Inactivation of glucose-6-phosphate dehydrogenase by glycation

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The increased glycation of structural proteins in diabetes has been intensely studied. Much less is known of the enzyme glycation. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme of considerable importance because it catalyses the first reaction in the hexose monophosphate shunt pathway and catalyses the formation of NADPH.

The in vitro inactivation of glutathione reductase, β-galactosidase, alkaline phosphatase and G6PD by glycation was previously demonstrated [1,2]. As both GSH level and G6PD activity are decreased in the lenses of diabetic rats and rabbits [3], the glycation of the enzymes might be responsible for some of the metabolic changes. In the present paper we studied the effect of various sugars on G6PD activity, as well as the effect of aspirin, paracetamol and ibuprofen on the inactivation.

The enzymic activity was estimated by the modified method of Langdon [4]. All the assays were done in triplicate.

The effect of various sugars on the activity of G-6-PD was investigated by incubating the enzyme with 5mM glucose or fructose or galactose, at 37°C, for 0 - 8 hours. Before incubation, the reaction mixture was sterilized through 0.2μm-pore-size filters and divided into sterilized glass vials.

The enzyme activity decreases in the presence of the sugars in a time dependent way. At one hour of incubation, the inhibition of the enzyme incubated with sugars is about twice as much as without; the percentage inhibition is similar with fructose, glucose or galactose. After 2 hours, the activity decreases more rapidly in the presence of fructose than of glucose or galactose (Fig. 1). At 8 hours of incubation the enzyme was inhibited by 84% by fructose, 37% by glucose and 40% by galactose; the enzyme incubated in the same condition without sugar being inactivated by 21%.

The protective effect of drugs against the enzyme inactivation by sugars is shown in Fig. 2. The enzyme was incubated in the presence/absence of 5mM fructose with or without 20mM aspirin or paracetamol, for 0 - 8 hours. The drugs give a small protection against inactivation; at 8 hours of incubation the aspirin protection is 12%, the paracetamol only 5%. The ibuprofen does not give any protection.

It was of interest to test the specificity of the glycated enzyme, and because one variant of G6PD (Kobe) has a high affinity for galactose &phosphate [5], we tried this as an alternative substrate.

At zero time Gal 6-P is oxidised at only about 1.6% the rate of G6P (data not shown). This proportion does not change significantly after incubation without sugar for 8h. Even the incubation with fructose has no effect on this proportion showing that there is no change in specificity associated with the slow inactivation by fructosylation. These results add to the view that glycation of enzymes could be a hazard in ageing tissues and in diabetes. Fructose is more damaging than the other sugars both in the present work on G6PD and in the previous studies of other proteins. The small protective effect against glycation provided by aspirin and paracetamol is consistent with their protection against cataract in the diabetic rat [6], and apparent protection against human cataract [7].

Supported by the MRC.