# A STRUCTURALLY MODIFIED LIVER ALDOLASE IN FRUCTOSE INTOLERANCE : IMMUNOLOGICAL AND KINETIC EVIDENCE.°

Yves Nordmann, Fanny Schapira
and Jean Claude Dreyfus

Institut de Pathologie Moléculaire
Centre Universitaire Cochin
24, rue du Faubourg St-Jacques, Paris, France

Received May 10, 1968

Fructose Intolerance is an inherited metabolic disease due to a decrease in the aldolase activity of the liver (Hers 1961, Froesch 1966). The enzymatic activity is not decreased to the same extent towards the two substrates of the enzyme. The activity towards fructose-1-phosphate (F-1-P) falls to 3 % of the normal value, while fructose-1-6-diphosphate (F-D-P) is split at 15-20 % of the normal range. As a result, the activity ratio of the liver enzyme on the two substrates F-D-P/F-1-P, which is normally 1, is about 6 in Fructose Intolerance. Measurement of its residual activity shows that aldolase in this disease resembles more the muscle aldolase (high activity ratio) than normal liver aldolase. In addition, previous work from our laboratory (Schapira and Dreyfus 1967) has shown that Fructose Intolerance aldolase is inhibited by ATP, which inhibits normal muscle aldolase, but not normal liver aldolase.

Two hypotheses could be made as to the nature of the enzymatic defect : 1) A lack of synthesis of the adult liver-type aldolase (aldolase B) with persistance of the enzyme normally present in embryonic liver which bears

° This work was supported by grants from "le Centre National de la Recherche Scientifique", "l'Institut National de la Santé et de la Recherche Médicale", "la Délégation Générale à la Recherche Scientifique et Technique et ses Comités Scientifiques (Fonds de Développement)", The Muscular Dystrophy Associations of America Inc., New York U.S.A., The National Cancer Institute.

many similarities to muscle-type aldolase (Aldolase A).

2) A structural anomaly of aldolase B which would be present but with a greatly decreased activity : the small amount of aldolase A normally present in the liver would remain intact and become the main aldolase present in Fructose Intolerance liver. Evidence is presented here that the second hypothesis is correct. The presence of a protein, sharing the immunological properties of aldolase B, could be demonstrated in the liver of two patients. Kinetic data confirmed the structural abnormality : a significant increase of Km values was found in four patients.

## MATERIALS AND METHODS

Samples of liver of children showing all the clinical and biological symptoms of Fructose Intolerance were obtained by needle or surgical biopsy. Samples of control liver and muscle were obtained by surgical biopsy. Foetal liver (3-4 months of foetal age) came from therapeutical abortion or post mortem. Rabbit muscle aldolase, prepared according to Taylor et al (1948) was recrystallized four times. Pure liver aldolase was prepared according to Pogell (1962) as modified by Rutter et al (1967) and Morse and Horecker (1966). Michaelis constant was estimated using F-1-P as substrate. Aldolase activity was measured by a modification of the colorimetric method of Sibley and Lehninger (1949).

Proteins were determined according to Lowry et al (1951). Antiserum to rabbit liver aldolase (anti B) and to rabbit muscle aldolase (anti A) were prepared in chickens by repeated injections of crystalline rabbit liver or muscle aldolase, with or without complete Freund adjuvant. One unit of anti aldolase B was arbitrarily defined as the amount of antibody which inhibits completely 0,06 international unit of F-D-P cleavage activity. Rabbit and human aldolases are equally inhibited by chicken antisera. Aldolases show no significant species specificity. By contrast they display a strict tissue

specificity (less than 2 % of cross reacting material between muscle and liver Aldolases).

The presence of cross reacting material (CRM) was examined by comparing the removal of neutralizing antibodies by Fructose Intolerance liver and by normal liver extract. A standard curve was obtained by adding increasing amounts of normal liver extract to a constant amount of anti B. After incubation and centrifugation, the supernatant was assayed for residual antibody by determining the neutralization of the F-D-P cleavage activity of pure aldolase B.

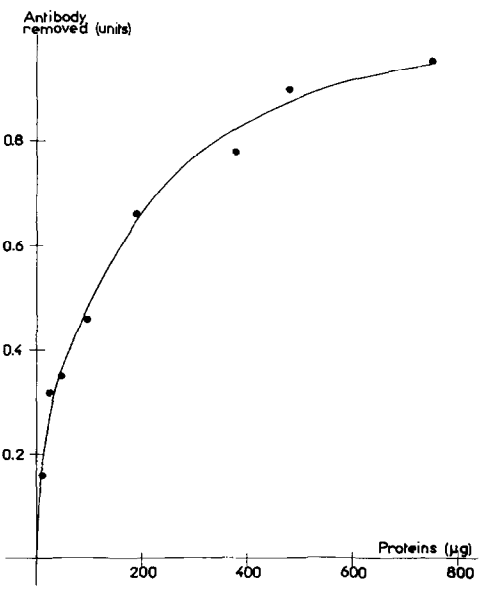


Fig. 1 - Removal of antibody by normal liver. Increasing amounts of liver proteins were incubated with 0,1 ml (1 Antibody unit) of anti B in a volume of 0,25 ml containing 0,05 M glycylglycine pH 7,5 and 2 % NaCl at 37° for 60 minutes and at 4° overnight. After centrifugation, supernatants were heated at 56° for 30 minutes to destroy aldolase activity and then centrifuged. The supernatants (0,1 ml) were analyzed for residual anti B by incubation with crystalline liver aldolase at 37° for 60 minutes and at 4° overnight and were then assayed for residual F-D-P cleavage activity. Controls included normal chicken serum. (This method has been described by Blostein and Rutter (1963) using anti-muscle aldolase).

