Free Rad. Res., Vol. 33, pp. 23–30
Reprints available directly from the publisher
Photocopying permitted by license only

© 2000 OPA (Overseas Publishers Association) N.V.
Published by license under
the Harwood Academic Publishers imprint,
part of The Gordon and Breach Publishing Group.
Printed in Malaysia.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

Fructose Induced Deactivation of Antioxidant Enzymes: Preventive Effect of Pyruvate

WEI ZHAO, P.S. DEVAMANOHARAN and SHAMBHU D. VARMA*

Departments of Ophthalmology and Biological Chemistry, School of Medicine, University of Maryland, Baltimore, MD 21201, USA

Accepted by Prof. B. Halliwell

(Received 13 October 1999; In revised form 23 November 1999)

Glycation initiated changes in tissue proteins, which are triggered by the Schiff base formation between the sugar carbonyl and the protein $-NH_2$, have been suggested to play an important role in the development of diabetes-related pathological changes such as the formation of cataracts. While the initial reaction takes place by the interaction of $>C=O$ of the parent sugars with the $-NH_2$ of proteins, reactive oxygen species (ROS) dependent generation of more reactive dicarbonyl derivatives from the oxidation of sugars also plays a significant role in these changes, altering the structural as well as functional properties of proteins. The purpose of this study was to examine whether the activities of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), catalase and superoxide dismutase (SOD) could be affected by the high levels of fructose prevalent in diabetic lenses. Incubation of the enzymes with this sugar led to a significant loss of their activities. GAPDH was inactivated within a day. This was followed by the inactivation of catalase (3–4 days) and SOD (6 days). The loss of the activities was prevented significantly by incorporation of pyruvate in the incubation mixture. The protective effect is ascribable to its ability to competitively inhibit glycation as well as to its ROS scavenging activity. Hence, it could play a significant role in the maintenance of lens physiology and cataract prevention.

Keywords: Antioxidant enzymes, deactivation, fructose, glycation, oxidation, pyruvate

INTRODUCTION

Hyperglycemia is one of the significant risk factors in the genesis of senile cataracts in human diabetics as well as in the formation of true diabetic cataracts in younger individuals.[1–3] The mechanism by which the elevated level of glucose contributes to such cataract development remains unsettled. One of the major proposed mechanisms suggests that it involves non-enzymatic glycation of lens crystallins.[4–6] This can also have an adverse effect on the structure of enzymatic proteins, leading to a loss of their activities.[7–10] Studies on the senile cataractous lenses isolated from human diabetics have shown a significant elevation in the levels of fructose, the end product of the polyol pathway.[11–13] Several studies in this and other laboratories

*Corresponding author. Department of Ophthalmology, School of Medicine, University of Maryland, Baltimore, MD 21201, USA. Tel.: 410-706-3395. Fax: 410-706-7057. E-mail: svarma2384@aol.com.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

have demonstrated that this metabolite is a more potent glycating agent than glucose.[14–16] It has also been demonstrated that among the various physiological sugars, fructose undergoes a more rapid auto-oxidation, generating reactive oxygen species (ROS).[17] These observations hence suggest that the accumulation of high levels of this sugar in the lens associated with diabetes and aging could participate in cataract formation via a direct ROS dependent oxidative stress as well as via glycation. The dissection of the relative importance of the two processes, however, is difficult because ROS are also involved in accelerating the glycation reaction.[18]

We have previously shown that pyruvate is an effective scavenger of H_2O_2 as well as of $O_2^{\bullet-}$, thereby protecting the lens against oxidative stress and consequent cataract formation, under *in vitro* as well as *in vivo* conditions.[19–22] Pyruvate has also been shown to protect the lens proteins against glycation by competitively inhibiting the initial reaction between the sugar carbonyl and the protein $-NH_2$.[23] The purpose of the present study was to determine the effect of fructose at levels prevalent in the diabetic lens on the activities of certain enzymes involved in protecting the tissues against oxidative damage, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), catalase and superoxide dismutase (SOD). The studies were also undertaken to assess the possibility of modulation of such deactivation, if any, by pyruvate, a compound produced in the glycolytic pathway and known to scavenge ROS.[19–22] The results demonstrate that the above defence enzymes are substantially inactivated on incubation with fructose and such deactivation is significantly preventable by pyruvate.

