# REGULATION OF HEPATIC *ALTRO* HEPTULOSE 1,7-BISPHOSPHATE LEVELS AND CONTROL OF FLUX THROUGH THE PENTOSE PATHWAY BY FRUCTOSE 2,6-BISPHOSPHATE

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## 1. Introduction

The regulation of carbon flux through the hepatic fructose bisphosphatase–phosphofructokinase substrate cycle is regulated, in part, by the level of a novel sugar phosphate fructose 2,6-$P_2$ [1–7]. Methods for its synthesis and its anomeric specificity have been reported [8,9]. We also reported that the level of fructose 2,6-$P_2$ could be regulated by a variety of hormones and agents (e.g., α-agonists, vasopressin and the $Ca^{2+}$ ionophore A23187) [10–12]. The existence of an enzyme, separate from phosphofructokinase, was described which could synthesize fructose 2,6-$P_2$ from fructose 6-P and Mg-ATP and the activity of the enzyme was inhibited following glucagon pretreatment of liver [11,13–15].

The role played by fructose 2,6-$P_2$ in controlling flux through the non-oxidative segment of the pentose pathway was thus investigated since *altro* heptulose 7-P* and *altro* heptulose 1,7-$P_2$ are also substrates for phosphofructokinase and fructose bisphosphatase, respectively [16–18]. *altro* Heptulose 1,7-$P_2$ is not considered to be an intermediate of the pentose phosphate pathway as seen in current text books, however recent studies have shown that it is present in liver tissue and can be formed by liver enzymes in vitro [19–21]. *altro* Heptulose 1,7-$P_2$ does play a key role in the path of carbon in photosynthesis [22]. The absence of measurable levels of erythrose 4-P monomer may also be accounted for by the presence of *altro* heptulose 1,7-$P_2$, since aldolase will condense erythrose 4-P and dihydroxyacetone-P to form this intermediate [23,24].

* *altro* heptulose is the systematic name for sedoheptulose

Here, the effect of glucose and glucagon on the levels of the two heptulose phosphates is shown, since these agents will produce increased and decreased levels, respectively, of fructose 2,6-$P_2$ [11]. Increased levels of fructose 2,6-$P_2$ would theoretically increase the concentration of *altro* heptulose 1,7-$P_2$, while decreased levels of fructose 2,6-$P_2$, as in the case of glucagon treatment, would lower its concentration. These predictions were realized suggesting that flux of carbon through reactions of the pentose pathway can be regulated by the levels of fructose 2,6-$P_2$.

## 2. Experimental

Livers from fed male Sprague Dawley rats (250 g body wt) were perfused by recirculation as in [25]. The perfusate contained either $10^{-8}$ M glucagon (Eli Lilly Co.), 50 mM glucose or no addition (control) and the perfusion continued for 15 min after which time the livers were freeze-clamped [26]. The sugar-P's were then isolated as their $Ba^{2+}$ salts from 40 g (wet wt) tissue (pooled from separate liver perfusions) from each experimental condition [2]. Following removal of $Ba^{2+}$ [2] the samples were applied to a column (28 × 1.8 cm) of Dowex 1 × 8 (200–400 mesh) ion-exchange resin in the borate form (Bio-Rad Labs, Richmond CA). Sugar P's were eluted with a linear gradient of 0.1–0.4 M ammonium tetraborate (250 ml of each), 5 ml fractions were collected. The column was then eluted further with 0.4 M ammonium tetraborate until all the sugar bisphosphates were removed. The heptulose P's were located and quantitated in the column profile by the cysteine–$H_2SO_4$ method [27]. The difference in absorbance at 505 and 550 nm was

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determined for each sample together with standards of *altro* heptulosan monohydrate [18].

## 3. Results

The enzymatic methods currently used to measure *altro* heptulose 7-P and *altro* heptulose 1,7-$P_2$ are not specific (see below) for each heptulose, hence the best approach to quantitate these heptuloses is to separate them by ion-exchange chromatography and quantitate them using the specific cysteine–$H_2SO_4$ method [27]. The enzymatic method in [28] not only estimates *altro* heptulose 1,7-$P_2$ but also D-*glycero* D-*ido* octulose 1,8-$P_2$, D-*glycero* D-*altro* octulose 1,8-$P_2$, fructose-1-P and fructose-1,6-$P_2$ which are also present in liver [20]. The enzymatic method in [29] for *altro* heptulose 7-P will also detect D-*glycero* D-*altro* octulose 8-P which has been detected in liver [20].

