Physiol. Res. 43: 61–64, 1994

SHORT COMMUNICATION

Glycation of Myofibrillar Proteins and ATPase Activity after Incubation with Eleven Sugars

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Received January 25, 1993
Accepted July 24, 1993

Summary

Rat skeletal muscle myofibrils were incubated in the presence of D-glucose, D-fructose, D-galactose, D-ribose, D-tagatose, D-arabinose, D-xylose, D-mannose, L-sorbose, L-rhamnose or DL-glyceraldehyde and myofibrillar ATPase activity as well as the extent of glycation was measured. The attachment of sugars to proteins during glycation was generally dependent on the percentage of a given sugar present in the open-chain form. Glycation resulted in the decrease of myofibrillar ATPase activity. This decrease was low after incubation of myofibrillar proteins with slowly glycating sugars (e.g. glucose) and high with fast glycating sugars (e.g. ribose or glyceraldehyde). ATPase activity was less reduced in the presence of β-mercaptoethanol.

Key words

Glycation – Myofibrils – ATPase activity – Sugars – Skeletal muscle

Glycation involves a relatively slow reaction of a reducing sugar with the aminogroup of a protein. It occurs *in vivo*, especially under certain pathophysiological conditions, such as diabetes mellitus, but it can also be studied *in vitro*. The reaction rates of reducing sugars with proteins differ one from another (Bunn and Higgins 1981), depending on the percentage of a given sugar present in the open-chain form. It is well documented that glycation impairs the function of some proteins and modulates enzymatic activity (Arai *et al.* 1987, Blakytny and Harding 1992, Brown and Knull 1992, Mira *et al.* 1991, Shilton and Walton 1991, Watanabe *et al.* 1992, Watkins *et al.* 1985). However, no systematic study has been performed on the relation between glycation with various sugars and its influence on enzymatic activity.

The aim of the present study was to see how myofibrillar ATPase activity is inactivated after glycation with sugar glycating proteins at different reaction rates over a wide range.

The myofibrils were isolated as described previously (Potter 1974) from three-month-old Wistar rats. The final pellet was resuspended in 80 mM KCl, 1 mM HEPES (pH 7.0) to a final concentration of 5.0 mg protein/ml.

The suspension of myofibrils was glycated by incubation in 80 mM KCl, 1 mM HEPES (pH 7.0), 3 mM sodium azide, 2 mM phenylmethylsulfonylfluoride at 25 °C for 48 h in the presence of sugar (0.01 M glyceraldehyde or 0.5 M other sugars indicated). Very low concentration of glyceraldehyde was used, as glycation with glyceraldehyde is much faster than with hexoses. Samples were dialysed extensively against 80 mM KCl, 1 mM HEPES (pH 7.0) for 48 h at 4 °C to remove unbound sugar. Some samples were incubated in the presence of additional 0.8 mM β-mercaptoethanol, to protect proteins against oxidation.

Mg^{2+} activated ATPase of myofibrils was determined in a reaction mixture containing 8 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 0.1 mM $CaCl_2$, 2 mM NaN_3, 20 mM Tris-HCl (pH 7.4) and 0.3 mg of myofibrillar protein/ml. Inorganic phosphate was assayed according to Fiske and Subbarow (1925). Protein concentration was determined after digestion with a catalyst mixture (Chibnall *et al.* 1943) by the

Conway microdiffusion technique (Conway 1957). To determine the amount of sugar covalently bound to the protein, the thiobarbituric acid method was used (Ney *et al.* 1981).

One triose, three pentoses and seven hexoses were used for glycation of myofibrils. Glycation caused a decrease of myofibrillar ATPase activity (Fig. 1). By comparing the decrease of ATPase activity and the extent of glycation it is evident that glycation diminishes ATPase activity. Glyceraldehyde reduces ATPase activity to a very low level, incubation of myofibrils with pentoses also reduces ATPase activity. Hexoses are the least effective.

