A HIGH SPECIFIC ACITVITY FORM OF MAMMALIAN LIVER ALDOLASE

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1. Introduction

The enzyme aldolase (D-fructose 1,6-bisphosphate D-glyceraldehyde 3-phosphate lyase, EC 4.1.2.13) is known to exist in vertebrates as three tetrameric isozymes, A_4 (muscle type), B_4 (liver type) and C_4 (brain type). A–C hybrids exist in tissues where both A and C subunits are present. The isozymes are distinguishable by their relative activities with the substrates fructose 1,6-bisphosphate (FBP) and fructose 1-phosphate (FlP), and by their electrophoretic mobilities [1].

The livers of most adult mammals contain predominantly aldolase B_4 [2,3]. However, aldolases containing A or C subunits are present in the livers of some adult, as well as foetal and immature animals [4]. The liver isozyme has been purified from a number of mammalian species. The technique of affinity elution with FBP has replaced other chromatographic methods [5]. Using FBP concentrations of 2.5 to 5 mM, homogeneous preparations of aldolase B_4 have been obtained [6–9]. These preparations are reported to have specific activities of 1–2 international units per mg with respect to FBP, compared with aldolase A_4 which has a specific activity of 14–18 international units per mg [9].

Aldolase A_4 cleaves FBP with a greater efficiency than it does FiP, having and FBP:FlP activity ratio of about 50 at 1 mM substrate concentration. Aldolase B_4, on the other hand, is reported to use both substrates with equal efficiency, having an FBP:FlP activity ratio of 1 [1].

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In this study, different concentrations of FBP have been used to elute liver aldolase from a phosphocellulose column and evidence is presented that the use of a low concentration of substrate results in the separation of a form of liver aldolase which has a high specific activity, but which is distinct from aldolase A_4.

2. Materials and methods

Rabbit and beef liver aldolases were purified by a modification of the substrate elution method [2]. Livers were excised and extracted at room temperature in a blendor with two volumes (v/w) of 50 mM Tris-HCl buffer, containing 5 mM EDTA and 50% saturated ammonium sulphate at pH 7.5. The supernatant (crude extract) was obtained by centrifugation at 16 000 *g* for 60 min at 4°C. It was calculated that the ammonium sulphate concentration of the crude extract was about 40% saturated. Sufficient solid ammonium sulphate was added to bring the concentration to 60% saturation. The 40–60% ammonium sulphate precipitate was collected by centrifugation at 16 000 *g* for 10 min at 4°C and resuspended in a minimal volume of 10 mM Tris-morpholinopropane sulphonic acid containing 0.2 mM EDTA, pH 8.0 (Tris-MOPS-EDTA buffer).

In some cases, the isolation was temporarily stopped at this stage and the ammonium sulphate precipitate was stored at −30°C for two to four days.

The ammonium sulphate precipitate was desalted on a column of Sephadex G-25, equilibrated in Tris-MOPS-EDTA buffer, pH 8.0, and treated batchwise with DEAE-cellulose equilibrated in the same buffer. The DEAE-cellulose was washed on a Buchner funnel. This step effected the removal of all of the haemo-

globin and other acidic proteins from the extract and represented a major step in the purification. All of the aldolase activity was recovered in the filtrate.

The pH of the filtrate was adjusted to 7.0 with 1 M MOPS and the sample was applied to a column of phosphocellulose (Sigma) equilibrated in Tris-MOPS-EDTA buffer, pH 7.0, using a column volume of 25 cm^3 per 100 g liver.

The column was washed with the equilibrating buffer and then with the same buffer containing 20 mM KCl. The first pool of aldolase was eluted with Tris-MOPS-EDTA-KCl buffer, pH 7.0, containing 0.2 mM FBP. Following another wash of Tris-MOPS-EDTA-KCl buffer, pH 7.0, a second pool of aldolase was eluted with Tris-MOPS-EDTA-KCl buffer, pH 7.0, containing 2.0 mM FBP.