The concentration of *altro* heptulose 7-P increases ~50% and 400%, respectively, in livers perfused with 50 mM glucose or $10^{-8}$ M glucagon when compared to control livers (fig.1). In the case of the 50 mM glucose perfusion, the small increase in *altro* heptulose 7-P concentration presumably results from the increased hexose 6-P concentration which occurs in this condition [11] together with the following reactions. Reaction (1) being catalyzed by transketolase and transaldolase [30]:

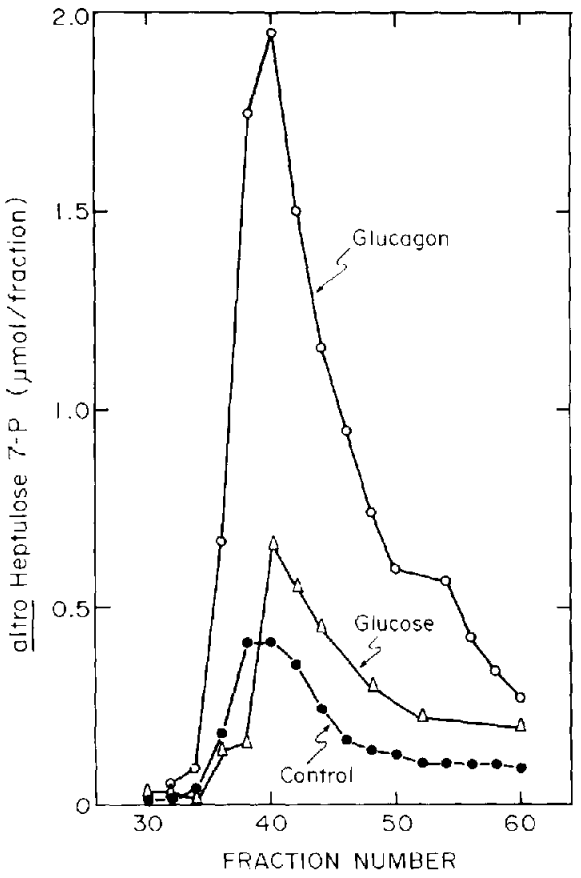


Fig.1. Elution profile of *altro* heptulose 7-P from control, glucose and glucagon treated livers. For other details see section 2. Authentic *altro* heptulose 7-P (Sigma Chemical Co.) co-elutes with the heptulose from each of the conditions (not shown).

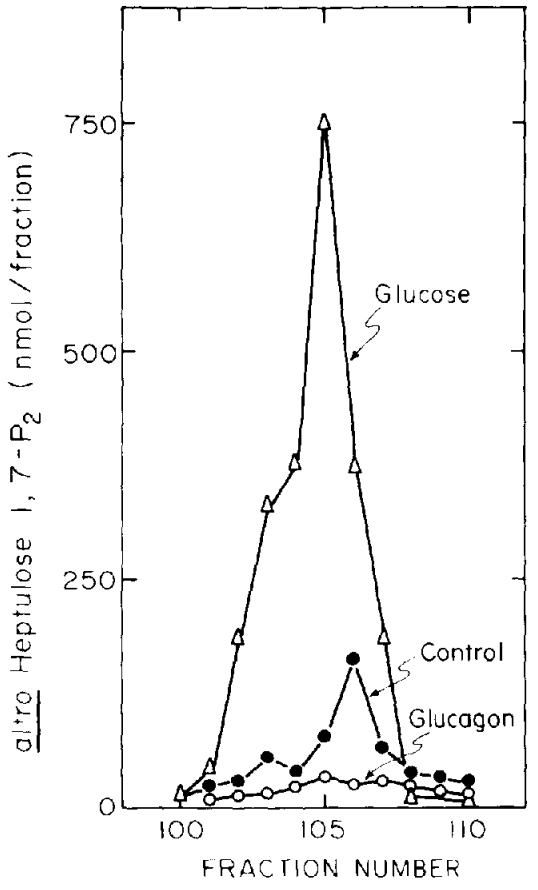


Fig.2. Elution profile of *altro* heptulose 1,7-$P_2$ from control, glucose and glucagon treated livers. For other details see section 2. The bisphosphate region of the profiles in fig.1 are shown. Authentic *altro* heptulose 1,7-$P_2$ (Sigma Chemical Co.) co-elutes with the heptulose from each of the conditions (not shown).

