLB *et al* [15] have reported for the beef liver arginase a molecular weight of 30 000 determined by gel filtration Since analytical ultracentrifugation of beef liver arginase yielded a molecular weight of about 130 000, it was suggested that the molecular weight determined by gel filtration corresponded to that of the dissociated enzyme However, as in the experiments of SOROF AND KISH[14], no reassociation studies were presented

Studies are presently in progress in our laboratory regarding the exact role of

manganese in the maintenance of the structure of the active enzyme and/or in the catalysis itself

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