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L-*myo*-Inositol-1-Phosphate Synthase Expressed in Developing Organ: Isolation and Characterisation of the Enzyme from Human Fetal Liver

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Abstract L-*myo*-inositol-1-phosphate synthase (MIPS; EC: 5.5.1.4) activity has been detected and partially purified for the first time from human fetal liver. Crude homogenate from the fetal liver was subjected to streptomycin sulphate precipitation and 0–60 % ammonium sulphate fractionation followed by successive chromatography through DEAE cellulose and BioGel A 0.5-m columns. After the final chromatography, the enzyme was purified 51-fold and 3.46 % of MIPS could be recovered. The human fetal liver MIPS specifically utilised D-glucose-6-phosphte and NAD^+ as its substrate and coenzyme, respectively. It shows pH optima between 7.0 and 7.5 while the temperature maximum was at 40 °C. The enzyme activity was remarkably stimulated by NH_4^+, slightly stimulated by K^+ and Ca^{2+} and highly inhibited by Zn^{2+}, Cu^{2+} and Hg^{2+}. The K_m values of

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MIPS for D-glucose-6-phosphate and NAD^+ were found to be as 1.15 and 0.12 mM respectively while the V_{max} values were 280 nM and 252 nM for D-glucose-6-phosphate and NAD^+ correspondingly. The apparent molecular weight of the native enzyme was determined to be 170 kDa.

Keywords Human fetal liver · Enzyme purification · L-*myo*-inositol-1-phosphate synthase · *Myo*-inositol · D-glucose-6-phosphate · L-*myo*-inositol-1-phosphate

Abbreviations

G-6-P	D-glucose-6-phosphate
MIPS	L-*myo*-inositol-1-phosphate synthase
MIP	L-*myo*-inositol-1-phosphate
ME	2-mercaptoethanol

Introduction

Myo-inositol is a polyol that is characterised as a six-carbon ring where each carbon is hydroxylated. A number of these sugar-alcohol isomers are biologically active, but *myo*-inositol is the most common [1–3]. It constitutes a critical component of membrane phospholipids, mediates osmoregulation and is an important precursor for the phosphoinositide signalling pathway [3]. Its phosphorylated derivatives participate in a multitude of key biochemical processes: they act as critical second messengers in signal transduction pathways [4], mediate phosphorylation of target proteins [5], participate in chromatin remodelling and gene expression [6, 7] and facilitate mRNA export from the nucleus [8].

The de novo generation of *myo*-inositol occurs by a universal mechanism that is conserved throughout the phylogenetic domain [9]. L-*myo*-inositol-1-phosphate synthase (MIPS; EC: 5.5.1.4) is a rate-limiting enzyme that catalyzes the first step in the biosynthesis of all *myo*-inositol containing compounds [10]. It converts D-glucose-6-phosphate (G-6-P) to L-*myo*-inositol-1-phosphate (MIP). The phosphate moiety in MIP is subsequently removed by inositol monophosphatase 1 (IMPase) to produce free *myo*-inositol [3].

Myo-inositol deficiency causes high accumulation of triacylglycerol, cholesterol and non-esterified lipids in mammalian liver. *Myo*-inositol deficient animals developed fatty liver and a minimum threshold level of free *myo*-inositol is required for protection against fatty liver formation [11, 13]. Hence, the metabolic understanding of *myo*-inositol status in any biological organ or system is primarily dependent on MIPS activity and its regulation.

Although studies on *myo*-inositol synthesis during fetal life has been done by Burton and Wells [13] and Adhikari and Majumder [14], so far, there has not been a single report regarding the same from developing human fetal liver. The developmental events of this organ occur during the prenatal period culminating in fully grown organ at the onset of postnatal life. The supply and synthesis of *myo*-inositol is obligatory in the developing human fetus in order to prevent irregularities of liver function [11, 12] and principally as the precursor of myelin in nervous system [3, 14, 15]. Naturally, it could be anticipated that de novo *myo*-inositol biosynthesis should start in humans not only in brain [14] but also in liver at fetal stage itself to install an intracellular protection against any fatty liver formation for postnatal life.

Therefore, the present investigation is confined to the study of MIPS, the key enzyme of *myo*-inositol biosynthesis, in human fetal liver which exhibits a high demand for *myo*-inositol for normal metabolism. For the first time, the enzyme has been isolated and characterised from developing human fetal liver.

