Locust Alanine Aminotransferase has Subunit Structure

S. D. MANE[1] and K. N. MEHROTRA[2]

Division of Entomology, Indian Agricultural Research Institute, New Delhi–110012 (India), 9 September 1975.

Summary. Ion exchange and gel chromatography of locust flight muscle alanine aminotransferase showed that the cytoplasmic enzyme occurs both as monomer and tetramer, whereas mitochondrial enzyme exists mainly as a dimer.

Insect alanine aminotransferase exists both in cytosol and mitochondria[3,4]. However, nothing is known about the nature of this enzyme, associating with different subcellular fractions. Recently it has become a subject of controversy in relation to the hormonal titres and protein synthesis in the metamorphosing insect on one hand, and the level of alanine aminotransferase on the other[5,6]. We believe that this has suggested the need for the investigations on the nature of this enzyme. Probably this might help in explaining some of the phenomena of hormonal mechanism and in vivo regulation of insect transaminases. This paper describes the chromatographic and molecular properties of alanine aminotransferase (EC 2.6.1.2.), and presents the first evidence on the occurrence of isomeric forms of this enzyme.

Flight muscle tissue from adult *Schistocerca gregaria*, 10–20 days old, was used. The enzyme activity was assayed by hydrazone method[7]. Protein was estimated by Folin's reagent method[8]. Analytical gel filtration was performed according to the standard procedure[9]. DEAE-cellulose chromatography of flight muscle soluble enzyme showed 3 active species as is evident by the appearance of 3 elution peaks (Figure 1, S). Of the 3, peak I was not retained on the ion exchanger and was eluted in equilibration buffer 0.05 *M*, pH 8.2,[1] as a bright yellow-green band. On the other hand, peaks II and III were adsorbed on the cellulose and were eluted both as bright yellow-brown bands only in 0.05 *M* phosphate buffer, containing 0.15 *M* and 0.25 *M* NaCl respectively. Mitochondrial enzyme when chromatographed in a similar way showed only 1 distinct peak (Figure 1, M) of activity, which corresponded to the peak II of the soluble fraction i.e; it was eluted as a bright yellow-brown band in a buffer containing 0.15 *M* NaCl. This chromatographic behaviour of enzyme suggests the differences in the protein structures of the various forms. We predicted two possibilities: they could be isozymes, or perhaps the subunits of the native enzyme which may show the structural

[1] Present address: Max-Plack-Institut für Biochemie, D-8033 Martinsreid bei München, BRD.

[2] We thank the Director of IARI for providing facilities, the ICAR for a fellowship to S.D.M. and Prof. Dr. H. REMBOLD for critical reading of the manuscript.

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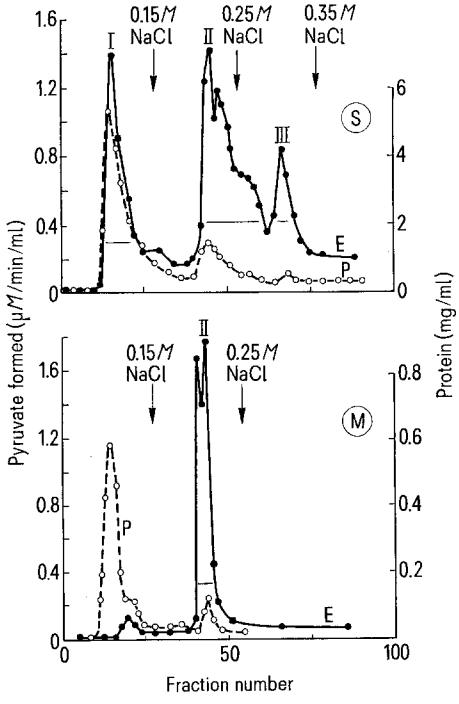

Fig. 1. Chromatography of locust flight muscle alanine aminotransferase on DEAE-cellulose column, 2.5 × 30 cm. S, soluble enzyme; M, mitochondrial enzyme.

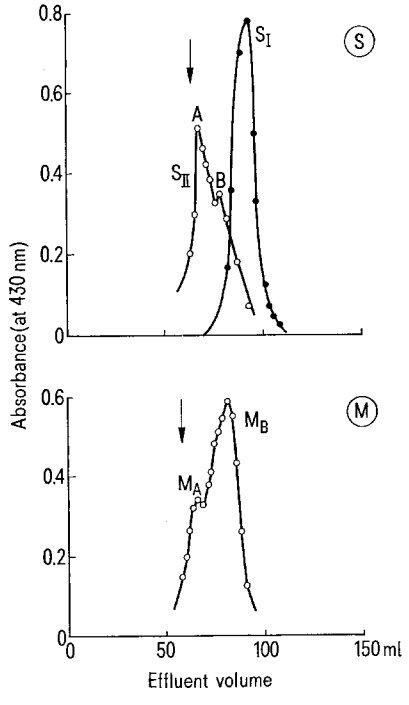

Fig. 2. Gel chromatography of alanine aminotransferase on Sephadex G-200. Arrow indicates void volume.

