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Quinone induced stimulation of hexose monophosphate shunt activity in the guinea pig lens: role of zeta-crystallin

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The response of the hexose monophosphate shunt (HMS) in organ-cultured guinea pig lens to 1,2-naphthoquinone and 5-hydroxy-1,4-naphthoquinone (juglone) has been investigated. Both these compounds, which are substrates of guinea pig lens zeta-crystallin (NADPH:quinone oxidoreductase), were found to cause increases in the rate of $^{14}CO_2$ production from 1-^{14}C-labelled glucose. Exposure of lenses to 15 μM 1,2-naphthoquinone or 20 μM juglone yielded 5.9- and 7-fold stimulation of HMS activity, respectively. Unlike hydrogen peroxide-induced stimulation of HMS activity, these effects were not abolished by preincubation with the glutathione reductase inhibitor, 1,3-bis(2-chloroethyl)-1 nitrosourea (BCNU). While hydrogen peroxide produced substantial decrements in lens glutathione (GSH) levels, incubation with quinones was not associated with a similar reduction in GSH concentration. Protein-bound NADPH content in quinone-exposed guinea pig lenses was decreased, with a concomitant increase in the amounts of free $NADP^+$. This finding supported the involvement of zeta-crystallin bound NADPH in the in vivo enzymic reduction of quinones. Hydrogen peroxide, on the other hand, caused decreases in the level of free NADPH alone, serving to confirm our earlier inference that quinone stimulated increases in the guinea pig lens HMS could be mediated through zeta-crystallin NADPH:quinone oxidoreductase activity.

Introduction

Zeta-crystallin is a taxon-specific crystallin constituting as much as 10% of total soluble protein in the lens of the guinea pig (*Cavia porcellus*). The fact that a mutation in the zeta-crystallin gene is associated with formation of an autosomal dominant congenital cataract in a line of strain 13/N guinea pigs [1] stimulated our interest in elucidating the functional role(s) of this protein in the lens. Earlier work from this laboratory [2] has demonstrated that zeta specifically binds NADPH and that the levels of both NADPH and $NADP^+$ are 30–60-fold higher in guinea pig lens than in lenses from most other animals [3,4]. 92–95% of the NADPH was found to be protein-bound and a correlation was established between NADPH levels and zeta-crystallin content in the guinea pig lens. The NADPH binding properties of zeta, together with its presence in small amounts in the liver and kidney [1] suggested that it, like some other taxon-specific crystallins, might be a functional enzyme [5]. This was confirmed when it was discovered that zeta is capable of catalytically reducing the electron acceptor 2,6-dichlorophenol-indophenol [6]. Further characterization of its enzymatic properties indicates that zeta is an NADPH: quinone oxidoreductase [7].

The presence of high levels of $NADP^+$, as well as NADPH suggests that redox cycling of these nucleotides takes place in guinea pig lenses. This possibility is supported by the high activities [2] of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.43), the enzymes known to reduce $NADP^+$ to NADPH. Limitations inherent in inferring the physiological significance of an enzyme solely from in vitro assays of its activity carried out under optimal conditions are self-evident. Thus, we have utilized the lens organ culture system to address the following questions in an effort to understand the possible relation between the high levels of (zeta-bound) NADPH and the NADPH generating dehydrogenases of the oxidative part of the hexose monophosphate shunt (HMS): (i) Are the high activities of glucose 6-phosphate dehydrogenase and 6-phos-

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Abbreviations: HMS, hexose monophosphate shunt; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; GSH, glutathione.

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phogluconate dehydrogenase indicative of an elevated HMS activity (basal and maximal) in guinea pig lens? (ii) What is the effect on HMS activity of the large amount of protein-bound NADPH present in guinea pig lens? (iii) Is the zeta/NADPH:quinone oxidoreductase active in the lens, and if so, would its catalytic function (which entails conversion of the zeta-bound NADPH to $NADP^+$) act as a mechanism to up-regulate the activity of the HMS?

Materials and Methods

Hartley guinea pigs and Sprague-Dawley rats (both aged 8 weeks) were obtained from Charles River Laboratory Animal Supplies, U.S.A. Hydrogen peroxide (30% solution) was from Sigma Chemicals (St. Louis, MO), while radiolabelled glucose (1-^{14}C, 6-^{14}C and 2-^{14}C, 7.9–8.5 mCi/mmol) was obtained from New England Nuclear (Boston, MA). BCNU was generously provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. 1,2-Naphthoquinone, 5-hydroxy-1,4-naphthoquinone (juglone), menadione and 9,10-anthraquinone were from Aldrich Chemicals (Milwaukee, WI). All other chemicals were of reagent grade.