MATERIALS AND METHODS

All the chemicals were purchased from Sigma Chemical Company, St. Louis, MO. U-^{14}C-fructose was obtained from New England Nuclear Company, Boston, MA.

Inhibition of GAPDH Activity by Fructose

The enzyme activity was determined by measuring spectrophotometrically the generation of NADH from NAD^+ in the presence of DL-glyceraldehyde-3-phosphate (DL-GAP) as substrate. Briefly, 10 U/ml rabbit muscle GAPDH (0.125 mg/ml protein, Sigma #28H7818) was incubated at 37°C with various concentrations of fructose (0–25 mM) in 50 mM sodium phosphate buffer, pH 7.2, for various time periods. Following the incubation, 20 µl of the incubated enzyme was added to a cuvette containing 2.88 ml of reaction mixture consisting of 0.015 M sodium pyrophosphate (pH 8.5), 0.03 M sodium arsenate, and 7.5 mM NAD^+. The initial absorbance at 340 nm was recorded. Then 0.1 ml of 15 mM DL-GAP was added to the cuvette and the resulting increase in the absorbance at 340 nm due to NADH formation recorded for 5 min. The deactivation of the enzyme on incubation with fructose was expressed as the percentage of the basal activity obtained from the samples incubated without fructose. Addition of the blank incubation mixture containing fructose without the enzyme when added to the assay mixture had no effect on the GAPDH activity.

In other experiments, the GAPDH and fructose mixture was incubated in buffer containing either catalase (5 U/ml), SOD (10 U/ml), DTPA (1 mM), pyruvate (1 mM), sodium chloride (1 mM), or heat-inactivated catalase (5 U/ml) and SOD (10 U/ml), and the enzyme activity measured subsequently, as described above.

In order to assess the possible involvement of the oxidation of –SH in GAPDH deactivation, experiments were carried out to determine –SH levels before and after its incubation with fructose using DTNB (2,2′-dithio-bis-5-nitropyridine) reagent.[24]

Binding of ^{14}C-Fructose to GAPDH

GAPDH (0.4 mg/ml) was incubated at 37°C in 50 mM sodium phosphate buffer, pH 7.2,

RIGHTSLINK

containing U-^{14}C-fructose as a tracer with increasing levels of the cold fructose (0–10 mM). The incubation was continued for 9 h. Incubation mixtures (0.3 ml) were then taken out and the proteins precipitated by adding 0.3 ml of cold 20% trichloroacetic acid (TCA). The precipitate was washed by centrifugation with 1 ml of 5% TCA three times. The residue was then dissolved in 100 µl of 1 M NaOH, transferred to a liquid scintillation cocktail and the amount of fructose bound to the protein was determined by measuring the radioactivity. The specific activity of fructose was also determined simultaneously.

To determine the inhibition of fructose binding to the enzyme by pyruvate, the enzyme was preincubated with pyruvate (0–10 mM) for 30 min prior to the addition of U-^{14}C-fructose. The mixture was then incubated for an additional 9 h and fructose bound to the protein determined as above.

Inhibition of Catalase and SOD by Fructose

Catalase activity was determined by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2. Briefly, 500 U of bovine liver catalase (200 µg/ml protein, Sigma #88F7185) were incubated with various concentrations of fructose (0–30 mM) in 0.05 M sodium phosphate (pH 7.2) at 37°C in a total volume of 1 ml. A blank experiment was also conducted with the buffer incubated with fructose. The incubated enzyme (20 µl) or blank was taken out at various incubation times and added to 2.98 ml of an assay mixture containing 14 mM H_2O_2 in 0.05 M sodium phosphate buffer, pH 7.2. The decrease in the absorbance at 240 nm was recorded for 5 min. Addition of the blank aliquots to the assay mixture had no effect on the measurement of the enzyme activity. The deactivation of catalase on incubation with fructose was expressed as the percentage of control where the enzyme was incubated without fructose.