2 Fructose 6-P ⇌ *altro* heptulose 7-P + xylulose 5-P (1)

while reaction (2) is catalyzed by transketolase:

Fructose 6-P + ribose 5-P ⇌ *altro* heptulose 7-P + erythrose 4-P (2)

Also increased flux through reactions of the oxidative segment of the pentose pathway, together with the transketolase reaction (reaction (3)) would contribute to the increased *altro* heptulose 7-P levels.

Ribose 5-P + xylulose 5-P ⇌ *altro* heptulose 7-P + glyceraldehyde 3-P (3)

In the glucagon treated liver, the increased *altro* heptulose 7-P concentration would be due to the same set of reactions as above, together with a decreased utilization of *altro* heptulose 7-P by phosphofructokinase, which would be inhibited in this condition due to the decreased level of fructose 2,6-$P_2$ [11]. Alternatively it could arise from *altro* heptulose 1,7-$P_2$ by the activation of fructose bisphosphatase which occurs after glucagon treatment [5]. Consistent with this, the level of *altro* heptulose 1,7-$P_2$ is decreased in the glucagon condition (fig.2).

The concentration of *altro* heptulose 1,7-$P_2$ is increased following glucose infusion (fig.2). This is consistent with fructose 2,6-$P_2$ activating phosphofructokinase which would convert *altro* heptulose 7-P to *altro* heptulose 1,7-$P_2$.

## 4. Discussion

Although there appears to be some debate regarding the mechanism of the non-oxidative pentose phosphate pathway in liver [31–34], there is very clear evidence for the existence in liver tissue of *altro*