Molecular weight of alanine aminotransferase from soluble and mitochondrial fractions (both after DEAE-cellulose chromatography) of locust flight muscle

Fraction	Ve/Vo	Molecular weight (daltons)	Multiplication factor
Soluble[a]			
S_I	1.59	63,000	1
S_{II-B} minor	1.34	139,500	2
S_{II-A} major	1.15	257,000	4
Mitochondrial			
M_B major	1.39	119,000	2
M_A minor	1.17	241,000	4

A calibrated column, 107 × 1.3 cm, was fitted to the regression equation, log M = 7.00452 — 1.3865. (Ve/Vo), where M is molecular weight, Ve and Vo are elution and void volumes respectively in milliliters.

[a] Soluble enzyme peak I and peak II were chromatographed.

differences at the quaternary level[10]. That the species we observed in the present investigations were indeed the isomeric forms of the enzyme finds support in our results on the molecular weight determination by gel chromatography on Sephadex G-200.

Our observations (Table and Figure 2) on the flight muscle soluble enzyme I and II indicated that these forms are monomer and tetramer, while the mitochondrial enzyme is a dimer. Further, the soluble enzyme peak II was associated with a dimeric minor enzyme protein which was not completely separated from the main (Figure 2, S). Similarly, mitochondrial fraction also showed incomplete chromatographic separation of the minor tetrameric enzyme protein from the main (Figure 2, M). This clearly suggests the occurrence of the dimer-tetramer complexes of enzyme in the cytosol as well as in the mitochondria. Although, the physiological significance of these structural subunits is not clear, it may nevertheless be added that they may play an important role similar to glycerophosphate or malate-oxaloacetate cycle in the proper maintenance of NAD/NADH ratio[11].

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Effects of Starvation and Ca^{++} on Glucose-Induced Accumulation of Cyclic 3′,5′-AMP in Pancreatic Islets[1]

B. Hellman

Department of Histology, University of Umeå, S–901 87 Umeå 6 (Sweden), 19 September 1975.

Summary. Exposure to glucose in the presence of 3-isobutyl-1-methylxanthine leads to accumulation of cAMP in islets microdissected from *ob/ob*-mice. This process is dependent on extracellular Ca^{++} but differs markedly from the glucose action on insulin release in the same in vitro system in disappearing after 18 h of starvation.

In analogy with the Sutherland hypothesis of hormone action through a second messenger, glucose initiation of insulin release has been supposed to occur as a result of receptor interaction leading to the formation of cyclic adenosine 3′,5′-monophosphate (cAMP)[2–5]. Although this concept has been challenged in several reports[6–11], it seems likely that accumulation of cAMP contributes to the self-potentiating action of glucose as an insulin secretagogue[9–11].

There has been a great deal of controversy as to whether exposure to glucose actually leads to accumulation of cAMP in the pancreatic islets[3–7,9–11]. Recent studies in this laboratory showed that perfusion with 20 mM glucose results in a significant increase of cAMP in islets microdissected from fed obese-hyperglycaemic mice (genotype *ob/ob*), provided that they are simultaneously exposed to a potent phosphodiesterase inhibitor[11]. The present study adds to the characterization of the glucose-induced accumulation of cAMP in these β-cell-rich pancreatic islets by demonstrating its dependence on the nutritional status of the animal and the presence of extracellular Ca^{++}.

Materials and methods. Female 7 month old *ob/ob*-mice, taken from a non-inbred colony, were used as the source of pancreatic islets containing more than 90% β-cells[12]. The animals had free access to water and were either allowed free access to food, or starved for 18 h before being killed by decapitation. Fresh pancreatic islets were microdissected free-hand and incubated at 37 °C with different concentrations of Ca^{++} in Krebs-Ringer bicarbonate medium containing 1 mg/ml albumin and equilibrated with O_2 + CO_2 (95:5). After 40 min of preliminary incubation, the amounts of insulin released were measured during 60 min of further incubation in medium containing 3 or 20 mM glucose and 1 mM 3-isobutyl-1-methylxanthine (IBMX). When analyzing the

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