The deactivating effect of fructose on SOD was also determined by incubation of the enzyme with and without fructose as described above. Briefly, 200 U of human erythrocyte SOD (80 µg/ml protein, Sigma #85F9345) were incubated with various concentrations of fructose (0–40 mM) in 0.05 M potassium phosphate (pH 7.8) containing 100 µM EDTA at 37°C. The incubation volume was 1 ml. At indicated times, 20 µl of the aliquot was taken out and added to 3 ml of a reaction mixture containing 0.05 M potassium phosphate (pH 7.8), 100 µM EDTA, 10 µM ferricytochrome c and 50 µM xanthine. The reaction was started by adding 0.03 U of xanthine oxidase and the rate of the reduction of ferricytochrome c monitored at 550 nm for 5 min. The deactivating effect of fructose was expressed as the percentage of the enzyme activity obtained from the sample incubated without fructose. Since fructose liberates small amounts of $O_2^{\bullet-}$ during incubation, a correction for its effect on the measurement of SOD activity was also made by running a separate experiment using buffer incubated with fructose alone. This correction ranged from 0 to 10%, (0 to day 6 of incubation).

To determine if pyruvate can prevent the fructose induced deactivation of the above antioxidant enzymes, incubations as described above were also conducted in the presence of sodium pyruvate (0–10 mM).

RESULTS

The possibility of deactivation of GAPDH upon its exposure to fructose at levels prevalent in certain diabetic tissues, such as the lenses isolated from diabetic individuals (cataract), is apparent from the data summarized in Figure 1. As indicated therein, the loss in activity was significant (~46%) in the presence of 10 mM fructose after 12 h incubation. The loss in activity without fructose was less than 10%. The decrease was proportional to the concentration of fructose as well as to the time of incubation. As shown in Figure 2, the deactivation of the enzyme that occurred following its incubation with fructose was prevented by pyruvate.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

RIGHTSLINK

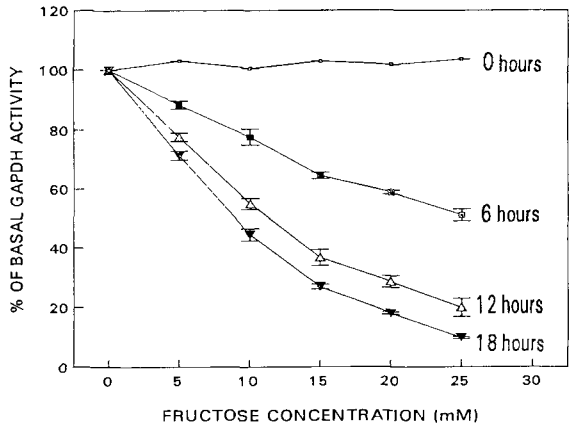

FIGURE 1 Loss of GAPDH activity on incubation with fructose: 10 U/ml of GAPDH were incubated in 50 mM sodium phosphate buffer, pH 7.2 with various levels of fructose (0–25 mM) at 37°C. At indicated times, the enzyme activity was determined spectrophotometrically as described in the methods. The results (mean ± SD) are expressed as the percent of controls wherein the enzyme was incubated without any fructose. $n = 3$.

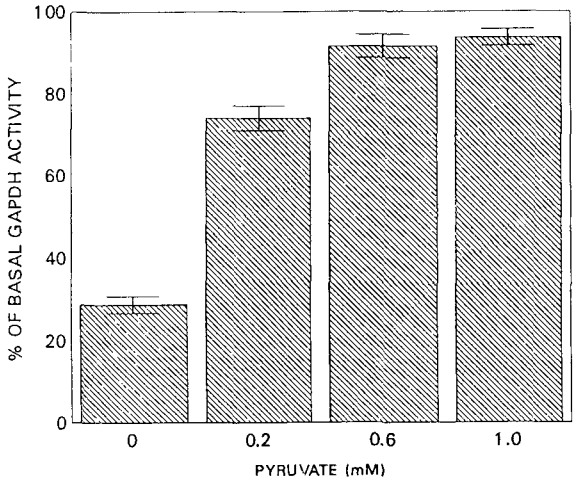

FIGURE 2 Protective effect of pyruvate against the loss of GAPDH activity on incubation with fructose: this was done by incubating the enzyme with fructose (20 mM) at 37°C for 12 h in the absence and presence of various concentrations of pyruvate as indicated. The protocol was similar to that described in Figure 1. $n = 3$.