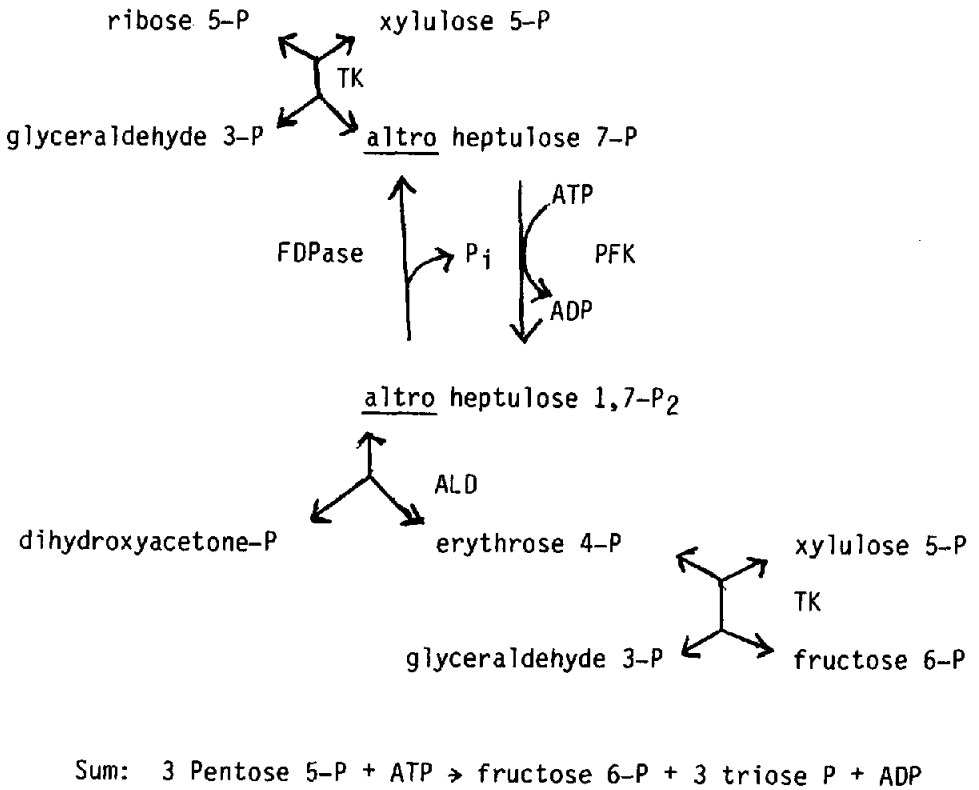


Fig.3. Alternate reaction sequence for the nonoxidative pentose pathway involving the enzymes FDPase and PFK. *Abbreviations:* FDPase, fructose 1,6-bisphosphatase; PFK, phosphofructokinase; TK, transketolase; ALD, aldolase.

heptulose 1,7-$P_2$ (here and [19,20]). Its concentration in control liver perfusions was ~10 nmol/g tissue (fig.2) which is similar to 5–7 nmol/g tissue found in [20]. This intermediate plays a rôle in the proposed L-type pentose pathway [35,36], and the existence of octulose phosphates [19,20] support the operation of this pathway. However, there does appear to be some doubt regarding the metabolic fate of arabinose 5-P another key intermediate in the L-type pentose pathway [33,34].

Another variant of the pentose phosphate pathway in liver is shown in fig.3 in which phosphofructokinase and fructose bisphosphatase play a rôle in the interconversion of *altro* heptulose 7-P and *altro* heptulose 1,7-$P_2$. Since there are no convincing control mechanisms demonstrated for controlling flux of carbon through the non-oxidative pentose pathway [37], the operation of the pathway shown in fig.3 would serve to regulate the interconversion of hexose and pentose phosphates since phosphofructokinase and fructose bisphosphatase are under hormonal control [1–7]. The labelling experiments performed in hepatocytes [36] are consistent with, but do not prove, the operation of the scheme in fig.3.

These data support the notion that fructose 2,6-$P_2$ can regulate the levels of *altro* heptulose 7-P and *altro* heptulose 1,7-$P_2$ in hepatocytes and that the pathway shown in fig.3 does operate in this tissue. Since no specific isotopic method is available to measure carbon flux through reactions shown in fig.3, no quantitation of the pathway after glucagon and glucose treatment can be made. The changes in *altro* heptulose 1,7-$P_2$ and *altro* heptulose 7-P levels however are at present the only way of showing changes in flux through the pathway. Thus glucose and hormones (e.g., epinephrine and vasopressin) which increase the level of fructose 2,6-$P_2$ [10,11] will stimulate flux through the pentose pathway shown in fig.3 in the direction of hexose phosphate, while agents which decrease its level (e.g., glucagon and β-agonists) will decrease flux through the pathway.

There appears to be a need to control the concentration of *altro* heptulose 7-P and *altro* heptulose 1,7-$P_2$ since *altro* heptulose 7-P is a competitive inhibitor of phosphoglucose isomerase ($K_i$ = 9 μM) and erythrose 4-P (which arises by aldolase cleavage of *altro* heptulose 1,7-$P_2$) is also a potent inhibitor of phosphoglucose isomerase ($K_i < 1$ μM) [38]. Erythrose 4-P also inhibits transketolase, ribose phosphate isomerase and triose phosphate isomerase [24]. Thus control of phosphofructokinase and fructose bisphosphatase by fructose 2,6-$P_2$ would regulate the levels of these inhibitors.

On a final note, since the interconversion of fructose 6-P and fructose 1,6-$P_2$ is similar to that of *altro* heptulose 7-P and *altro* heptulose 1,7-$P_2$ by the enzymes phosphofructokinase and fructose bisphosphatase one might speculate that by analogy the enzyme which synthesizes fructose 2,6-$P_2$ from fructose 6-P will also convert *altro* heptulose 7-P to *altro* heptulose 2,7-$P_2$ and that this sugar phosphate, if formed, may also regulate phosphofructokinase and fructose bisphosphatase. This intriguing possibility is under investigation.

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