Materials and Methods

Fetal Material

Human fetal samples of different gestation periods were obtained from normal mothers undergoing hysterectomy and ligation as a part of medical termination of pregnancy at the District Hospital, Darjeeling, West Bengal, India. All the mothers were multiparous and their anthropometric measurements conformed to Indian Council of Medical Research Standards for healthy Indian women. The study was permitted by the Chief Medical Officer of Darjeeling and the investigators had no say in the termination of pregnancy. Fetal liver were dissected aseptically and stored at −20 °C within 0.5 h of operation, and the liver tissue minus the gall bladder were kept frozen.

Isolation of MIPS from Human Fetal Liver

Human fetal liver samples (8–20 weeks of gestation period) were minced and suspended in 2 volumes of chilled 0.154 M KCl containing 0.2 mM ME. All experimental steps were carried out at 0–4 °C except when otherwise stated. The samples were homogenised in a glass homogeniser for 5 min. The crude homogenate was centrifuged at 10,000×*g* for 20 min in a Plasto Crafts Superspin-R centrifuge. The supernatant was collected and dialysed against 500 volumes of the extraction medium for 4 h. The dialysed fraction (low-speed supernatant) was recovered from the dialysis bag and used as the enzyme source for screening experiments.

Partial Purification of MIPS from Human Fetal Liver

To purify MIPS, the low-speed supernatant obtained from liver tissues (16–20 weeks of gestation period) was subjected to 2 % streptomycin sulphate treatment with constant stirring. The mixture was kept in an ice bucket for 30 min and then centrifuged at 11,400×*g* for 20 min. The streptomycin sulphate-treated supernatant (streptomycin sulphate fraction) was kept for 2 to 3 min in a water bath maintained at 60 °C. It was immediately cooled to 0 °C and maintained at that temperature for 30 min, centrifuged at 11,400×*g* for 20 min and the supernatant collected (heat-treated fraction).

The heat-treated supernatant was fractionated with 0–60 % ammonium sulphate, and it was kept in an ice bucket for 30 min and then centrifuged at 11,400×*g* for 20 min. The pellet obtained was dissolved in a minimal volume of 50 mM Tris-acetate buffer (pH 7.0) containing 0.2 mM ME and dialysed for 12 h against 500 volumes of the same buffer with one change. On completion of dialysis, the ammonium sulphate fraction (ammonium sulphate fraction) was recovered from the dialysis bag.

The dialysed ammonium sulphate fraction from the previous step was adsorbed on DEAE cellulose and the preparation was loaded on a glass column. The adsorbed proteins were eluted from the column with a linear gradient of 0.0–0.5 M KCl (Fig. 1a). The active DEAE cellulose fractions (DEAE cellulose fraction) loaded onto a column of BioGel A 0.5 m, and

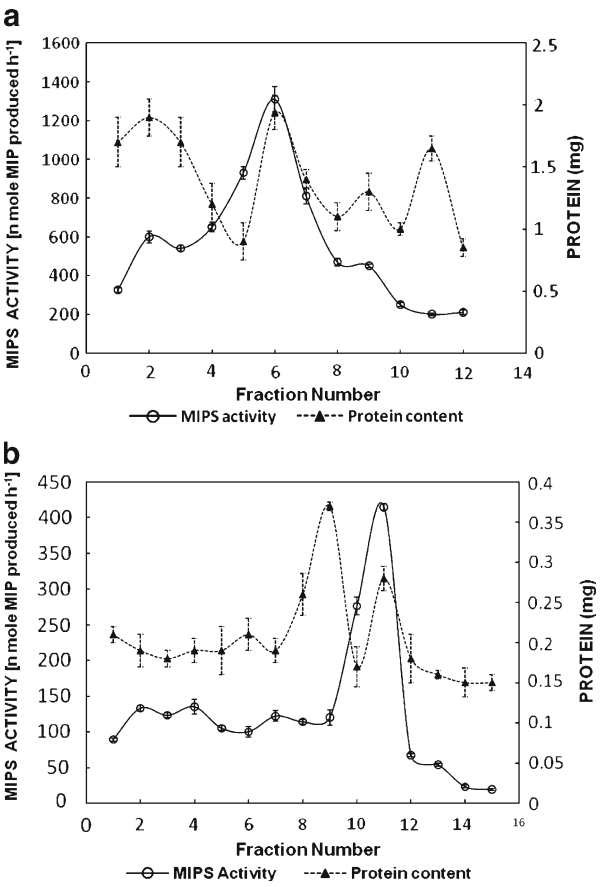

Fig. 1 **a** Elution profile of human fetal liver MIPS chromatographed through DEAE cellulose column (MIPS activity expressed as n mole *myo*-inositol-1-phosphate produced (1.0 ml fraction)$^{-1}$h^{-1}). **b** Elution profile of human fetal liver MIPS chromatographed through BioGel A 0.5-m column (MIPS activity expressed as nmol *myo*-inositol-1-phosphate produced (0.75 ml fraction)$^{-1}$h^{-1})