To determine if the enzyme deactivation could also be related to fructation by the reaction between the enzyme protein $-NH_2$ and the keto group of the fructose or its dicarbonyl derivative, we measured the incorporation of this sugar into

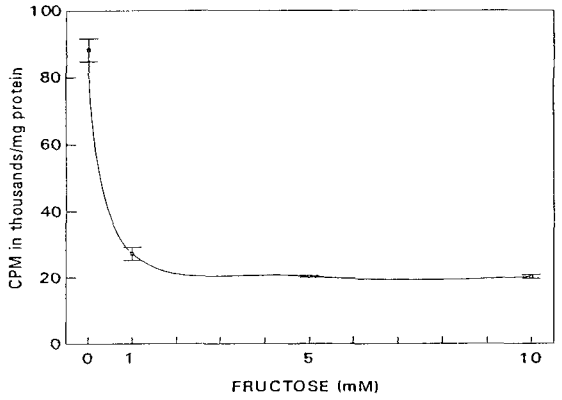

FIGURE 3 ^{14}C-fructose binding to GAPDH: GAPDH (0.4 mg/ml) was incubated with U-^{14}C-fructose in 50 mM sodium phosphate buffer, pH 7.2 containing cold fructose (0–10 mM) for 9 h at 37°C and its binding determined as described in the Materials and Methods. Mean ± SD, $n = 3$.

the enzyme by incubating it with U-^{14}C-fructose. As shown in Figure 3, significant incorporation of fructose into the enzyme was observed. Addition of cold fructose (1 mM) resulted in about 60% inhibition of the process. The precise reason for only a partial inhibition of ^{14}C-fructose binding with the cold fructose is not yet clear. Several kinetic and structural factors could be involved; such as the reversibility of the initial glycation reaction, conversion of the fructated protein to Amadori and AGE, and simultaneous generation of $O_2^{\bullet-}$ from fructose.

Whether this binding of fructose to GAPDH could be inhibited by pyruvate was examined in a subsequent experiment. In this experiment, the enzyme was pre-incubated with pyruvate and then challenged with fructose as described above. The results summarized in Figure 4 demonstrate that the binding of fructose was indeed inhibited significantly, the extent of inhibition being about 50% with levels of pyruvate of 0.2–1 mM. These levels were similar to the levels of pyruvate showing a substantial prevention against the loss of the enzyme activity caused by its incubation with this sugar.

In a recent study, we have demonstrated that the loss of fructose-induced glucose-6-phosphate

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

RIGHTSLINK

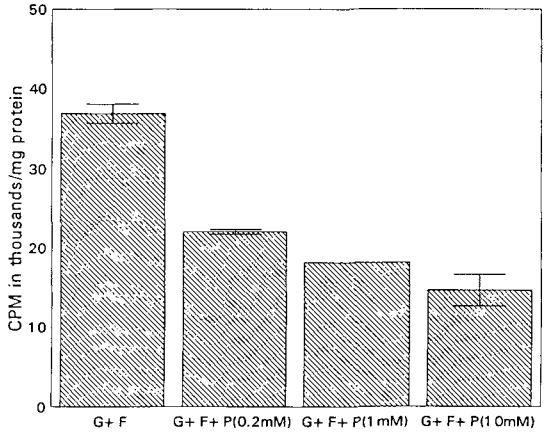

FIGURE 4 Inhibition of fructose binding to GAPDH by pyruvate: GAPDH (0.4 mg/ml) was incubated with fructose (10 mM) containing its U-^{14}C-analogue in 50 mM sodium phosphate buffer, pH 7.2 at 37°C for 9 h in the absence and presence of pyruvate (0–10 mM) and the amount of fructose bound to the enzyme was determined as described in the method. $n=3$. G = GAPDH, F = fructose, P = pyruvate.

