BIOCHEMICAL LETTERS

Effect of fructose 1-phosphate on the activation of liver glycogen synthase

The intravenous administration of fructose causes within minutes a massive accumulation of fructose 1-phosphate in the liver (about 10 $\mu\text{mol/g}$ of tissue), with secondary changes in various metabolic pathways [1]. As far as the effect on glycogen metabolism is concerned, contradictory results have been obtained by various groups of authors (see [2]). Ciudad *et al.* [3] reported a simultaneous activation of glycogen synthase and of glycogen phosphorylase after the addition of fructose to isolated hepatocytes from fasted rats. In this Journal, Gergely *et al.* [2] presented an explanation at the enzyme level for the latter observations.

(i) Data with crude liver preparations [2] and with purified enzymes [4] indicated that fructose 1-phosphate causes a marked inhibition of phosphorylase phosphatase. The latter effect is expected to result in a higher steady-state concentration of phosphorylase *a*.

(ii) The activation of glycogen synthase is strongly inhibited by phosphorylase *a* [5], and see Fig. 1a). However, fructose 1-phosphate was reported to release synthase phosphatase from inhibition by phosphorylase *a* [2]. Hence the accumulation of fructose 1-phosphate in the liver after fructose injection could explain the simultaneous activation of phosphorylase and of synthase.

The group in Leuven has also regularly observed an inhibitory effect of added fructose 1-phosphate (10–20 mM) on the inactivation of phosphorylase in gel-filtered liver extracts, but failed to detect the reported release [2] of synthase phosphatase activity from the inhibitory effect of phosphorylase *a*. Analysis of the experimental conditions revealed as the only apparent difference the type of fructose 1-phosphate, which was either a bis-cyclohexylammonium salt* in Debrecen [2] or a sodium salt in Leuven, both obtained from Sigma Chemical Co. (as well as the barium salt). Therefore, the effects of the commercial fructose 1-phosphate salts were compared with those of compounds that were transformed from their original form into sodium or potassium salts by passage through Dowex⁺ columns (H^+ form) and subsequent neutralization with either NaOH or KOH. The general experimental conditions were as described by Gergely *et al.* [2]. Fructose 1-phosphate was assayed as described [6].

As shown in Fig. 1, the inactivation of phosphorylase in a gel-filtered extract was about equally inhibited by the addition of 20 mM of any tested preparation of fructose 1-phosphate (the bis-cyclohexylammonium salt and the

Fig. 1. Effect of various salts of fructose 1-phosphate on the inactivation of glycogen phosphorylase (○) and on the activation of glycogen synthase (●) in a gel-filtered rat liver extract

A post-mitochondrial supernatant was prepared and filtered through a column of Sephadex G-25 as described [2]. The filtrate was supplemented with 5 mM- $(\text{NH}_4)_2\text{SO}_4$ and 1 mM-magnesium acetate, plus buffer (a), or 20 mM-cyclohexylamine, neutralized with HCl (b), or 20 mM-fructose 1-phosphate (c–f); the latter were a commercial sodium salt (c), or a commercial bis-cyclohexylammonium salt (d), or a sodium salt, prepared from the bis-cyclohexylammonium salt (e), or a sodium salt prepared from a commercial barium salt (f). The supplemented filtrates were then incubated at 25 °C and samples were withdrawn at the indicated times for the assay of phosphorylase *a* and of synthase *a* [2]. Total phosphorylase (*a*+*b*) was 3.0 units/ml and total synthase was 220 m-units/ml.

