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Latency and solubilization of the mitochondrial aspartate transaminase of rat cerebral cortex

Several enzymes present in mitochondrial preparations isolated from mammalian liver[1–4], heart[5,6] and brain[7] are known to exhibit, when assayed under conditions preserving mitochondrial structural integrity, only a portion of the activity that becomes apparent after treatment of these preparations by disruptive procedures. That the use of such procedures fails at times to release the fully active enzyme in soluble form, as judged by non-sedimentation after centrifugation at high speeds, has been shown repeatedly[1,4,5,8,9]. We have recently observed that L-aspartate: 2-oxoglutarate aminotransferase (aspartate transaminase, EC 2.6.1.1) present in mitochondrial fractions of rat cerebral cortex[10,11] exhibits latency and, furthermore, that vigorous treatments are required to render the measurable transaminase activity of such preparations maximal.

Centrifugal fractions of rat cerebral cortex consisting of "heavy" + "light" mitochondria were isolated in 0.25 M sucrose as previously described[12]. Aspartate

TABLE I

EFFECT OF PRETREATMENTS OF HOMOGENATE AND MITOCHONDRIAL FRACTIONS OF RAT CEREBRAL CORTEX ON THE ACTIVITY OF ASPARTATE TRANSAMINASE

Expt.	Pretreatment	Fraction	
		Homogenate (units/g)	Mitochondria (units/g)
1*	None	3570	760
	1 h, 37° + digitonin	4210***	1770***
	20 h, 4°	3400	1080
	43 h, 4°	3170	1360
2*	None	—	454
	Digitonin	—	2520
	1 h, 37°	1730	—
	1 h, 37° + digitonin	2520	
3**	None	—	408
	Digitonin	—	715
	1 h, 37°	—	1500
	1 h, 37° + digitonin	—	1975

* Ti e equivalent: 100 mg/ml.
** Ti e equivalent: 167 mg/ml.
*** A rtate transaminase, specific activity: homogenate, 13.3; mitochondrial fraction, 20.9.
Digiton 0.33 mg/ml.

transaminase activity was determined according to KARMEN[13]. A unit of enzyme activity is defined as μmoles of DPNH oxidized per h. In all experiments the specific activity (units/mg of protein) of mitochondrial aspartate transaminase exceeded that of the enzyme in the homogenate by at least 1.5 times. Homogenates or suspensions of mitochondria (for effects of dilution, see below) were assayed immediately after isolation and again after incubation at 37° for 1 h in the presence of digitonin (0.33 mg/ml), a treatment initially thought to ensure attainment of maximal total

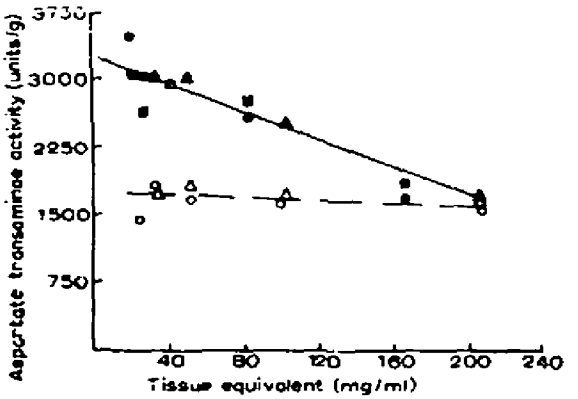

Fig. 1. Dilution-dependent effect of digitonin on cerebral aspartate transaminase. Serial dilutions of a rat cerebral cortex homogenate were made by adding to a constant volume of homogenate suitable volumes of ice-cold 0.25 M sucrose or 0.25 M sucrose + digitonin (final concentration, 0.33 mg/ml). Aspartate transaminase activity was determined after 10 min at 0° (see ref. 13). Symbols indicate successive experiments. open, −digitonin; closed, + digitonin.

activities of aspartate transaminase. Table I summarizes the effects of pre-incubation (37°) with and without digitonin, and of ageing, on the activity of the enzyme. Ageing at 4° (Expt. 1) resulted in a graded increase of mitochondrial activity together with a slight decrease of the activity of unfractionated homogenates. Pre-

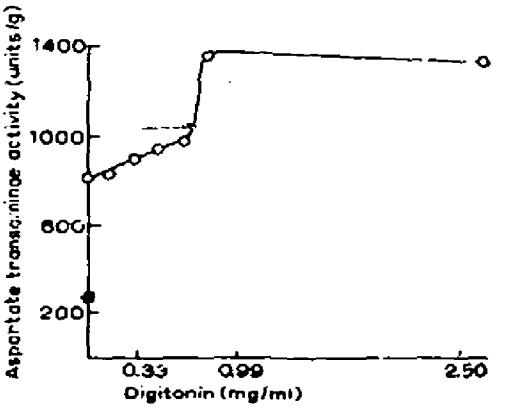

Fig. 2. The activation of rat cerebral-cortex mitochondrial aspartate transaminase by digitonin. To 1-ml aliquots of a suspension (167 mg/ml) of rat cerebral -cortex mitochondria in 0.25 M sucrose (of aspartate transaminase activity denoted by •), was added 0.2 ml of 0.25 M sucrose or 0.25 M sucrose + digitonin to give final concentrations of detergent as indicated. The tubes were incubated for 1 h at 37° before aspartate transaminase activity was assayed[13].

incubation of homogenates (100 mg/ml) in the presence of digitonin (Expt. 2) resulted in an increase of aspartate transaminase activity; this effect was found to be dependent on the dilution of the pre-incubated homogenate (Fig. 1) and was not observed when the detergent was omitted. Similarly, mitochondrial aspartate transaminase activity was found to be enhanced by digitonin in an equally dilution-dependent manner (Expts. 2 and 3).