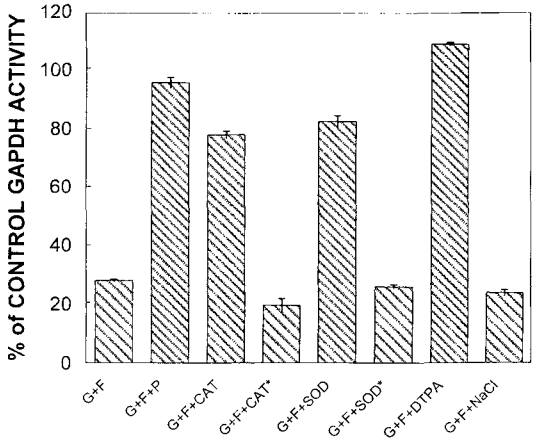

FIGURE 5 Protective effect of pyruvate and antioxidants against the loss of GAPDH activity on incubation with fructose: this was done by incubating the enzyme with fructose (20 mM) at 37°C for 9 h in the absence and presence of various antioxidants as indicated. The protocol was similar to that described in Figure 1. $n=3$. G = GAPDH, F = fructose, P = pyruvate, CAT = catalase, CAT* = inactive catalase, SOD = superoxide dismutase, SOD* = inactive SOD, DTPA = diethylene triaminepentaacetic acid, NaCl = sodium chloride.

TABLE I Oxidation of –SH group in GAPDH: prevention by antioxidants

	GAPDH	GAPDH+ fructose	GAPDH+ fructose+ SOD	GAPDH+ fructose+ catalase	GAPDH+ fructose+ pyruvate	GAPDH+ fructose+ DTPA
% of –SH*	100	48.5 ± 6.2	84.1 ± 5.4	94.0 ± 5.5	93.7 ± 7.8	111.8 ± 12.5

*–SH content in GAPDH is expressed as the percentage of the basal –SH group present in the enzyme incubated without fructose. GAPDH (0.8 mg/ml) was incubated with 20 mM fructose in the absence and presence of SOD (10 U/ml), catalase (5 U/ml), pyruvate (1 mM) and DTPA (1 mM). The incubation was carried out at 37°C for 9 h and the –SH content was determined by reaction with DTNB. The loss in the –SH content of the enzyme incubated without any fructose was less than 10%, when compared to fresh enzyme preparation. Mean ± SD, $n=3$.

dehydrogenase (G6PDH) activity is also attributable to the ROS generated during the sugar auto-oxidation.[17] Hence, further studies were undertaken to examine whether the loss of GAPDH activity associated with its incubation with fructose could be prevented by some ROS scavengers such as catalase and SOD, and DTPA, an agent that inhibits $OH^{\bullet}$ formation by chelating reactive metal ions. As shown in Figure 5, all the compounds were found to be protective. Heat-inactivated catalase and SOD did not protect. The results hence suggest that GAPDH inactivation is predominantly caused by ROS, which can be generated directly during oxidation of fructose[17] as well as of the fructated protein.[25] Both are known to generate $O_2^{\bullet-}$, albeit the Amadori is supposed to be more potent in this regard. NaCl, used as a negative control, had no effect.

As shown in Table I, the –SH content of GAPDH also decreased on incubation with fructose. The decrease was preventable by SOD, catalase, DTPA, as well as pyruvate. The latter also prevented the enzyme deactivation.

Subsequent studies were undertaken to determine the possible deleterious effect of fructose on the activity of the primary antioxidant enzymes