the proteins were eluted with the extraction medium (Fig. 1b). The pooled active fractions (BioGel A 0.5-m fraction) were concentrated and used as the final preparation of the partially purified enzyme.

Assay of MIPS Activity

MIPS assay was carried out following the method of Barnet et al. [16] as modified by Adhikari et al. [17]. The incubation mixture contained 50 mM Tris-acetate (pH 7.0), 14 mM NH_4Cl, 0.8 mM NAD, 0.5 mM ME, 5 mM G-6-P and an appropriate protein aliquot (10–100 μg), in a total volume of 0.5 ml. The enzymatic incubation was carried out for 60 min at 37 °C, and the reaction was stopped by adding 0.2 ml of 20 % chilled TCA. Two such sets (Set I—periodate and Set II—non-periodate) were run simultaneously each having one blank (without enzyme), one 0-min control (in which 0.2 ml of 20 % (*w*/*v*) chilled TCA was added before adding the enzyme) and two experimental tubes. The quantity of the enzymatic product was estimated by periodate oxidation followed by the estimation of inorganic phosphates. After completion of the enzyme incubation, the reaction mixture was treated with 0.7 ml of 200 mM sodium metaperiodate ($NaIO_4$) and incubated for 60 min at 37 °C. Then 1.4 ml of freshly prepared 1 M Na_2SO_3 was added in case of Set I

to destroy excess of $NaIO_4$. In Set II, water was added instead of $NaIO_4$ and Na_2SO_3 to maintain the volume.

Inorganic phosphate was liberated from *myo*-inositol-1-phosphate during oxidation. Product-specific release of inorganic phosphate was estimated by subtracting the corrected value of Set II from that of Set I. Inorganic phosphate was estimated by the method of Chen et al. [18] with slight modifications as per Adhikari et al. [17]. A freshly prepared P_i—reagent (2.8 ml) containing H_2SO_4 (6 N), ascorbic acid (10 %, *w*/*v*), chilled ammonium molybdate (2.5 %, *w*/*v*) and H_2O (1:1:1:2) was added to the reaction mixture and incubated for 60 minutes at 37^0 C. After incubation, the blue colour developed was measured at 820 nm in a Beckman DU-64 spectrophotometer. The inorganic phosphate released was estimated with the help of a standard curve prepared from different known quantities (0–100 μg) of phosphorous (using K_2HPO_4).

Protein was determined according to the method of Bradford [19]. The protein content in fractions obtained from column chromatography was also determined by measuring absorbance at 280 nm.

As 1 mol of *myo*-inositol-1-phosphate contains 1 mol of inorganic phosphate, mole number of inorganic phosphate estimated was equal to the number of *myo*-inositol-1-phosphate produced. The specific activity of MIPS was defined as nmol MIP produced $(mg)^{-1}$ protein h^{-1}.

Protein Electrophoresis

Polyacrylamide gel electrophoresis of the BioGel fraction was performed under non-denaturing conditions in 1 mm gel following the method of Bollag et al. [20]. The protein sample (40 μg/lane) was loaded on a gel system (size of the slab gel was 8.0×8.0 cm with seven identical lanes) containing 10 % separating gel and electrophoresed in a Biotech regular slab gel apparatus at 4 °C at 100 V. For the MIPS activity assay from gel, one of the replicate gels was sliced into 5-mm fragments. Each fragment was extracted with 200 μl of 50 mM tris-acetate buffer (pH 7.5) at 0 °C for 30 min, and the extracts were assayed for MIPS activity.

M_r Determination

The apparent molecular weight of the fetal liver MIPS, obtained from the PAGE carried out with active BioGel A 0.5-m fraction was determined in GEL LOGIC 100 IMAGING SYSTEM (Kodak) using Molecular imaging software Kodak MI-V.4.0.5. The instrument was calibrated previously with the marker proteins of known molecular weights namely Myosin rabbit muscle (205 kDa), phosphorylase-b (97.4 kDa), BSA (68 kDa), ovalbumin (43 kDa) and carbonic anhydrase (29 kDa).

