Hypothesis

COUPLING BETWEEN FRUCTOSE 1,6-BISPHOSPHATASE AND *MYO*-INOSITOL SYNTHASE

An hypothesis for 'rescue synthesis' of *myo*-inositol

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1. Introduction

myo-Inositol (hexahydroxycyclohexane) has long been known as one of the important growth-promoting chemicals for the living cells. A number of reports have suggested its importance in micro-organisms [1–3], plant cells and tissues in culture [4], mammalian cells [5] and animals [6]. As phosphomonoester, *myo*-inositol serves as precursor for phytic acid [7–11], an important phosphate reserve in plants, which on hydrolysis provides inorganic phosphate and carbon skeleton necessary for the growth of the germinating seed [12,13]. In addition, its involvement as a lipotropic factor [14–16], as cofactor of enzymes and mitochondrial functions [17,18] and mediators of cellular responsiveness as phosphoinositides [19] have long been recognized.

Deficiency of this compound induces remarkable changes in the metabolic makeup of the cell, manifested in the abnormal accumulation of lipids [20], alterations of sugar transport [21], and changes in the membrane structure and properties [22,23]. Acute starvation for *myo*-inositol has been reported to lead to loss of cell viability, a phenomenon known commonly as 'inositol-less death' [22–24].

Synthesis of *myo*-inositol from its precursor glucose 6-phosphate (G-6-P) is accomplished by an enzyme system L-*myo*-inositol 1-phosphate synthase (*myo*-inositol synthase, EC 5.5.1.4) and by the specific L-*myo*-inositol 1-phosphatase. Though reported from numerous sources, e.g., animal, plant and microbial, complete purification of *myo*-inositol synthase from animal sources [25,26] and *Saccharomyces cerevisiae* [27] has only recently been achieved. The reaction mechanism of the enzyme, an internal oxidoreductase requiring NAD, is yet to be determined in detail.

Since G-6-P, the only known de novo precursor of *myo*-inositol, is the pivotal metabolite for essential cellular functions, a competition for substrate is always in operation between *myo*-inositol synthesis and other pathways of utilisation of G-6-P. A precise control over this competition would be necessary to enable a cell to have uniform utilization for the two sets of pathways for synthesis of an adequate amount of *myo*-inositol, a compound of vital importance for cell survival.

How does the living cell accomplish this? Based mostly on biochemical and genetic studies with *myo*-inositol-deficient systems, a model is proposed here suggesting a coupled operation of the gluconeogenic enzyme, fructose 1,6-bisphosphatase and *myo*-inositol synthase, as the probable regulatory event for cell survival under conditions of *myo*-inositol deficiency.

2. Induction of *myo*-inositol deficiency and biochemical changes associated with it

One classical approach for establishing the essentiality of a nutrient is to deplete the system of the nutrient in question and study the biochemical changes associated with it in the deficient cells. Such studies for *myo*-inositol are discussed below.

2.1. *Deficiency induced by nutrient manipulation*

Depletion of *myo*-inositol in a system may be achieved by feeding an animal with a *myo*-inositol-free diet or growing cells in synthetic media without inositol. Care must be taken so that the special dietary condition does not cause any complicated metabolic disorder unrelated to *myo*-inositol deficiency. Animals fed with a *myo*-inositol-deficient diet developed

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acute lipodystrophy, which can be corrected by *myo*-inositol supplementation [14,28,29]. Lactation-induced fatty liver formation was discovered in [30] while studying the effect of *myo*-inositol deprivation in pregnant and lactating rats, which was prevented by dietary supplementation or termination of lactation. It was concluded [30] that a possible threshold level of free *myo*-inositol (~0.15 μmol/g lipid free tissue) is required to prevent fatty liver formation in this system. In [20,31] neutral lipid accumulation in rat liver was demonstrated by induction of dietary *myo*-inositol deficiency within a short period of time without interfering with the growth of the experimental animal.

