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EFFECT OF L-USNIC ACID ON A THERMICALLY DENATURED UREASE

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INTRODUCTION

Usnic acids are powerful enzyme inactivators, being demonstrated for DNAase (1), glutamate dehydrogenase (2) and urease (3). This function is a common characteristic of phenolics (4), causing aggregation states which increase molecular weight of proteins. Recently, VICENTE et al. (5) have shown that urea, substrate of urease, causes itself an aggregation of protein cooperating with the L-usnic acid-depending aggregation. The aggregates, commonly inactive, show a molecular weight higher than 800,000.

The inactive aggregates reverse to active protein when the aggregation provoked by L-usnic acid is carried out in the presence of L-cystein (6). Therefore, we should reject the hypothesis referring to the aggregation state at the only responsible for the enzymatic inactivation since active aggregates of high molecular weight have been found.

The present paper studies the changes in the quaternary structure of urease produced by L-usnic acid depending on the tertiary structure of enzyme.

MATERIAL AND METHODS

It has been used for this work crystalline urease (type III, Sigma Chemical Co.) preincubated with L-cystein and L-usnic acid in the concentration indicated in every case, as it has been previosly described (3). The specific activity has been determined according Conway's method (7) estimating the quantity of protein according Warburg and Christian's (8)

method. The molecular weight of different aggregates have been determined by filtering the samples through a Sepharose 6B column of 21 cm in height and 3 cm in diameter, which was balance with 75 mM phosphate buffer, pH 6.9, and comparing eluates with a filtration diagram made under the same conditions either for tyroglobulin, glutamate dehydrogenase, α-urease and catalase (all from Sigma Chemical Co.).

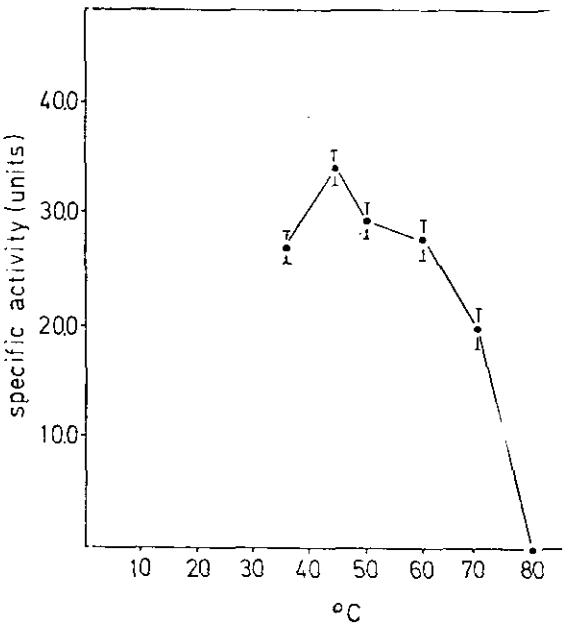

Figure 1.—Thermic inactivation of urease (10 mg in 10 of final volume).

Results and discussion

10 mg urease in a final of 10 ml are thermically denatured whithout a precipitation of protein has been carried out. Figure 1 shows the results obtained from thermic deactivation process and figure 2 shows the results of filtering this protein under different treatings through Sepharose 6B. On it, it may be observed a continous appearance of two main molecular forms, one of 820,000 molecular weight (eluted at 10 ml filtration) and another, the main peak, of 690,000 molecular weight

(eluted at 60 ml), which ever is the chemical treating the protein thermically inactivated has suffered previously to filtration.

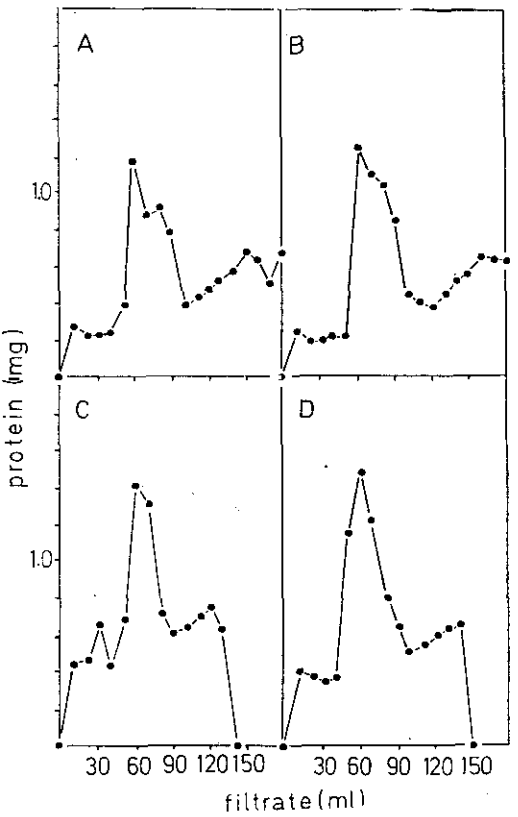

Figure 2.—Diagram of filtration of a thermically inactivated urease: A) whithout chemical treating; B) incubated for 5 minutes at 37° C with L-usnic acid (35 μ moles/ml); C) with L-cystein (50 μ moles) and D) incubated with both L-usnic acid and L-cystein.

The enzyme is thermostable enough, since being one hour at 70° C provokes the loss of a third part of specific activity. The total thermic, inactivation is achieved by incubation for one hour at 80-90° C. In this way, it is produced an aggregation state of thermic origin by which molecular weights similar to the ones obtained from the chemical inac-

tivation process may be obtained (5, 6). In this agrregation state, neither the inactivator nor the aminoacid show considerable effects, since the peaks obtained by filtering through Sepharose 6B are identical to the ones found for the thermal denatured enzyme when both compounds are absent from it. Finally, it could be deduced that the tertiary structure is absolutely necessary for the inactivation process by L-usnic acid to be carried out.

Summary

L-usnic acid acts as an urease inactivator causing modifications on the quaternary strutucture of the protein. Enzyme tertiary structure is absolutely necessary for the inactivation process.

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