Results

MIPS Activity in Human Fetal Liver during Development

MIPS assay was performed using the dialysed low-speed supernatant of fetal liver of different gestation periods. Table 1 presents the result obtained from such experiment. From Table 1, it is clear that no MIPS activity is initiated in fetal liver up to 12 weeks of gestation period, beyond which appreciable MIPS activity is evident which increases sharply as the gestation period advances.

Table 1 *Myo*-inositol-1-phosphate synthase activity during development in the human fetal liver

Gestation period (weeks)	Average body weight of samples (g)	Average liver weight of samples (g)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})
8–12	12.50	1.65	0.00
12–16	50.00	6.40	21.64±2.02
16–20	350.00	14.00	36.57±2.98

Purification of MIPS

The enzyme, MIPS was isolated and purified from human fetal liver. The summary on the purification of MIPS is given in Table 2. Chromatographic profiles of proteins resolved from $(NH_4)_2SO_4$ fraction of the crude homogenate of human fetal liver are shown in Fig. 1a. The BioGel A 0.5-m chromatography (Fig. 1b) has revealed that the MIPS from human fetal liver was retained and eluted in a single sharp peak with the extraction buffer. In the present study, an overall purification of the enzyme to about 51-fold with about 3.5 % recovery based on total activity was achieved.

Characterisation of the Partially Purified Enzyme

Requirements for Different Assay Components for Human Fetal Liver MIPS Activity

The fetal liver MIPS, when assayed in presence of all the components of the reaction mixture recorded maximal activity. When the substrate, G-6-P was not added to the incubation mixture, enzymatic synthesis of MIP could not be detected. Exclusion of NAD^+ from the assay components caused about 87 % drop in the enzyme activity. About 20 % activity was lost when tris buffer was omitted from the complete reaction mixture. Deduction of NH_4Cl and ME reduced the enzyme activity by about 40 and 11 %, respectively (Table 3).

Table 2 Summary of partial purification of L-*myo*-inositol-1-phosphate synthase from human fetal liver (values are mean±SE, $n=3$)

Purification step	Protein content (mg/ml)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})	Total activity (nmol MIP produced h^{-1})	Recovery (%)	Purification (fold)
Homogenate	1,462.00±48.74	38.45±1.96	56,213.90±71.94	100.00±6.25	1.00±0.12
Low-speed supernatant	1,044.20±30.60	43.20±1.31	45,109.44±89.16	80.24±3.24	1.12±0.09
Streptomycin sulphate fraction	860.22±50.46	44.36±2.00	38,159.35±85.09	67.88±1.16	1.15±0.33
Heat-treated fraction	775.59±13.20	48.00±3.32	37,228.32±90.41	66.22±1.27	1.24±0.13
Ammonium sulphate fraction	384.75±9.39	89.37±4.50	34,385.10±15.79	61.16±1.67	2.32±0.73
DEAE cellulose fraction	4.38±0.11	637.50±54.36	2,792.25±34.20	4.96±0.10	16.57±0.73
BioGel A 0.5-m fraction	0.99±0.07	1,964.70±60.09	1,945.05±73.31	3.46±0.26	51.09±1.63

Table 3 Requirements of different assay components for human fetal liver L-*myo*-inositol-1-phosphate synthase activity (values are mean±SE, n=3)

Assay condition	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})	Percent activity
Complete set	354.60±3.58	100.00±4.51
Minus D-glucose-6-phosphate	0.00	0.00
Minus tris-buffer	288.72±5.70	81.42±1.86
Minus NAD^+	47.50±2.05	13.39±1.06
Minus ammonium chloride	212.00±4.04	69.78±2.08
Minus 2-mercaptoethanol	317.63±1.48	89.57±3.40
Heat-killed enzyme	0.00	0.00

Substrate Specificity

The partially purified human fetal liver MIPS has been found to specifically utilise G-6-P as the substrate for MIP production. Other hexose phosphates viz., D-fructose-6-phosphate, D-mannose-6-phosphate and D-galactose-6-phosphate at identical concentration were all ineffective as the substrate for human fetal liver MIPS (Table 4).