The biochemical mechanism underlying neutral lipid accumulation in *myo*-inositol-deficient *Saccharomyces carlsbergensis* has been detailed in [32]. In these cells grown in glucose, *myo*-inositol deficiency induced a cellular increase in fructose 1,6-bisphosphate (Fru-P_2) and a lower citrate level resulting in an enhancement of the acetyl CoA carboxylase activity concomitant with the accumulation of neutral lipids [32]. The involvement of Fru-P_2 in this phenomenon was observed in [33,34] showing that no lipid accumulation was observed when *myo*-inositol-deficient cells of *Saccharomyces carlsbergensis* are grown with ethanol, glycerol or lactate as carbon source. Most likely, use of these carbon sources activated the gluconeogenic enzymes like Fru-P_2ase resulting in a drop of cellular Fru-P_2 followed by subsequent loss of lipid accumulation.

2.2. *Induction of deficiency by genetic means*

Because of endogenous synthesis, induction of *myo*-inositol deficiency by nutrient manipulation can not make an organism auxotrophic for it. Ideally, this may be obtained by inducing mutations at the *myo*-inositol synthase gene locus. This was achieved in [24] raising 52 inositol-requiring mutants in *Saccharomyces cerevisiae* by treatment with ethyl methane sulfonate. These mutants fall into 10 independent genetic loci designated *ino*-1 – *ino*-10. Of the mutants, 36 behave as alleles of the *ino*-1 locus (the structural gene for inositol synthase [27]; fully characterized [35]). Under conditions of inositol starvation, all the representative mutants (with the exception of the class V mutant) exhibit dramatic loss of cell viability (inositol-less death) which can be prevented by inositol supplementation or by disruption of protein synthesis by cycloheximide [24]. Complete abolition of inositol synthesis by mutagenesis was not possible in this organism and the mutants exhibited a spectrum of residual synthase activity, from 0.004% to ~2.5% that of the wild-type [36]. However, these mutants are potential tools for studying the biochemical lesions originating from inositol starvation. Similar mutant cell lines have been reported from animal sources [37,38].

3. The yeast inositol auxotroph, *ino* 5-12

Of the 10 different classes of mutants isolated in [24], the mutant *ino* 5-12, the only representative of the complementation class V, exhibits some unique characteristics [24] which can be summarized as follows:

(i) While all the other *ino*-mutants grow on glucose, this mutant grows on ethanol or glycerol and glucose exerts inhibitory effects on its growth.

(ii) The residual inositol synthase activity of *ino* 5-12 is highest among all the mutants and is ~2.5% that of the wild-type.

(iii) Though an inositol auxotroph, this mutant does not exhibit the 'inositol-less death' characteristic of all the other *ino* mutants.

The inability of *ino* 5-12 to grow on glucose may be explained as due to (i) defects in the glucose transport system and/or (ii) lack of hexokinase/glucokinase activity. However, this does not explain the inhibitory effect of glucose on its growth or its capability to grow on ethanol or glycerol. Though not yet verified experimentally, it is more likely that the mutant is a gluconeogenic organism and that the glucose effect, in fact, reflects the known catabolite inactivation of the gluconeogenic enzymes of the system [39–42]. Prevention of 'inositol-less death' in this mutant without external supplementation suggests that the residual synthase activity provides the required amount of inositol necessary for survival.

4. Mammalian brain Fru-P_2ase: Probable relation with regulation of inositol synthesis

From sections 2.1 and 2.3 it becomes apparent that Fru-P_2 and the gluconeogenic enzyme Fru-P_2ase

have some relation to the inositol biosynthetic reactions. A similar phenomenon had been observed during our investigations on the phospholipid-mediated G-6-P accumulation in a low-speed supernatant of rat brain [43]. The locus of this phospholipid effect was delineated at the fructose phosphate interconversion step leading subsequently to the discovery of Fru-P_2ase in this non-gluconeogenic organ [44]. The unequivocal demonstration of Fru-P_2ase in brain was later confirmed in [45]. However, the physiological role played by this enzyme in brain is open to speculation. It has been suggested [43] that this enzyme may have a regulatory role in brain inositol synthesis. Mammalian brain is estimated to have a high titre of free *myo*-inositol (~5–8 mM), half of which is enzymatically synthesized in situ [46,47]. Since this organ is essentially a glycolytic one [48], a control mechanism in the utilization of G-6-P is expected to operate for providing sufficient precursor for this high rate of synthesis. The accumulation of G-6-P, by

