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# Fructose-1, 6-bisphosphatase in human fetal brain and liver during development

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**Abstract.** Activity of fructose-1,6-bisphosphatase (EC 3.1.3.11), one of the key gluconeogenic enzymes, was measured in human fetal brain and liver during development. Fructose-1,6-bisphosphatase was distributed throughout the different regions of the brain. In contrast to the partially purified enzyme from the brain, the liver enzyme was dependent on $Mg^{2+}$ for maximal activity, EDTA, citrate, oleate and linoleate were stimulatory, whereas 5'-AMP inhibited the activity of the liver enzyme.

**Keywords.** Human fetus; brain and liver fructose bisphosphatase; fructose-phosphate inter-conversion; *myo*—inositol synthesis.

## Introduction

During development, the human fetus depends on the mother for the supply of glucose. The glucose homeostasis of the fetus not only aids development but also contributes to extrauterine adaptation of the fetus, in which neonatal energy metabolism supported by gluconeogenesis plays a decisive function.

In order to understand the possible role of gluconeogenesis during the development of human fetus, the present investigation on the nature and properties of fructose-1,6-bisphosphatase (EC 3.1.3.11), a key gluconeogenic enzyme was undertaken. The two organs considered for this study were the fetal liver and brain. Although the brain is not ordinarily considered to be a gluconeogenic organ, it was taken into consideration because of the unequivocal demonstration of the enzyme therein (Majumder and Eisenberg, Jr., 1977). The present communication deals with the preliminary studies on fructose bisphosphatase during development of human fetal liver and brain.

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## Materials and methods

Human fetuses of different gestation periods (determined by considering the last menstrual period) were obtained from- normal mothers undergoing hysterotomy and ligation in the SSKM hospital attached to the Institute of Post Graduate Medical Education and Research, Calcutta, as a part of the Medical Termination of Pregnancy Clinic. The project was cleared by the Ethical Sub-Committee of the Institute of Post Graduate Medical Education and Research. The fetuses were put on ice immediately after the operation and the liver and brain were dissected within 30 min,after the operation.

Fructose- 6-bisphosphate, sedoheptulose-l,7-bisphosphate, glucose-6-phosphate, fructose-6-phosphate, and sodium β-glycerophosphate were purchased from Sigma Chemical Company, St. Louis,Missouri, USA. Sodium salts of oleate and linoleate (Applied Science, USA) were kind gifts of Dr. Eisenberg, Jr. of the National Institutes of Health, Bethesda, USA. Partially purified fructose bisphosphate was isolated from human fetal brain of different stages of gestation following essentially the procedure of Majumder and Eisenberg, Jr. (1977) described for the enzyme from rat brain. A 25% homogenate of a portion of tissue from different parts of brain in 0.15 M KCl containing 0.01 M $NaHCO_3$ was centrifuged at 10,000 *g* for 30 min in a refrigerated Remi K-24 centrifuge. The supernatant was heated for 3 min at 60°C and again centrifuged at 15,000 *g* as before. The heated supernatant was adjusted to pH 4.8 with 5 M acetic acid and immediately centrifuged at 10,000 *g.* The supernatant thus obtained was adjusted to pH 7.5 (termed acid fraction), dialysed against 0.005 M Tris-HCl, pH 7.5 and used as the enzyme source.

A somewhat different procedure was adopted for the isolation and partial purification of the enzyme from human fetal liver. A 10,000 *g* supernatant was obtained after centrifuging a 25% 0.15 M KCl/0.01 M $NaHC0_3$ homogenate of fetal liver. This was centrifuged at 100,000 *g* for 1 h in a MOM (Hungary) Ultra-centrifuge. The supernatant obtained was heated for 3 minutes at 60°C and the coagulated protein centrifuged off at 20,000 *g* in a Remi K-24 (India) refrigerated centrifuge. The supernatant thus obtained (termed heat fraction) was dialysed against 0.005 M Tris-HCl, pH 7.5 and used as the enzyme in this study.

Assay for fructose bisphosphatase activity was carried out by the procedure of Majumder and Eisenberg, Jr. (1977) by measuring the released inorganic phosphate (Ames 1966). The standard incubation mixture contained the following (all in μmol ml); Tris-HCl buffer (pH 7.5/9.5) 100; $MgSO_4$, 5; fructose-1, 6-bisphosphate, 0.1-0.5; and enzyme, appropriate amount. It was incubated at 37°C for an hour, deproteinized by the addition of 5% trichloroacetic acid and the supernatants assayed for inorganic phosphate. Parallel sets were run using fructose-6-phosphate as substrate for determination of any non-specific phosphatase activity.

Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

## Results and discussion

A comparison of the fructose bi-phosphatase activity at different stages of gestation revealed appreciable change in the activity in both fetal brain and liver during development (table 1). The relative enzyme activity at the neutral and

**Table 1.** Fructose-l,6-bisphosphatase activity of human fetal brain & liver at different gestation periods.

| Gestation period (in weeks) | Body wt (in *g*) | Fructose bisphosphatase activity (µmol $P_i$ liberated/ h /mg protein) | | | |
|---|---|---|---|---|---|
| | | Brain | | Liver | |
| | | pH 7.5 | pH 9.5 | pH 7.5 | pH 9.5 |
| 8 to 12 | 4.4 | 0.183 | 0.241 | — | — |
| | 8.3 | 0.496 | 0.496 | 0.78 | — |
| | 10.3 | 0.32 | 0.38 | — | 0.58 |
| 13 to 16 | 15.0 | — | — | 0.20 | 0.38 |
| | 15.7 | 0.241 | 0.274 | — | — |
| | 16.4 | — | — | 0.16 | 0.47 |
| | 34.3 | 0.132 | 0.164 | — | — |
| | 37.0 | — | — | 0.49 | 0.60 |
| | 38.8 | — | — | 0.17 | 0.25 |
| | 47.1 | 0.158 | 0.274 | — | — |
| | 50.0 | 0.158 | 0.174 | — | — |
| | 73.3 | 0.248 | 0.064 | — | — |
| | 103.0 | 0.151 | 0.191 | — | — |
| | 108.0 | 0.281 | 0.248 | — | — |
| 17 to 20 | 113.0 | — | — | 0.33 | 0.33 |
| | 172.0 | 0.106 | 0.145 | — | — |
| | 265.0 | — | — | 0.165 | 0.204 |
| 21 to 24 | 310.0 | 0.125 | 0.091 | — | — |
| | 392.0 | 0.15 | 0.0 | 0.90 | 0.28 |
| | 524.0 | 0.053 | 0.32 | 0.19 | 0.49 |
| | 560.0 | 0.44 | 0.52 | 0.10 | 0.43 |
| 28 to 32 | 1553.0 | — | — | 0.56 | 0.57 |
| Adult | — | 1.18 | 0.96 | — | — |

The enzyme activity was determined in the 10,000 *g* supernatant as described in Methods and expressed as µmol $P_i$ liberated per mg protein per hour after correction for the Fructose-6-Phosphatase activity, if any. Other details are in Methods.

— Not detected

alkaline range varied at different stages of development, although there was a tendency towards even distribution at the two pH values. The fructose-6-phosphatase activity of the different samples, if at all, was about 20% that of fructose bisophosphatase activity. Variation was noticed among the specific activity values for the brain and liver enzymes. However, in case of brain, the activity of the enzyme during fetal life was much lower than that of the adult organ but higher than the adult rat brain (Majumder and Eisenberg, Jr., 1977).

Table 2 describes fructose bisphosphatase activity isolated from the fetal brain of different gestation periods at different stages of purification as outlined under Methods. It was evident that, in all the specimens, except for that of 21-24 weeks

**Table 2.** Fructose-l,6-bisphosphatase and fructose-6-phosphatase activity during purification of human fetal brain obtained at different stages of development.

| | Gestation period | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8-12 weeks | | | | 13-16 weeks | | | | 17-20 weeks | | | | 21-24 weeks | | | |
| Stage of purification | Fru-$P_2$ase at pH | | F-6-pase at pH | | Fru-$P_2$ase at pH | | F-6-pase at pH | | Fru-$P_2$ase at pH | | F-6-pase at pH | | Fru-$P_2$ase at pH | | F-6-pase at pH | |
| | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 | 7.5 | 9.5 |
| 10,000 *g* supernatant | .158 | .158 | 0 | 0 | .158 | .190 | .026 | .026 | .183 | .222 | .077 | .077 | .183 | .181 | .058 | .090 |
| Heat fraction | .664 | .412 | 0 | 0 | .148 | .200 | 0 | .016 | .032 | .280 | .064 | .232 | .116 | .174 | 0 | .032 |
| Acid fraction | 1.32 | 1.32 | 0 | 0 | .412 | .438 | 0 | 0 | .741 | 0 | .125 | .061 | .174 | .206 | 0 | 0 |

Fru-$P_2$ase and F-6-Pase activities were determined as described in Methods and expressed as μmol $P_i$ liberated per mg protein per hour. Procedures for partial purification are described in Methods.

Fru-$P_2$ase — fructose-l,6-bisphosphatase
F-6-pase — fructose-6-phosphatase.

of gestation, there was a progressive increase in the specific activity during purification. Better purification was achieved with specimens between 8-12 weeks of gestation, a stage also characterized by no detectable fructose-6-phosphatase activity. The ratio of the activities at pH 7.5 and pH 9.5 was close to 1. It may be worth mentioning here that although mammalian liver fructose bisphosphatase is reported to exhibit two activities at pH 7.5 and pH 9.5 as a result of proteolytic modification (Horecker *et al.*, 1975) the rat brain enzyme failed to show two peaks of activity over a pH range of 6 to 10 (Majumder and Eisenberg, Jr., 1977). Human fetal brain fructose bisphosphatase exhibited a similar property. Scanning of

different parts of the fetal brain e.g. cerebrum, midbrain and cerebellum showed that the enzyme was distributed in all these regions. Little variation in the distribution was noticed in samples between 8 to 24 weeks of gestation (table 3).