Coenzyme Specificity

MIPS from most sources require NAD^+ as an essential coenzyme for the oxidation-reduction reaction. To find out whether the human fetal liver MIPS uses NAD^+ as its specific coenzyme or not, experiments were performed whereby the enzyme activity was determined in presence of 0.3, 0.6 and 0.9 mM NAD^+ and $NADP^+$ in two parallel experimental sets. It was determined that NAD^+ could not be substituted by $NADP^+$ at any concentration. However, there was a basal activity of the enzyme in the experimental set with $NADP^+$ or the ones completely lacking both NAD^+ and $NADP^+$ (Table 5).

Effect of Monovalent and Divalent Cations

Effect of different metal ions was tested using chloride salts of metals (at 5 mM concentration). Of the monovalent cations tested, K^+ had a little stimulatory effect, Na^+ had no effective role and NH_4^+ was strongly stimulatory which increased the enzyme activity to

Table 4 Substrate specificity of human fetal liver L-*myo*-inositol-1-phosphate synthase activity (values are mean±SE, n=3)

Compound	Concentration (mM)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})	Percent activity
D-glucose-6-phosphate	5.0	273.48±21.19	100.00±11.02
D-fructose-6-phosphate	5.0	0.00	0.00
D-mannose-6-phosphate	5.0	0.00	0.00
D-galactose-6-phosphate	5.0	0.00	0.00

Table 5 Coenzyme specificity of L-*myo*-inositol-1-phosphate synthase activity from human fetal liver (values are mean±SE, *n*=3)

Coenzyme	Concentrations (mM)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})
NAD+	0.0	81.24±5.44
	0.3	218.84±7.85
	0.6	268.50±17.04
	0.9	276.63±13.04
NADP+	0.0	86.31±2.15
	0.3	94.05±9.50
	0.6	78.46±4.16
	0.9	88.00±9.46

about 1.8-fold. In case of divalent cations, it was revealed that Mg^{2+} and Mn^{2+}exhibited no effect but Ca^{2+} acted as an appreciable activator of the enzyme. The heavy metals Zn^{2+} inhibited the enzyme by 23 %, Cu^{2+} by 56 % and Hg^{2+} strongly inhibited the enzyme causing 70 % loss of activity (Table 6).

Enzyme Stability

The human fetal liver MIPS was found to be moderately stable and the *s*tability of the enzyme varied at different levels of purification. While the $(NH_4)_2SO_4$ fraction remained active for 20–25 days when stored at −20 °C, the DEAE cellulose fraction remained active for 15–20 days and the BioGel A 0.5-m purified fractions maintained its activity only up to 5–7 days at identical temperature. Addition of common enzyme stabilisers like sucrose, glycerol, dithiothreitol or ME failed to increase the shelf-life of the enzyme (Table 7). In addition repeated freeze–thaw caused drastic loss of the enzyme activity.

Enzyme Concentration and the Time Linearity

The human fetal liver MIPS exhibited enzyme activity linearity up to 250 μg of protein concentration under optimal assay conditions (Fig. 2). The rate of enzyme reaction

Table 6 Effect of monovalent and divalent cations on human fetal liver L-*myo*-inositol-1-phosphate synthase activity (values are mean±SE, *n*=3)

Cation	Concentrations (mM)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})	Percent activity
Control	0	160.85±5.43	100.00±9.83
K^+	5	187.28±16.69	116.43±9.25
Na^+	5	156.63±9.83	97.37±8.10
NH_4^+	5	294.15±10.17	182.17±15.55
Mg^{2+}	5	173.42±5.98	107.81±3.05
Mn^{2+}	5	170.00±17.58	105.68±5.80
Ca^{2+}	5	200.06±12.08	124.37±10.15
Zn^{2+}	5	123.76±8.84	76.94±3.11
Cu^{2+}	5	70.55±8.46	43.86±2.79
Hg^{2+}	5	48.13±4.83	29.92±2.22

Table 7 Effect of different enzyme stabilisers on human fetal liver L-*myo*-inositol-1-phosphate synthase activity (values are mean±SE, n=3)

Enzyme stabiliser	Concentration (mM)	Storage period (days)	Specific activity (nmol MIP produced $(mg)^{-1}$ protein h^{-1})
No stabiliser	0	5	261.46±13.74
		10	204±20.63
2-mercaptoethanol	100	5	266.17±21.56
		10	218.31±17.06
Dithiothreitol	100	5	259.04±18.30
		10	201.85±16.33
Sucrose	100	5	264.94±13.08
		10	189.65±11.72
Glycerol	100	5	252.57±9.25
		10	176.44±22.36

proceeded linearly up to 75 min with G-6-P as the substrate and NAD^+ as the coenzyme. Beyond this, although there was a slight increase in catalytic activity, it appears to reach plateau at this time point (Fig. 3).