The effect of increasing concentrations of digitonin on mitochondrial aspartate transaminase was studied using a mitochondrial suspension containing approx. 165 mg of tissue equivalent per ml (Fig. 2). Under these conditions, maximal aspartate transaminase activity was reached at about 2.5 g/l of digitonin. When mitochondrial suspensions were pre-incubated (37°, 1 h) either in the absence or in the presence of, respectively, 0.38 mg/ml and 2.8 mg/ml of digitonin and were then centrifuged (140 000 × *g*, 20 min) to assess the extent of aspartate transaminase release into soluble form, the results shown in Table II (Expt. 1, A) were obtained. Whereas solubilization of aspartate transaminase was nil after pre-incubation without digitonin (Expt. 1, B), more than half of the particulate activity could be released after pre-incubation with 2.8 mg/ml of digitonin. Mechanical treatment (Virtis homogenizer, 3 min, 0°, rheostat setting = 60) of the supernatant fluid obtained after centrifugation resulted in partial enzyme inactivation. Addition of crystalline malate dehydrogenase (EC 1.1.1.37) before the Virtis treatment had no effect.

In view of the recent report by GAUL AND VILLEE[8] on the failure of sodium deoxycholate to bring about total solubilization of liver mitochondrial aspartate

TABLE II

SOLUBILIZATION OF MITOCHONDRIAL ASPARTATE TRANSAMINASE OF RAT CEREBRAL CORTEX

Mitochondrial suspensions (3 ml) were pre-incubated for 1 h at 37° with or without digitonin, as indicated and were centrifuged at 140 000 × *g* for 20 min to give supernatant S_1 (Expts. 1 and 2, B) and pellet P_2 (Expt. 2, C). The latter was suspended in 3 ml of 0.25 M sucrose and the aspartate transaminase activity was determined on a 0.01-ml aliquot. Sodium deoxycholate (10% w/v, 0.5 ml) was added; the transaminase activity of the cleared suspension was again determined (Expt. 2, D) and the remainder was centrifuged at 140 000 × *g* for 30 min to yield supernatant S_2.

	Digitonin during preincubation		
Expt. 1	*0 mg/ml*	*0.38 mg/ml*	*2.8 mg/ml*
	Units transaminase/g		
A. Mitochondrial suspension	645	692	955
B. S_1	0	136	545
C. S_1, mechanically treated	0	0	470
D. S_1 + crystalline malate dehydrogenase	0	....	—
Expt. 2			
A. As A, Expt. 1	272	475	905
B. S_1	0	219	680
C. P_2 in 0.25 M sucrose	420	520	590
D. P_2 in 0.25 M sucrose + 1.42% sodium deoxycholate	2160	2040	2040
E. S_2	2000	2360	2160

transaminase, this detergent was made use of in an attempt to detach cerebral mitochondrial aspartate transaminase from its structural matrix. Table II (Expt. 2, D) illustrates the activating effect of 1.42% sodium deoxycholate on the enzyme. Unlike liver mitochondrial aspartate transaminase, all of the activated, particulate enzyme activity could be recovered in the soluble supernatant fluid after centrifugation of the deoxycholate-treated suspensions at 140 000 × *g* for 30 min (Expt. 2, E).

The discrepancies in enzyme recovery (Table II, Expt. 2, B + C/A) have not been resolved to date. It should be noted, however, that good enzyme recoveries have been obtained in preliminary studies on the intracellular localization of aspartate transaminase in rat cerebral cortex, in which the enzyme activity of all subcellular fractions was determined in the presence of 1.42% deoxycholate.

Our results on the latency of cerebral mitochondrial aspartate transaminase are in general agreement with the observations of MAY *et al.*[10], as well as with the results of MCARDLE *et al.*[11]. At this time, therefore, the preferred interpretation of the effect of the detergents is that of a lytic action upon those structural mitochondrial lipoproteins which impede optimal interactions between enzyme and substrate in the intact mitochondrion.

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The oxidation of 4(5)-imidazolone-5(4)-propionic acid to hydantoin-5-propionic acid by xanthine oxidase

Administration of radioactive L-histidine to the monkey, human and rat leads to the excretion of L-[^{14}C]hydantoin-5-propionic acid in the urine. From 4–8% of the administered radioactive is excreted in the urine during the first 12 h after in-