* The product did carry the correct label 'di-monocyclohexylammonium salt', in contrast to the misleading entry 'dicyclohexylammonium salt' in the Sigma catalogue. A similar inaccuracy has recently led to misinterpretations in another area of metabolism (see [9]).

sodium salt, as such or prepared either from the bis-cyclohexylammonium salt or from a barium salt). The stimulatory effect of fructose 1-phosphate on the activity of glycogen synthase *b* [2] was also independent of the type of cation (results not shown). In contrast, the effect of 20 mM-fructose 1-phosphate on the activation of glycogen synthase was highly dependent on the type of cation (Figs. 1*c*–1*f*); only the bis-cyclohexylammonium salt allowed synthase phosphatase to be moderately active in the presence of phosphorylase *a*; all the other preparations merely prolonged the latency, as expected from the slower inactivation of phosphorylase. It is noteworthy that 20 mM-cyclohexylamine inhibited the inactivation of phosphorylase without ‘deinhibiting’ the activation of glycogen synthase (Fig. 1*b*). In another experiment the bis-cyclohexylammonium salt was transformed into a potassium salt, which also behaved like the sodium salt (results not shown). We have not further investigated the mechanism of the discrepant effect of the bis-cyclohexylammonium salt of fructose 1-phosphate, but we checked that its stability in the gel-filtered extract was similar to that of the commercial sodium salt (less than 5% disappearance after a 20 min incubation).

Gergely *et al.* [2] also observed that the activation of glycogen synthase in unfiltered liver extracts from fructose-injected rats occurred without latency. Since gel filtration restored the sequential inactivation of phosphorylase and activation of glycogen synthase, the ‘deinhibition’ in the unfiltered extract was attributed to the presence of fructose 1-phosphate [2]. However, the latency is generally much less pronounced in unfiltered extracts from control animals, probably because of the post-mortem accumulation of substances like AMP and Mg^{2+} [5]. We have therefore compared the enzyme activity changes during incubation of unfiltered liver extracts from four fructose-injected and four glucagon-injected rats; the rate of inactivation of phosphorylase and the activation pattern of glycogen synthase were closely similar in the two conditions (results not shown). In a separate experiment fructose 1-phosphate was added to a control liver extract at concentrations (1–3 mM) that are expected to occur in a 20% (w/v) liver extract after fructose injection. No effects were observed at these concentrations.

We conclude that the accumulation of fructose 1-phosphate in the liver may at least in part explain a fructose-induced increase in the concentration of phosphorylase *a*. Other mechanisms may contribute as well [7,8]. The accumulation of fructose 1-phosphate does not explain, however, the fructose-induced activation of glycogen synthase that occurs in some experimental conditions. In general, the present data argue against the use of complex organic salts when the biological effects of metabolites are to be examined.

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Buffer capacity of intracellular Ca^{2+} indicators

The intracellular Ca^{2+} indicators designed by Tsien (1980) and Grynkiewicz *et al.* (1985) are now standard biochemical tools. Quin2, the first probe to become generally available and the most widely used, does involve the disadvantage of a substantial buffer capacity. Although there need be less buffering with more recent probes, reviewers still note the lack of attention to this point (DeLisle & Williams, 1986).

Some experiments with quin2 in synaptosomes illustrates a straightforward measurement of indicator buffer capacity. The synaptosomes were prepared from guinea-pig cerebral cortex (Gray & Whittaker, 1962; Hajos, 1975) and suspended in a Tris-buffered (pH 7.4)

balanced salt solution containing 2 mM- $CaCl_2$. The 3H_2O -permeable, $[^{14}C]$ sucrose-impermeable space of synaptosomes prepared by the more convenient procedure of Hajos (1975), $4.2 \pm 0.3 \mu\text{l}/\text{mg}$ of protein, mean $\pm$ S.E.M., $n = 6$, closely matched values for intrasynaptosomal volumes in the original preparation ($4 \mu\text{l}/\text{mg}$ of protein at physiological tonicities; Marchbanks, 1975). Following quin2 loading (Ashley *et al.*, 1984) particulate suspensions of up to $350 \mu\text{g}$ of protein/ml were maintained at 37°C and stirred continuously. Total intrasynaptosomal chelator concentrations were found by comparing the Ca^{2+} -saturated fluorescence of synaptosomes with Ca^{2+} -saturated free acid standards (conveniently prepared by mild alkaline hydrolysis of the ester). The ratio of Ca^{2+} -complexed to free quin2 ($[CaQ]/[Q]$) was also measured fluorimetrically (Tsien *et al.*, 1982) with the precautions noted by Ashley