Effect of Incubation Temperature and pH

When studied between the temperature ranges of 0 to 60 °C the activity of human fetal liver MIPS was minimal both at 10 and 50 °C. The enzyme was remarkably active between the temperature ranges of 20–40 °C with temperature maxima at 40 °C (Fig. 4). The human fetal liver MIPS exhibited optimum activity at a pH range of 7.0–7.5. Well-defined decline in catalytic activity of the enzyme was recorded below pH 6.5 or above pH 8.0 (Fig. 5).

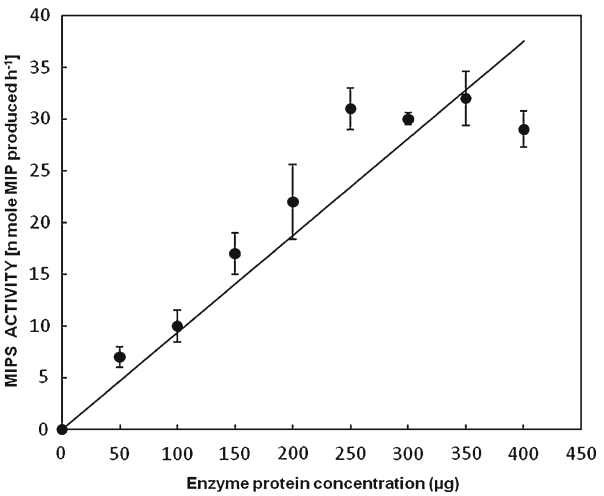

Fig. 2 Effect of variable enzyme protein concentrations on human fetal liver MIPS activity (MIPS activity expressed as nmol *myo*-inositol-1-phosphate produced h^{-1})

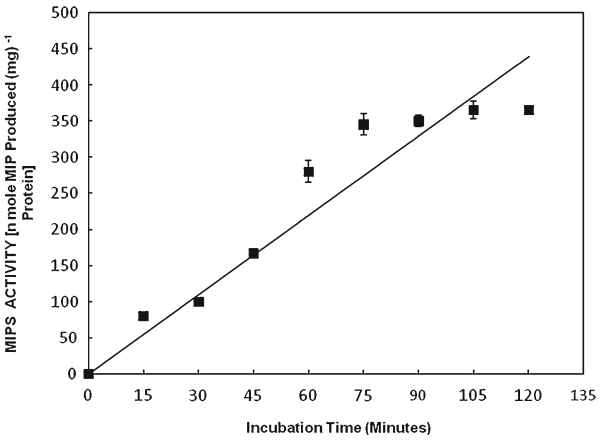

Fig. 3 Progress of human fetal liver MIPS reaction with time (MIPS activity expressed as nmol *myo*-inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1})

Determination of K_m and V_{max} Values

Kinetic studies were carried out using G-6-P (substrate) in the range of 0.0–8.0 mM. The reaction rate was found to increase with respect to G-6-P up to a concentration of 3 mM. The K_m value for G-6-P calculated from Lineweaver–Burk double reciprocal plot was 1.15 mM and the V_{max} value was calculated as 280 nM. Between concentrations of 0.0–1.0 mM of NAD^+ (coenzyme), the activity of purified enzyme was found to increase up to 0.4 mM concentration. With more increase in coenzyme concentration the activity could not be increased. The coenzyme saturation curve is hyperbolic in nature. The K_m value for NAD^+ was 0.12 mM and the V_{max} value was calculated as 252 nM from the Lineweaver–Burk double reciprocal plot.

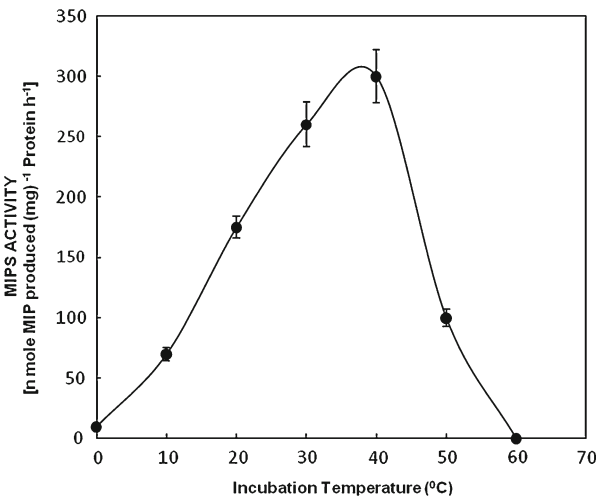

Fig. 4 Effect of different incubation temperature on human fetal liver MIPS activity (MIPS activity expressed as nmol *myo*-inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1})