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

RIGHTSLINK

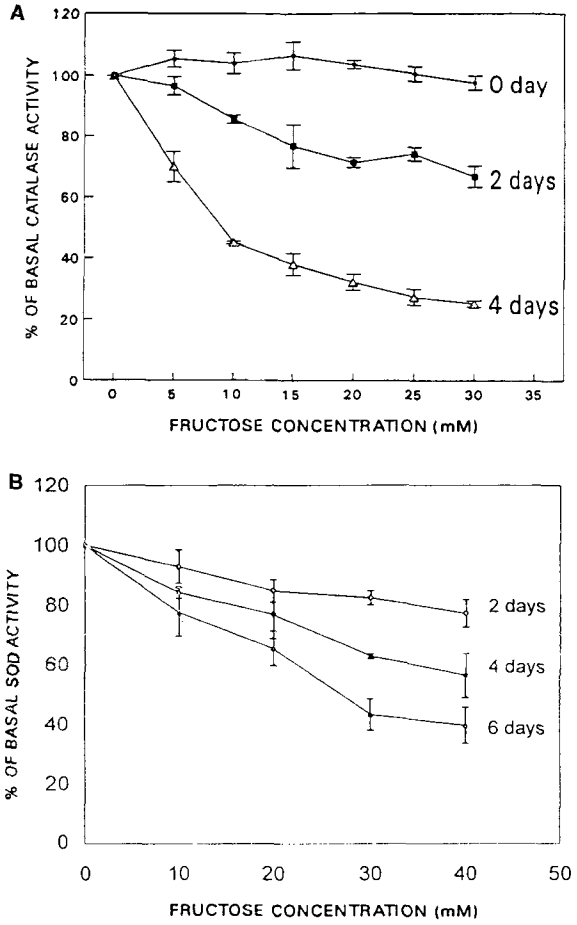

FIGURE 6 Loss of antioxidant enzyme activity on incubation with fructose: A. catalase; B. SOD. At indicated times, the enzyme activity was determined spectrophotometrically as described in the methods. The results (mean ± SD) are expressed as the percent of controls wherein the enzyme was incubated without any fructose. $n = 3$.

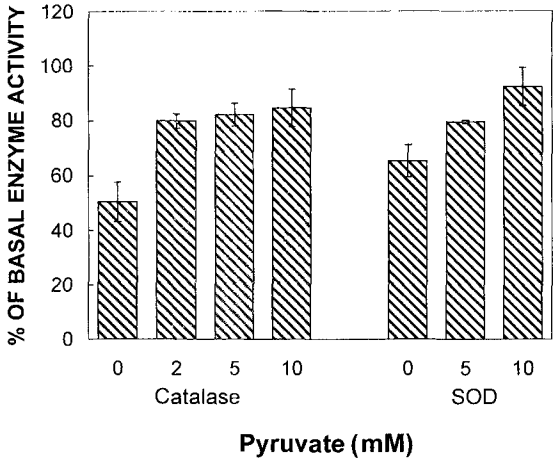

FIGURE 7 Protective effect of pyruvate against loss of antioxidant enzyme activity on incubation with fructose: this was determined by incubating the enzymes with fructose (20 mM) at 37°C in the absence and presence of pyruvate as indicated. Catalase was incubated for 3 days and SOD for 6 days. The protocol was similar to that described in Figure 6. $n = 3$.

themselves and the possibility of preventing such damage by pyruvate. As summarized in Figure 6, catalase as well as SOD did lose their activities significantly on incubation with fructose. The extent of the deactivation depended on the fructose concentration as well as the time of incubation. Fresh fructose when added to the assay mixture had no significant effect on the assay procedure. However, blanks consisting of the incubation mixture containing the buffer and fructose without the enzyme seemed to increase the rate of the reduction of ferricytochrome c in the SOD assay by approximately, 10%. A correction for this was hence made. Pyruvate protected against the deactivation of these antioxidant enzymes caused by fructose (Figure 7).

DISCUSSION

Several studies suggest that oxidative stress concomitant to the generation of ROS is potentially involved in the pathogenesis of age-related diseases. Recently, it has been demonstrated that auto-oxidation of fructose generates ROS in the presence of trace metals.[17,25] In the present study, we demonstrate that excessive accumulation of fructose in the tissue can lead to the deactivation of several important metabolic and antioxidant enzymes. GAPDH is an important enzyme in the glycolytic pathway. Since lens is a relatively anaerobic tissue, the activity of this enzyme is critical in the maintenance of tissue bioenergetics and lens transparency. Maintenance of its Cys^{149} in the reduced state is essential for GAPDH catalytic activity.[26] The importance of the Cys –SH for the

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

activity of the enzyme is proven by its deactivation on storage in air or reaction with H_2O_2 and its reactivation by treatment with reducing thiols.[27] Hence, the ROS generated from auto-oxidation of fructose was considered to be potentially damaging. Indeed, it was found that –SH content in GAPDH incubated with fructose does decrease. In line with this, the activity of this enzyme also decreased substantially on incubation with this sugar at levels prevalent in the senile cataracts isolated from diabetic individuals. That the primary effect of fructose is exerted via the ROS generation was apparent from the preventive effect of SOD, catalase and DTPA.

The protective effect of SOD could be attributable to its effectiveness in scavenging superoxide, which is produced by the reaction of trace metals with oxygen. The protective effect of catalase and DTPA is consistent with the hypothesis that the actual damaging agent is $OH^{\bullet}$ produced by Haber–Weiss reaction. Interestingly, however, the data from the present study suggest that these antioxidant enzymes are also susceptible to fructose-induced deactivation. Such deactivation of these antioxidant enzymes would lead to excessive ROS levels in the tissues. Fortunately, the deactivation of these protective enzymes by fructose took a longer time, in comparison to G6PDH[17] and GAPDH.

