CRYSTALLIZATION OF 2-OXOGLUTARATE L-ASPARTATE TRANSAMINASES FROM MITOCHONDRIAL AND SOLUBLE FRACTIONS OF BEEF LIVER*

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Recently we have highly purified 2-oxoglutarate L-aspartate transaminase from each of mitochondria and the soluble fraction of beef livers and found that there were marked differences in some properties of these two isozymes (Morino and Wada, 1962). In continuation of the previous work, both of these enzymes have been obtained in crystalline form.

This communication describes the crystallization of 2-oxoglutarate L-aspartate transaminases of mitochondrial and extramitochondrial origin, respectively, and some of their properties.

The standard assay conditions were as described in the previous paper (Morino and Wada, 1962). The mitochondrial and soluble fractions were obtained from 20 per cent homogenates (in 0.25 M sucrose solution containing 0.0001 M Versene) of fresh beef livers by differential centrifugation, essentially according to the method described by Hogeboom (1955).

The Mitochondrial Enzyme

The protocol of purification procedures was shown in Table I. The details will be described elsewhere.

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Table I. Purification of the Mitochondrial Enzyme

(Starting from about 500 g. (wet weight) of mitochondrial pellets)

Step	Volume	Protein/ml	Specific activity*	Recovery
	ml	mg		%
1. Heated Extract (72°)	2200.0	3.2	3.96	100
2. 1st Ammonium sulfate (60 to 75%)	48.0	25.6	13.0	57
3. 2nd Ammonium sulfate (55 to 65%)	25.0	22.0	24.0	47
4. Hydroxylapatite column eluate concentrates	11.0	14.0	66.0	37
5. 1st Crystallization	9.0	13.0	69.0	29
6. 2nd Crystallization	9.0	11.0	69.0	25
Supernatant from 2nd crystallization	12.0	1.2	67.0	4

*μ mole of oxaloacetate formed per minute per mg of protein

The concentrates of the column eluate (Step 4) were adjusted to 0.05 M with potassium phosphate buffer, pH 6.0, and to 0.02 M with α-ketoglutarate. Just after the addition of finely powdered ammonium sulfate to a slight turbidity (about 60 % saturation), the solution was freed from amorphous precipitates by brief centrifugation. Then to the clear supernatant was gradually added the ammonium sulfate powder under constant stirring on a magnetic stirrer in the cold. Usually thin rod-shaped crystals appeared after several hours. Recrystallization was carried out by repeating the same process as in the first crystallization. The recrystallized enzyme was completely homogeneous on ultracentrifugation. The sedimentation constant ($S_{20,w}$) and diffusion constant ($D_{20,w}$) of the enzyme were 4.70×10^{-13}(cm/sec) and 4.67×10^{-7}(cm^2/sec), respectively. The molecular weight was calculated to be approximately 100,000 assuming a partial specific volume of 0.75. On free boundary electrophoresis, the enzyme migrated towards cathode at pH 6.8 as almost a single boundary. The pyridoxal

phosphate content of the enzyme was found to be 2 moles per one molecule of the enzyme, as determined by the method described by Wada and Snell (1961).

The Enzyme of the Soluble Fraction

The yield and specific activity at each step of purification were summarized in Table II. The details of the procedures will be described elsewhere.

Table II. Purification of the Enzyme of the Soluble Fraction

(Starting from about 10 kg of beef livers)

Step	Volume	Protein/ml	Specific Activity	Recovery
	ml	mg		%
1. Sucrose Extract (84°)	47000	10.2	0.25	100
2. 1st Ammonium sulfate (50 to 65%)	3000	29.0	1.07	78
3. Heat treated	3000	3.4	7.70	66
4. 2nd Ammonium sulfate (40 to 50%)	75	15.0	45.0	42
5. Acetone (0 to 50%)	20	17.0	117.0	33
6. Hydroxylapatite column eluate concentrates	10	12.0	190.0	19
7. 1st Crystallization	8	12.0	188.0	15
8. 2nd Crystallization	8	11.0	187.0	14
Supernatant from 2nd crystallization	10	0.5	160.0	0.8

The concentrates of the column eluate (Step 6) were adjusted to 0.05 M with potassium phosphate buffer, pH 8.0. The enzyme crystallized through the same process as in the case of the mitochondrial enzyme except that α-ketoglutarate was not added and the pH was adjusted to around 8 by the addition of dilute sodium hydroxide solution during the addition of ammonium sulfate powder. It has not yet been possible to obtain the crystals of the soluble enzyme under the condition where the mitochondrial enzyme has crystallized. The recrystallized enzyme was completely homogeneous on ultracentrifugation. The $S_{20,w}$ and $D_{20,w}$ of

the enzyme were 5.40×10^{-13}(cm/sec) and 4.32×10^{-7}(cm^2/sec), respectively. The molecular weight was calculated to be approximately 120,000 assuming a partial specific volume of 0.75. On electrophoretic analysis, the soluble enzyme migrated towards the anode as a single boundary under the same conditions as with the mitochondrial enzyme. These electrophoretical findings are in consistency with the earlier data reported by several authors (Boyde and Latner, 1962; Boyd, 1962; Katsunuma et al., 1962). The pyridoxal phosphate content was found to be 2 moles per one molecule of the enzyme.

Differences in Some Other Properties

The kinetical data of both enzymes were worked out by following the excellent procedure described by Velick and Vavra (1962). The values of Michaelis constants for substrates thus measured were summarized in Table III.

Table III. Michaelis Constants for Substrates.

Enzyme	α-ketoglutarate	L-aspartate	L-glutamate*	Oxaloacetate*
Mitochondrial	2.0	0.4	20	0.02
Soluble	0.3	5.0	20	0.05

* The equipments employed by us were not sufficiently sensitive to give reliable values of initial velocities at a range of concentration of oxaloacetate lower than 0.05 mM. Therefore, the values listed in the table are approximate ones.

Some comments would be given here on the interpretation of the kinetical data obtained by us in preliminary experiments. In the case of the enzyme of the soluble fraction, the double reciprocal plots of initial velocities versus concentrations of one substrate gave a family of parallel lines as a function of the concentration of the second substrate. Such kinetical result is quite in consistency with the one reported by Velick and Vavra (1962) on the pig heart enzyme. With the mitochondrial enzyme, on the contrary, a kinetical analysis revealed that the

similar plots did not give a family of parallel lines but a family of lines which intersect in a left-hand quadrant. This finding would indicate that there occur some complexes between enzyme and at least two substrates at a time during the enzymic reaction. It also might support the possibility (Morino and Wada, 1962) that there are two active sites on the surface of the mitochondrial enzyme, one binding 4-carbon substrates and the other binding the 5-carbon substrates, respectively. A further analysis on kinetical data are now under way.

An immunological difference between these isozymes was also found in a preliminary experiment in which sera from rabbits immunized against the enzyme of the soluble fraction inhibited and precipitated the enzyme of the same origin in a specific way but did not show any sign of reaction with the mitochondrial enzyme. A further immunochemical study is now in progress.

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