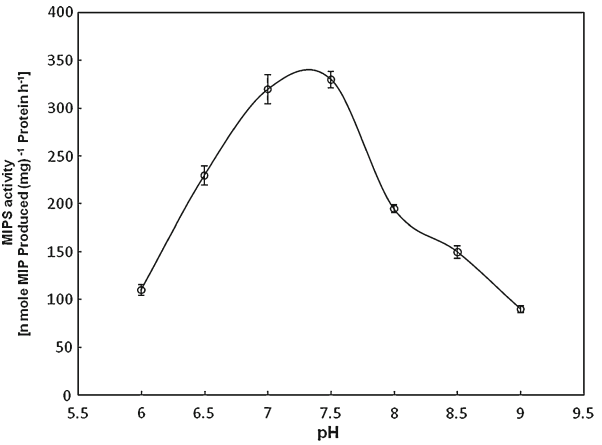

Fig. 5 Effect of different pH on human fetal liver MIPS activity (MIPS activity expressed as nmol *myo*-inositol-1-phosphate produced $(mg)^{-1}$ protein h^{-1})

PAGE Profile and Corresponding Enzyme Activity

The MIPS activity was determined from 5-mm gel slices after the BioGel A 0.5-m fraction of fetal liver was electrophoresed under non-denaturing conditions. Only one band demonstrated enzyme activity (Fig. 6).

Molecular Weight

The apparent molecular weight of human fetal liver MIPS was determined following the procedure as described in the materials and methods. It was found that the molecular weight of the enzyme was approximately 170 kDa.

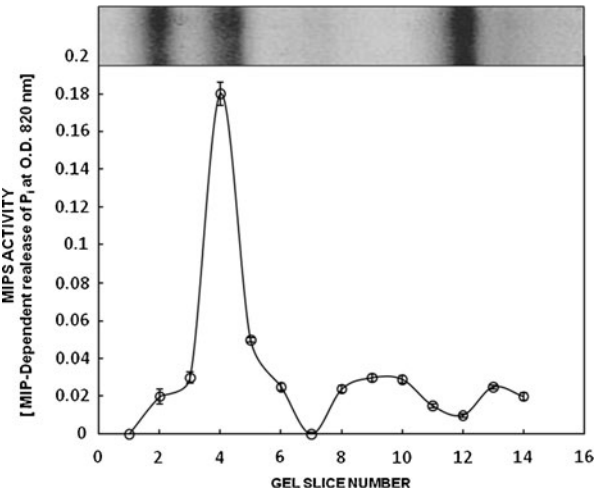

Fig. 6 PAGE profile showing the MIPS activity of the corresponding band when assayed with 5-mm gel slice

Discussion

MIPS has been isolated and characterised from a wide range of organisms including archea [21], bacteria [22], protozoa [23], plants [24, 27–35] and animals. In the animal systems, MIPS activity has been reported in the brain of insect, fish, amphibia, reptile and bird by Biswas et al. [36]. Mammalian MIPS was first purified by Maeda and Eisenberg Jr. from rat testes [37] and Mauck et al. [38] from bovine testis. Loewus et al. [39] found the enzyme in ram epididymis and Wong et al. [40] have shown the same from vascular elements including cerebral capillaries of bovine brain. Adhikari and Majumder [14] reported MIPS enzyme from adult rat and fetal human brains. So far, human fetal MIPS activity has only been reported from brain exhibiting the maximum titre of activity between the gestation period of 8 and 12 weeks. This suggests that inositol metabolism is active in human fetal brain even during development [14, 15].

During this study the enzyme, MIPS has been found to operate from the 12–20 weeks of gestation period suggesting that the fetal organ (liver) is preparing for an autonomous life in terms of *myo*-inositol generation too from this period itself. The enzyme from human fetal liver was absolutely specific for G-6-P. In the absence of this substrate, no MIPS activity was detected. Though the enzyme exhibits its optimal activity in presence of coenzyme NAD^+, still it could maintain about 13 % of the total activity when NAD^+ was not added externally. This NAD^+ independent activity is probably due to the presence of endogenous NAD^+ in the enzyme system like other sources [41, 42].