The concentration of fructose is relatively high also in the normal lens as compared to most other body tissue.[12] This has been attributed to a relatively higher activity of the polyol pathway in this tissue, which converts glucose first to sorbitol and then to fructose.[28] The kinetic parameters of the enzyme in human lens are such that the conversion of sorbitol to fructose is faster than the formation of sorbitol from glucose.[13] This results in a lower accumulation of sorbitol and a higher level of fructose. The situation is exacerbated further in diabetes, when fructose concentration in the human lens can be as high as 15 mM.[11–13] Hence it is conceivable that high levels of fructose in the diabetic lens can participate in the enhancement of the multifactorial cataractogenic process. In view of the above observations, it is likely that it may do so by generating ROS as well as by glycating the structural and enzymatic lens proteins. The preventive effect of pyruvate against oxidative damage to the enzymatic and nonenzymatic proteins as well as against their glycation is hence interesting from the viewpoint of cataract prevention. The compound has previously been shown to prevent oxidation induced cataract formation initiated by sodium selenite administration.[22] In addition, it has been shown to attenuate cataract formation in rats fed with high amounts of galactose.[29,30] The preventive effect of pyruvate can hence be attributed to its scavenging of ROS and consequent inhibition of the conversion of the initial glycated protein to AGE, as well as to the inhibition of the conversion of fructose to its dicarbonyl derivative (osone). The latter are much more potent glycating agents. These finding therefore lend further support to the hypothesis that pyruvate could be useful in maintaining the action of metabolic as well as primary antioxidant enzymes necessary to prevent tissues against pathophysiological damage caused by excessive ROS generation in diabetic and nondiabetic situations.

Acknowledgments

The authors are thankful to the financial support of NIH through the grant #EY0129-23* and for the support of Research to Prevent Blindness (RPB), New York, through a departmental grant. S.D.V. is also a receipient of a RPB Senior Scientist Investigator Award.

References

[1] N.V. Neilson and T. Vinding (1984) The prevalence of cataracts in insulin-dependent and non-insulin-dependent diabetes mellitus. An epidemiological study of diabetics treated with insulin and oral hypoglycaemic agents OHA. *Acta Ophthalmology* **62**: 595–602.
[2] R. Klein, B.E.K. Klein and S.E. Moss (1984) Visual impairment in diabetes. *Ophthalmology* **91**: 1–9.
[3] B.E.K. Klein, R. Klein and S.E. Moss (1988) Prevalence of cataracts in a population based study of persons in the diabetes mellitus. *Ophthalmology* **92**: 1191–1196.