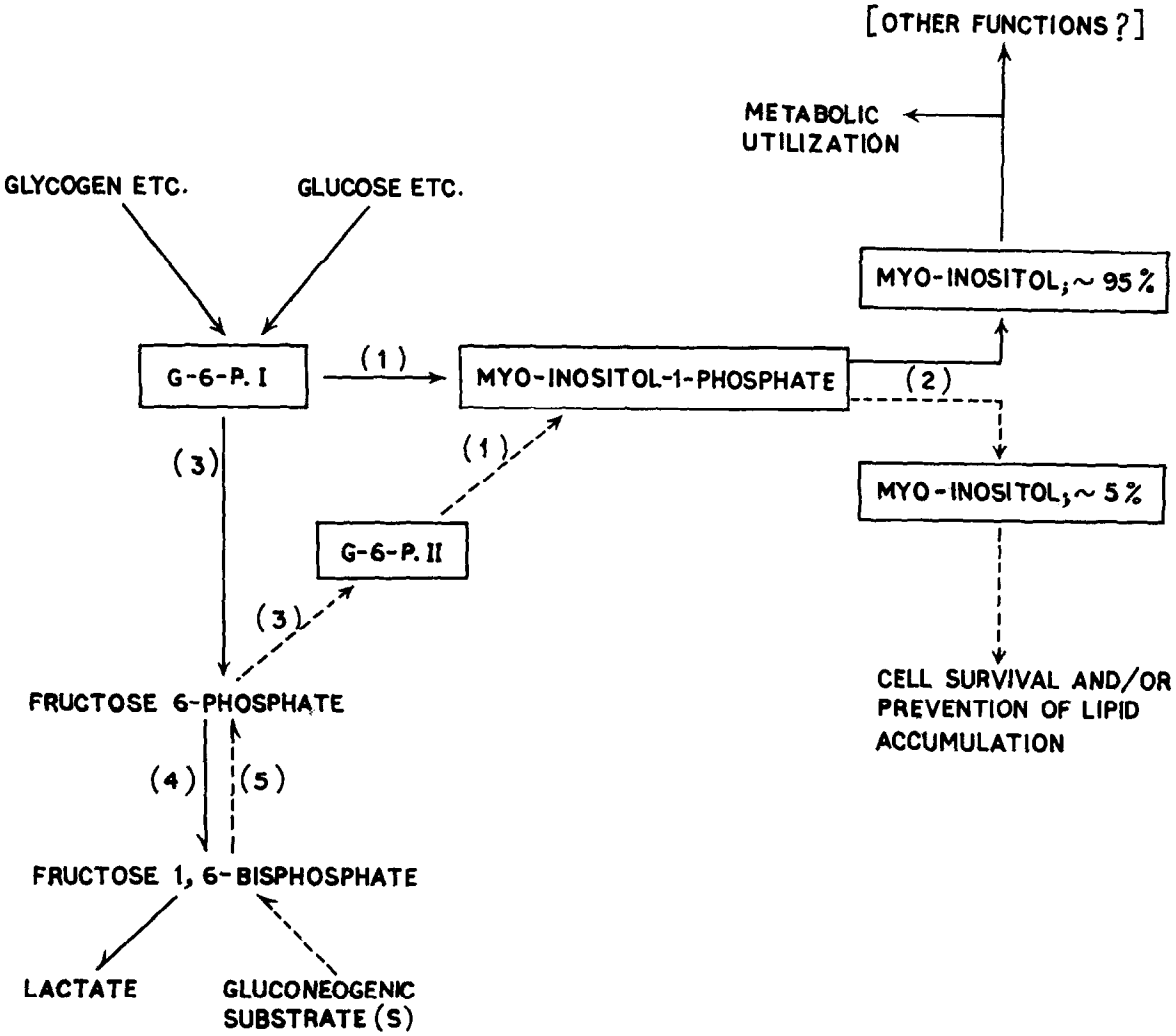

Fig.1. Probable events related to the postulated 'normal' (——) and 'rescue' (– – –) synthesis of *myo*-inositol. Numbers in parenthesis indicate enzymes of the reactions as follows: (1) *myo*-inositol synthase; (2) *myo*-inositol 1-phosphatase; (3) phosphohexoisomerase; (4) phosphofructokinase; (5) fructose 1,6-bisphosphatase.