Aldolase activity was measured spectrophotometrically [10]. Protein was measured assuming an absorbance of 0.89 at 280 nm for a 1 mg/ml solution of liver aldolase [11].

The aldolase pools eluted from the phosphocellulose were concentrated by ultrafiltration and dialysed into 50 mM Tris-HCl buffer, pH 7.5 to remove the substrate. Samples were stored at liquid nitrogen at a protein concentration of 10 mg/ml or, for a short time, as an ammonium sulphate suspension. In some cases, bovine serum albumin was added to increase the protein concentration.

Gel electrophoresis was carried out in vertical slabs consisting of 10% starch in buffer. Following electrophoresis, the gels were sliced horizontally. Protein was stained with 0.25% Coomassie brilliant blue R in 20% ethanol and 10% acetic acid. It was found that, in the buffer systems used, the activity of purified aldolase B_4 could not be stained.

3. Results and discussion

Using a low level (0.2 mM) and a high level (2.0 mM) of FBP, two pools of aldolase were obtained and these were designated I and II respectively. The results of a typical isolation of rabbit liver aldolase and the properties of aldolases I and II are summarized in table 1.

Aldolase I, obtained from rabbit liver in several purifications, had a specific activity of 10–20 international units per mg and had an FBP:FlP activity ratio of 1.8–2.2. Aldolase II had a specific activity of 1–4 international units per mg and had an FBP:FlP activity ratio of 0.8–1.0. A single isolation from beef liver yielded two aldolases with specific activities and activity ratios falling within the same ranges.

Thus the specific activity of aldolase I was in the same range as that of aldolase A_4, although its FBP:FlP activity ratio was much lower. Aldolase I was clearly separable from rabbit muscle aldolase A_4 by starch gel electrophoresis in several buffer systems. Moreover, aldolases I and II appeared to be separable from each other in some electrophoretic systems. The starch gel results on a given preparation were complicated, as either one, two or three bands could be

Table 1
Results from purification of aldolase from 460 g of rabbit liver

Purification step	Total FDP activity (iu)	Total protein (mg)	Specific activity (iu/mg)	FBP:FlP activity	Yield (%)
40–60% ammonium sulphate precipitate	190	6000	0.03		
DEAE-cellulose supernatant	182	2020	0.09		
Phosphocellulose pool I	90	4.5	20.0	2.02	47
Phosphocellulose pool II	25	6.7	3.7	0.96	13

Values for aldolase pools I and II were determined after concentration of the samples.

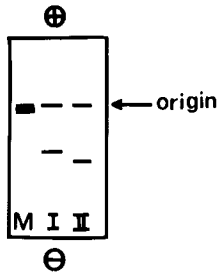

Fig.1. Starch gel electrophoresis of liver aldolases I and II and rabbit muscle aldolase (M) in a pH 7.0 buffer containing imidazole with a MOPS-EDTA discontinuity. Protein stained with Coomassie blue.

obtained with either liver aldolase, according to the buffer system used. The more complex gel patterns cannot be explained on present evidence but fig.1 illustrates a clear separation of aldolase I, aldolase II and aldolase A_4 in a pH 7.0 buffer system containing imidazole with a MOPS-EDTA discontinuity.

The results suggest that at least two forms of aldolase B_4 are present in the mammalian liver and one of these has a specific activity much greater than that previously reported. Heterogeneity of liver aldolase has been previously reported [12–14] but this heterogeneity was probably of a different nature: no form with a specific activity greater than about 2 was observed.

The FBP:FlP ratio of aldolase I could be lowered from 2 to 1, the observed value for aldolase II, by freezing once in liquid nitrogen. This was due to a 50% decrease in FBP activity, the FlP activity being unaltered. Several more freezing and thawing cycles had no further effect on activity towards either substrate. Both activities in aldolase II were stable to freezing and thawing. Determination of the structural relationship between aldolases I and II may clarify the relationship between their activities with FBP and FlP which appear to be at least partly separable.

Preliminary kinetic investigations have indicated that both liver aldolases have a much greater affinity for FlP than previously suggested [15], having K_M values of the order of 1 μM.

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