RIGHTSLINK

[4] V.J. Stevens, C.A. Rouzer, V.M. Monnier and A. Cerami (1978) Diabetic cataract formation and potential role of glycosylation of lens proteins. *Proceedings of National Academy of Sciences USA* **75**: 2918–2922.
[5] V.M. Monnier, V.J. Steven and A. Cerami (1979) Non-enzymatic glycosylation, sulphydryl oxidation and high molecular weight aggregation of lens proteins in experimental sugar cataracts. *Journal of Experimental Medicine* **50**: 1098–1117.
[6] M.S. Swamy and E.C. Abraham (1987) Lens protein composition, glycation and high molecular weight aggregation in ageing rats. *Investigative Ophthalmology and Visual Science* **28**: 1693–1701.
[7] E. Ganea and J.J. Harding (1995) Molecular chaperones protect against glycation-induced inactivation of glucose-6-phosphate dehydrogenase. *European Journal of Biochemistry* **231**: 181–185.
[8] H. Yan and J.J. Harding (1997) Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. *Biochemical Journal* **328**: 599.
[9] R. Blakytny and J.J. Harding (1992) Glycation (non-enzymic glycosylation) inactivates glutathione reductase. *Biochemical Journal* **288**: 303.
[10] M.M. Heath, K.C. Rixon and J.J. Harding (1996) Glycation-induced inactivation of malate dehydrogenase protection by aspirin and a lens molecular chaperone, α-crystallin. *Biochimica Biophysica Acta* **1315**: 176–184.
[11] S.D. Varma and J.H. Kinoshita (1974) Sorbitol pathway in diabetic and galactosemic rat lens. *Biochimica Biophysica Acta* **328**: 632–640.
[12] J.F. Kuch Jr. (1961) The formation of fructose in the ocular lens. *Archives of Ophthalmology* **65**: 100–106.
[13] J.A. Jedziniak, L.T. Chylack Jr., H.M. Cheng, M.K. Gillis, A.A. Kalustian and W.H. Tung (1981) The sorbitol pathway in the human lens: aldose reductase and polyol dehydrogenase. *Investigative Ophthalmology and Visual Science* **20**: 314–336.
[14] G. Suarez, J. Maturana, A.L. Oronsky and C.R. Suarez (1991) Fructose-induced fluorescence generation of reductively methylated glycated bovine serum albumin: evidence for nonenzymic glycation of Amadori adducts. *Biochimica Biophysica Acta* **1075**: 12–19.
[15] H.F. Bunn and P.J. Higgins (1981) Reaction of monosaccharides with protein: possible evolutionary significance. *Science* **213**: 222–224.
[16] K. Raza and J.J. Harding (1991) Non-enzymic modification of lens proteins by glucose and fructose: effects of ibuprofen. *Experimental Eye Research* **52**: 205–212.
[17] W. Zhao, P.S. Devamanoharan and S.D. Varma (1998) Fructose induced deactivation of glucose-6-phosphate dehydrogenase and its prevention by pyruvate: implications in cataract prevention. *Free Radical Research* **29**: 315–320.
[18] K.M. Namiki, M. Oka, M. Otsuka *et al.* (1994) Chemiluminescence developed at an early stage of Maillard reaction. In: Labuza *et al.* (Eds.), *"Maillard Reactions in Chemistry, Food and Ageing"* Royal Society of Chemistry Publishers, England, pp. 88–94.
[19] M.A.F. Holleman (1904) Note on the action of oxygenated water on alpha-ketoacids and 1,2-diketones. *Recl. Trav. Chi. Pays-bas Belg* **23**: 169–172.
[20] S.D. Varma, P.S. Devamanoharan and S.M. Morris (1995) Prevention of cataracts by nutritional and metabolic antioxidants. *Critical Reviews in Food Science & Nutrition* **35**: 111–129.
[21] S.D. Varma, P.S. Devamanoharan and S.M. Morris (1990) Photoinduction of cataracts in lens *in vitro*. Prevention by pyruvate. *Experimental Eye Research* **50**: 805–812.
[22] S.D. Varma, S. Ramachandran, P.S. Devamanoharan, S.M. Morris and A.H. Ali (1995) Prevention of oxidative damage to rat lens *in vitro* by pyruvate: possible attenuation *in vivo*. *Current Eye Research* **14**: 643–649.
[23] S.D. Varma, P.S. Devamanoharan and A.H. Ali (1997) Formation of advanced glycation end products in diabetes. Prevention by pyruvate and alpha keto glutarate. *Molecular and Cellular Biochemistry* **171**: 23–28.
[24] G.L. Ellman (1959) Tissue sulphydryl groups. *Arch. Biochem. Biophys.* **82**: 70–77.
[25] J.V. Hunt, R.T. Dean and S.P.C. Wolf (1988) Hydroxyl radical production and autooxidative glycosylation: glucose auto-oxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and aging. *Biochemical Journal* **256**: 205–212.
[26] C. Walsh (1979) Enzymatic Reaction Mechanisms. W.H. Freeman and Company, New York, pp. 322–330.
[27] C. Little and P.J. O'Brien (1969) Mechanism of peroxide-inactivation of the sulphydryl enzyme glyceraldehyde-3-phosphate dehydrogenase. *European Journal of Biochemistry* **10**: 533–538.
[28] R. Van Heyningen (1959) Formation of polyols by the lens of the rat with sugar cataracts. *Nature* **164**: 194–196.
[29] M. Henein, P.S. Devamanoharan, S. Ramachandran and S.D. Varma (1992) Prevention of galactose cataracts by pyruvate. *Lens and Eye Toxicity Research* **9**: 25–36.
[30] S.D. Varma, P.S. Devamanoharan, A.R. Rutzen, A.H. Ali and M. Henein (1999) Attenuation of galactose-induced cataract by pyruvate. *Free Radical Research* **30**: 253–263.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/21/11
For personal use only.

RIGHTSLINK