a reciprocal phospholipid effect on the phosphofructokinase (inhibition)/Fru-P_2ase (stimulation) system, may be the ideal regulatory mechanism by which this may be achieved. The Fru-P_2ase by itself may thus be responsible for sequestering G-6-P for synthesis of *myo*-inositol at a time of cellular demand.

5. 'Rescue synthesis' of *myo*-inositol: The model

The proposed model for coupling between Fru-P_2ase and *myo*-inositol synthase visualizes two distinctly different pools of G-6-P in a cell for utilization as *myo*-inositol precursor (fig.1). The first one (designated G-6-P. I) contributing the major (95–97%) cellular *myo*-inositol may be derived from usual substrates like glucose, glycogen etc; this synthesis may be termed 'normal synthesis'. The second pool of G-6-P (designated G-6-P. II) derived entirely from gluconeogenic substrates or others via Fru-P_2, synthesizes a minor (max. 3–5%) portion of *myo*-inositol, vital for cell survival and/or prevention of lipid accumulation under conditions of inositol starvation. It is this second type of synthesis to which I would like to ascribe the term 'rescue synthesis' which would require a co-ordinate operation of Fru-P_2ase and *myo*-inositol synthase.

The suggestive participation in brain of Fru-P_2ase in regulation of inositol synthesis may in fact, be related to this phenomenon of 'rescue synthesis'. It may be argued that at a time of cellular demand this enzyme in brain may be called upon for rerouting the G-6-P from the first pool (i.e., G-6-P. I) to the second pool (i.e., G-6-P. II) via Fru-P_2 for utilization solely as *myo*-inositol precursor.

Furthermore, in *myo*-inositol-deficient *Saccharomyces carlsbergensis* grown in ethanol [33,34], the mechanism of prevention of lipid accumulation might involve an activation of Fru-P_2ase coupled to production of threshold-level of *myo*-inositol by 'rescue synthesis'.

Experimental verification of the proposed coupling between Fru-P_2ase and inositol synthase might necessitate combined genetic and biochemical studies. To this end, the yeast mutant *ino* 5-12, with its unique properties already discussed, meets all requirements for potential test organism. The inositol synthase in this mutant produces ~2.5% of cellular inositol compared to that of the wild-type yeast [36] an amount of inositol obviously sufficient to maintain conditions for cell-survival, and may be considered as an estimate of 'rescue synthesis' in this organism. In accordance with the proposed model, the mutant *ino* 5-12 may be viewed as an organism in which the postulated 'normal synthesis' is entirely missing due to mutagenesis and the mutant survives by virtue of 'rescue synthesis' only. The synthase in *ino* 5-12 (the 'rescue synthase'?) thus may turn out to be an enzyme quite different from the wild-type synthase (the 'normal synthase'?). An inkling of this suggestion is found in the observation that, when challenged against the antibody raised against the purified wild-type inositol synthase, extracts of *ino* 5-12 failed to show any detectable cross-reactive ~62 000 M_r protein, the characteristic monomer of the wild-type synthase. This sensitive immunoprecipitation technique has been successfully employed for identification of the structural gene for inositol synthase [27] and for screening a large number of inositol mutants of *Saccharomyces cerevisiae* [35,49]. This finding, by itself, is suggestive of a multiple nature of the enzyme in yeast presumably designed for two different physiological functions.

Complete elucidation of the mechanism underlying the proposed hypothesis would require a rigorous biochemical as well as genetic analysis of the yeast mutant *ino* 5-12, probably the best-suited organism for this purpose.

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