**Table 3.** Distribution of fructose-l,6-bisphosphatase activity in different regions of human fetal brain, from different stages of development.

| Gestation period (in weeks) | Body weight (in g.) | Fructose-1,6-bisphosphatase activity (μmol $P_i$/hour/mg. protein) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cerebrum | | Midbrain | | Cerebellum | |
| | | pH 7.5 | pH 9.5 | pH 7.5 | pH 9.5 | pH 7.5 | pH 9.5 |
| 8-12 | 10 | 0.61 | 0.26 | 0.19 | 0.49 | — | — |
| 13-16 | 45.5 | 0.12 | 0.31 | 0.13 | 0.35 | 0.48 | 0.41 |
| | 58 | 0.28 | 0.23 | 0.29 | 0.29 | 0.24 | 0.11 |
| 17-20 | 157 | 0.29 | 0.49 | 0.28 | 0.88 | 0.49 | 0.63 |
| | 207 | 0.22 | 0.22 | 0.30 | 0.61 | 0.49 | 0.92 |

10,000×g supernatant from different regions of fetal brain, of different stages of gestation was used as enzyme source. Fructose-bisphosphatase activity was corrected for Fructose-6-phosphatase activity, if any.

Some properties of the fructose bisphosphatase partially purified from fetal brain (acid-fraction) and liver (heat-fraction) were studied. Substrate specificity of the enzymes from the two sources, using various sugar phosphates, such as fructose-l,6-bisphosphate,sedoheptulose-1,7-bisphosphate,glucose-6-phosphate, fructose-6-phosphate and β-glycerophosphate (each 0.5 mM) revealed that both the enzymes were unable to hydrolyze glucose-6-P, fructose-6-phosphate and ß-glycerophosphate. The brain enzyme hydrolyzed sedoheptulose-1, 7-bisphosphate to 20% of the fructose-1, 6-bisphosphate activity whereas 37% of the activity was recorded for liver enzyme with the same substrate.

Further studies revealed that while the liver enzyme was dependent on divalent cation for maximal activity, the brain enzyme had no requirement for it. $Mn^{2+}$ at 5 mM was optimally effective and could be partially replaced by $Mn^{2+}$ (2mM). The liver enzyme was stimulated by EDTA (0.1 mM) and inhibited by 5'-AMP (0.5 mM). Citrate (0.5 mM), oleate (0.5 mg/ml) and linoleate (0.5 mg/ml), increased the activity of the liver enzyme approximately by 2,1.5 and 2.3 fold respectively. This property was also shared by the rabbit liver enzyme (Baxter *et al.*, 1972, Carlson *et al.*, 1973). All these compounds had little effect on the brain enzyme activity.

Studies on the gluconeogenic enzymes during fetal life were mainly undertaken in human, sheep, guinea-pig and baboon (Aurricchio and Rigillo, 1960; Ballard and

Oliver, 1965; Jones and Ashton, 1972; Levitsky *et al*., 1976). For obvious reasons, a detailed study of these enzymes in the human fetus, in particular, was lacking and subhuman primates were often chosen for this purpose (Robinson *et al*., 1980; Sherwood *et al.,* 1980). Most of these studies dealt with determination of activities of different gluconeogenic enzymes in cell extracts from liver or kidney. In the present study we have restricted ourselves to the demonstration of the fructose bisphosphatase activity and the partial purification of the enzyme from both the organs upto a stage where it exhibits absolute specificity towards hydrolysis of fructose bisphosphate. The preliminary characterization of the liver enzyme reported here, showed that with respect to the divalent cation requirement, EDTA stimulation and 5'-AMP inhibition, it was similar to the enzyme from other mammalian sources and recently reported adult human liver enzyme (Dzugaj and Kochman, 1980).

Though not considered gluconeogenic (Scrutton and Utter, 1968), existence of fructose bisphosphatase in brain was suggested by [$^{14}$C]-pyruvate and [$^{14}$C]-glutamate incorporation studies in guinea-pig brain slices (Phillips and Coxon, 1975) and subsequently its presence in rat brain was demonstrated (Majumder and Eisenberg, Jr., 1977; Hevor and Gayet, 1978). Majumder and Eisenberg, Jr. (1976-1977) suggested that in brain, this enzyme along with the phosphofructokinase, may be regulated by fatty acids and phospholipids for controlled utilization of glucose-6-phosphate for synthesis of *myo*-inositol by the *myo*-inositol synthase reaction. Keeping this in view, a probable coupling between the two enzymes has recently been suggested (Majumder, 1981). Since during its development, the human fetal brain requires a high pool of *myo*-inositol for synthesis of polyphosphoinositides of the myelin (Eichberg and Dawson, 1965; Eichberg and Hauser, 1973),this system was thought to be ideal for this study. As preliminary to this we have demonstrated the presence of a fructose bisphosphatase in human fetal brain during development and studied some of their properties. Partial purification of *myo*-inositol synthase from rat brain was reported (Maeda and Eisenberg Jr 1980) and human fetal brain has been found to have appreciable synthase activity (Adhikari and Majumder, unpublished observation). Further studies would require a detailed characterization of the purified enzyme from both liver and brain as well as that of *myo*-inositol synthase. Work in this direction is now in progress.

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