The same type of experiment was performed with muscle, foetal liver, and Fructose Intolerance liver extracts. In the latter case the amount of available tissue (10mg as a mean) allowed to assay only one dilution. Plotting the

results on the standard curve allowed to compute the amount of CRM in each tissue studied after determination of protein concentration.

## RESULTS AND DISCUSSION

Three observations could be made :

1) The liver of Fructose Intolerance patients contains a protein immunologically related to normal liver aldolase. Fig. 1 and Table I show that Fructose Intolerance liver extracts contain 25 to 30 % of the normal content of immunological aldolase protein. This is in contrast with an enzymatic activity of only 3 % of the normal value (using F-1-P as the substrate). Foetal liver displays about 12-15 % of adult activity with F-1-P and 14 % of immunologically reacting material. Fructose Intolerance liver, therefore, possesses 4-5 times less enzymological activity than foetal liver, and twice as much immunological CRM.

Table I. Percentage of protein cross reacting (CRM) with liver type aldolase in human tissues (see fig. 1 for experimental details).

| Human tissues | Proteins mg/0,1 ml | Removal of neutralizing antibody (units) (x) | Normal liver proteins mg/0,1 ml (xx) | CRM Percentage (xxx) |
|---|---|---|---|---|
| Muscle | 1 | 0,05 | 0,02 | 2 |
| Foetal liver | 0,43 | 0,40 | 0,06 | 14 |
| Fructose Intolerance | | | | |
| Liver a | 0,75 | 0,66 | 0,18 | 24 |
| b | 0,80 | 0,71 | 0,24 | 30 |

(x) One unit : Amount of antibody inhibiting 0,06 unit of F-D-P cleavage activity

(xx) Amount of normal liver proteins producing an equivalent removal of antibody

(xxx) $\frac{\text{column 3}}{\text{column 1}} \times 100$

2) Aldolase B of Fructose Intolerance liver has abnormal enzymological properties. Table II shows that the Km towards F-1-P is increased about six times = 1.6 to $2.2.10^{-2}$M as opposed to a normal value of 2.2 to $3.5.10^{-3}$M.

Table II. Activity and Km values for F-1-P

| Liver enzyme | | Activity (x) (International Units) | Km |
|---|---|---|---|
| Control | (Normal (post mortem) | 2.7 | $3.5.10^{-3}$ |
| | )Glycogenosis (biopsy) | 8.5 | $2.5.10^{-3}$ |
| | (Hepatitis (biopsy) | 5.5 | $3.0.10^{-3}$ |
| Foetal | 3 months | 0.7 | $3.5.10^{-3}$ |
| | 3 months | 1.8 | $3.0.10^{-3}$ |
| | 4,5 months | 0.83 | $2.2.10^{-3}$ |
| Fructose Intolerance | | | |
| | Led.. | 0.160 | $1.6.10^{-2}$ |
| | Aud.. Th. | 0.178 | $1.9.10^{-2}$ |
| | Aud.. J. | 0.240 | $2.0.10^{-2}$ |
| | Lap.. | 0.260 | $2.2.10^{-2}$ |

(x) One unit = µM F-1-P metabolized per minute and per gramme at 38°C.

3) Most of the residual aldolase activity of Fructose Intolerance liver is due to aldolase A. In two cases the neutralizing effect of both anti A and anti B was assayed. The activity of Fructose Intolerance liver extract on F-D-P is inhibited by anti A (35 % in one case, 60 % in an other case), but very poorly by anti B (15 %). These results confirm our previous studies on aldolase inhibition by ATP : the remaining activity towards F-D-P is almost entirely due to unchanged aldolase A, which is inhibited by anti A and by ATP. We may conclude that in Fructose Intolerance the synthesis of liver type aldolase persists but that the enzyme has an abnormal structure. The disease, therefore, is due to a mutation of the structural gene of the liver-type aldolase. Few attempts have been made to detect quantitatively an inactive enzyme protein in human genetic diseases. No immunological detection was obtained in glycogen storage diseases of muscle due to a lack of phosphorylase (Robbins 1960, Rowland et al 1963) or phosphofructokinase (Layzer et al 1967). Qualitative detection of catalase was positive in acatalasemia (Micheli and Aebi 1965). In conclusion, the biochemical lesion of Fructose

Intolerance consists in a structural alteration of one of the isozymes of Aldolase, i.e. Liver type Aldolase. This alteration results in the synthesis of a protein which is nearly inactive, but keeps its immunological specificity.

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