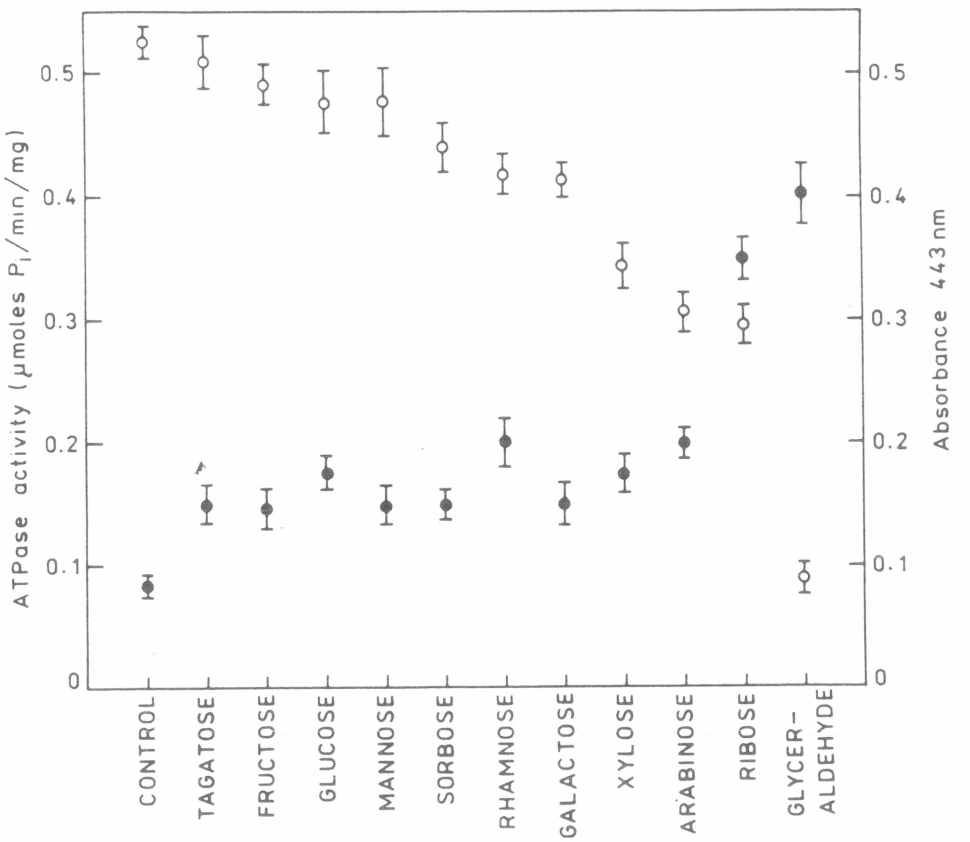

Fig. 1
The effect of myofibrillar proteins glycation on ATPase activity. Myofibrillar ATPase activity (open circles); the extent of glycation assayed with the thiobarbituric acid method and measured at 443 nm (full dots). Means and ranges of three experiments are indicated.

The inhibition of myofibrillar ATPase activity by various sugars in our experiment corresponds to the reactivity of sugars (Overend *et al.* 1961) which increases as follows: glucose < mannose < galactose < xylose < arabinose < ribose < glyceraldehyde (the reactivity of other sugars was not stated).

Myofibrillar ATPase activity was determined at high Ca^{2+} concentrations. Under these conditions the values obtained correspond to actin-myosin interaction. The lowering of myofibrillar ATPase as the result of glycation is probably due to the fact that the main constituent of myofibrils, myosin, is glycated. This assumption is in agreement with results of Brown and Knull (1992) who showed that ATPase activity of subfragment-1 of myosin is reduced after glycation. Other contractile proteins are also glycated *in vitro* (Brown *et al.* 1992, Syrový and Hodný 1993). It would be desirable to use pure myosin instead of myofibrils, but myosin, which is soluble only at high ionic strength, becomes viscous and gelatinous during glycation.

It can also be seen in Fig. 1 that the amount of covalently bound sugar, measured by colorimetry with thiobarbituric acid, is not exactly proportional to the reduction of ATPase activity. This may be due to the fact that several products are formed during glycation and no assay including the thiobarbituric acid method is specific for a single population of glycated protein only (Furth 1988). Moreover, glycation products formed from protein and various sugars do not yield the same colorisation after the reaction with thiobarbituric acid (Syrový 1993).

Table 1
The effect of β-mercaptoethanol on myofibrillar ATPase activity and glycation of myofibrillar proteins performed with various sugars. All values are the means of three experiments.

	ATPase activity (μmoles P_i/mg/min) Glycation		Glycation expressed as absorbance at 443 nm Glycation	
	without	with 0.8 mM β-mercaptoethanol	without	with 0.8 mM β-mercaptoethanol
Control	0.525	0.698	0.131	0.106
Tagatose	0.511	0.674	0.150	0.126
Fructose	0.488	0.639	0.145	0.119
Glucose	0.476	0.619	0.175	0.147
Mannose	0.476	0.614	0.149	0.120
Sorbose	0.438	0.560	0.150	0.127
Rhamnose	0.418	0.520	0.201	0.181
Galactose	0.416	0.524	0.150	0.128
Xylose	0.344	0.430	0.175	0.144
Arabinose	0.309	0.383	0.200	0.173
Ribose	0.290	0.356	0.351	0.321
Glyceraldehyde	0.088	0.107	0.403	0.382

Table 1 illustrates the effect of β-mercaptoethanol on myofibrillar ATPase activity and glycation performed with various sugars. The ATPase activity of myofibrils glycated in the presence of β-mercaptoethanol is higher when compared with samples incubated with the same sugar in the absence of β-mercaptoethanol. The presence of 0.8 mM β-mercaptoethanol reduces glycation assayed by the thiobarbituric acid method. In the presence of 1 mM dithiothreitol the same effect was essentially observed as with β-mercaptoethanol (results not shown). The increase of myofibrillar ATPase activity after incubation with sugars in the presence of β-mercaptoethanol is probably due to the fact that β-mercaptoethanol protects the SH groups of myosin or other proteins against oxidation (the control myofibrillar ATPase activity is also higher after incubation with β-mercaptoethanol). A protecting effect of β-mercaptoethanol on ATPase activity may also be due to the lower oxidation of sulphydryl groups by dicarbonyl compounds. The observed decreased glycation with a given sugar in the presence of β-mercaptoethanol is in accordance with the assumption that glycation causes protein conformational changes, that increased susceptibility of thiols to oxidation occurs and that β-mercaptoethanol prevents oxidation. It was observed that reducing agents (dithiothreitol and reduced glutathione) reverse the formation of aggregates during glycation (Stevens *et al.* 1978, Swamy *et al.* 1993) and that glutathione inhibits galactosylation (Huby and Harding 1988).

In conclusion, it was found that especially pentoses influence myofibrillar ATPase activity in the course of glycation. This might be of biologiacal significance, because in the living organism, especially under pathological conditions, not only hexoses but also pentoses may act as the glycating agents (Cohen 1987).

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Reprint Requests

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