The human fetal liver MIPS activity increases in a linear manner with respect to the concentration of enzyme protein in the assay mixture at least up to 250 μg under optimal assay conditions. The enzyme activity shows similar time linearity at least up to 75 min of incubation at 37 °C. The temperature maxima of human fetal liver MIPS was 40 °C which is slightly higher than the MIPS of human fetal brain [14], but similar to that of recombinant *Drosophila melanogaster* MIPS [25]. The enzyme was active over the pH range of 6.5 to 8.5 with optimal activity at pH 7.5 which was comparable to the MIPS from rat mammary gland [43, 44], human fetal brain and adult rat brain [14]. Higher acidic or alkaline pH was not suitable for optimum MIPS activity, in any known system [3]. However, in the present study, we tried to examine the MIPS activity even at pH 4.5, 5.0, 5.5 (in acidic range) and 9.5 and 10.0 (in alkaline range). But no activity could be detected at both the extremes of pH scale (i.e., pH 4.5 and 10.0). It was further observed that MIPS activity below pH 6.0 (i.e., at pH 5.0 and 5.5) dropped down to about 4.3 and 7.8 % respectively which was physiologically negligible in comparison to its maxima at pH 7.0–7.5 (100 %). On the other hand, only 3.6 % activity could be detected at pH 9.5 which was again physiologically insignificant.

Human fetal MIPS activity was found to increase with respect to substrate concentration up to 3 mM. The K_m value for G-6-P of this enzyme at 1.15 mM is comparable to that for the yeast enzyme [30] but quite far away from that of rat testis enzyme at 3.89 mM [37] and human fetal brain at 4.4 mM [14]. The K_m value for NAD^+ was 0.12 mM which was quite contrary to that from the rat testis enzyme having a value of 17.9 μM but similar to that of some plant MIPS from *Oryza sativa*, *Spirulina platensis* and *Euglena gracilis* [24]. The V_{max} value of G-6-P was 280 nm which may be considered somewhat comparable to that from yeast having a value of 167 nm [30] and human fetal brain having a value of 170 nM [14]. V_{max} for NAD^+ for the human fetal liver MIPS was 252 nM compared with the same from fetal brain MIPS having V_{max} of 170 nM [14].

Of the monovalent cations, Na^+ had no role, K^+ showed slight stimulatory effect while NH_4^+ exhibited significant stimulation of the human fetal liver enzyme. The NH_4^+ induced increase in MIPS activity was similar to that from other sources. Naturally, the human fetal

liver MIPS may belong to a type-III aldolase requiring NH_4^+ for its optimal activity [28]. Similarly, divalent cations exhibited activities same as reported earlier from other sources [24, 44, 45]. Among these, Ca^{2+} showed about 25 % increase in the enzyme activity, which is unique for fetal liver MIPS and not yet been reported from other sources [3]. The strong enzyme inhibition due to heavy metals suggests that one or more free sulphydryl groups are present within the active site of the enzyme [26]. Like all other eukaryotes, the human fetal liver MIPS requires NH_4^+ for its optimal activity in contrast to the divalent cation requiring MIPS of prokaryotes [22, 23]. The human fetal liver MIPS activity was inhibited by the addition of EDTA in a concentration dependent manner (results not shown) unlike the MIPS from rat testis [37], human fetal brain [14] and bovine testis [38]. However, this type of inhibition is a general character of MIPS of plant origin [27–35].

The native molecular weight 170 kDa for fetal liver MIPS seems lower when compared with other animal sources like *D. melanogaster* (271 kDa) and bovine testis (218 kDa), etc. [25, 38] having four protein sub-units, suggesting only three sub-units for this fetal liver MIPS. This is consistent with earlier report [1, 3].

Lypodystrophic effect was observed in rats due to the deficiency of *myo*-inositol and its supplementation ameliorates the situation. *Myo*-inositol deficient gerbils suffered from intestinal lipodystrophy [11, 12], high accumulation of cholesterol and triacylglycerol in mammalian liver [11]. Lack of *myo*-inositol may eventually precipitate as fatty liver formation [13]. Thus, this study on *myo*-inositol metabolism in the human fetal liver and its regulation unveils one of the unique metabolic features of hepatic-protection under the circumstances of certain life-style and metabolic diseases like fatty liver disease, liver fibrosis, liver-cirrhosis, steatohepatitis, etc.

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