Experiments for assessing oxidation of glucose by guinea pig lenses, were carried out as follows. The lenses were incubated individually in 3.0 ml of Medium 199 containing 5.54 mM glucose and Hanks' salts, without phenol red, in 20 ml glass vials closed with a silicone septum-lined, plastic screw cap (Wheaton Scientific, Millville, NJ). Polypropylene center wells (Kontes, Vineland, NJ) containing 200 μl of CO_2 trapping reagent (NCS, Amersham, Arlington Heights, IL) were attached to the wall of the vials with adhesive tape. Prior to incubation, the medium was allowed to equilibrate with a mixture of CO_2 (5%) and air. After a 4-h incubation at 37°C, the reaction was stopped by injecting 1 ml of 1 M H_2SO_4 through the septum into each vial. The vials were left in the incubator overnight to ensure that all the liberated CO_2 would be absorbed by the NCS. The vials were then uncapped, center wells detached and transferred to vials containing 15 ml of Hydrofluor (National Diagnostics, Manville, NJ) for scintillation counting. Disintegrations per min were converted to μmol CO_2 per 4 h by accounting for the specific activity of the glucose used (approx. 5500 counts/min per μmol for [1-^{14}C]- and [2-^{14}C]glucose and 21 000 counts/min per μmol for [6-^{14}C]glucose for guinea pigs, corresponding figures for experiments with rat lenses are 19 000 counts/min per μmol and 57 000 counts/min per μmol). Blank vials without a lens were set up in the same manner as outlined. In experiments designed to study the effect of quinones on the oxidation of glucose, lenses to which ethanol (the vehicle in which quinones were dissolved) was added, were included as additional controls. The effect of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on oxidation of glucose was studied by preincubating lenses in M-199 containing 0.5 mM BCNU for a period of 4 h, before transferring them to vials containing [1-^{14}C]- or [6-^{14}C]glucose. Incubation was then continued for 4 h as described above. Experiments with rat lenses were conducted in the same manner except that the volume of medium used with each was 2.0 ml.

The concentration of glutathione (GSH) was determined with 5,5'-dithio-bis-(2-nitrobenzoic acid) [8], while $NADP^+$ and NADPH were estimated spectrophotometrically by an enzymatic recycling method [3] as described by Rao and Bhat [9]. Free nucleotides (both NADPH and $NADP^+$) were separated from protein-bound nucleotides by subjecting the 20 000 $\times g$ supernatant of 10% lens homogenates to filtration through a Centricon 30 microconcentrator. Free NADPH was calculated as the difference between total and protein-bound NADPH and bound $NADP^+$ as the difference between the total and free $NADP^+$.

Results

Previous studies, especially by Giblin and co-workers [10–12], have demonstrated the effect of H_2O_2 in stimulating the HMS in cultured rabbit lenses and have shown that this stimulation results from the utilization of NADPH by the glutathione redox cycle as the H_2O_2 is enzymatically detoxified. For our initial studies we compared the HMS from guinea pig and rat lenses with respect to their basal rates and their ability to be stimulated by H_2O_2. The rat lens was used for comparison since it lacks both zeta-crystallin and the high amount of NADPH that is characteristic of the guinea pig lens. Table I indicates that the basal rates of glucose oxidation are low in the rat lens, (the CO_2 derived from [1-^{14}C]- and [6-^{14}C]glucose in control rat lenses being 14.5% and 57%, respectively, of corresponding control guinea pig lens) and it is seen that this lens possesses very little capacity to sustain an H_2O_2-induced increased rate of [1-^{14}C]glucose oxidation. The ratio of C-1/C-6, which climbs from a basal value of 2.9 to 6.6 at 0.02 mM peroxide, abruptly declines to 59% of control values at 0.04 mM H_2O_2. This trend is also obvious on examining the ratios of total NADPH/$NADP^+$, which increase from 1.0 to 1.8 and then fall back to basal values over the same concentration range of hydrogen peroxide. Oxidation at the C-2 position of glucose is known to contribute to shunt activity via the conversion of excess pentose phosphate to hexose phosphate [13]. Values for oxidation of [2-^{14}C]glucose have not been included here, because the values obtained were not significantly above background levels. Incubating guinea pig lenses in the presence of 0.06 mM hydrogen peroxide pro-

TABLE I

Effect of exposure to H_2O_2 on oxidation of glucose and the NADPH to $NADP^+$ ratio in organ-cultured lenses from rat and guinea pig

Figures represent mean ± S.D. of four individual observations each. Ratios NADPH/$NADP^+$ were calculated by estimating total $NADP^+$, NADPH content (nmols/g lens). Glucose oxidation reported as μmols $^{14}CO_2$/g lens per 4 h, was assessed as outlined in text.

H_2O_2 (μM)	μmols CO_2/g lens per 4 h			
	1-^{14}C	6-^{14}C	1-^{14}C/ 6-^{14}C	NADPH/ $NADP^+$
Rat lenses				
0 (control)	0.10 ± 0.003	0.04 ± 0.003	2.9	1.0
10	0.13 ± 0.010	0.03 ± 0.006	4.1	1.5
20	0.15 ± 0.004	0.02 ± 0.001	6.6	1.8
40	0.08 ± 0.007	0.05 ± 0.003	1.7	1.0
Guinea pig lenses				
0 (control)	0.67 ± 0.025	0.06 ± 0.005	10.7	2.0
50	1.80 ± 0.084	0.08 ± 0.007	22.8	2.0
60	1.90 ± 0.088	0.08 ± 0.009	25.4	1.8
70	1.41 ± 0.063	0.06 ± 0.003	24.9	1.7
80	0.69 ± 0.030	0.06 ± 0.005	10.7	1.7

duced a 2.8-fold increase in the rate of oxidation of [1-^{14}C]glucose, as compared to control lenses (0.133 vs. 0.047 μmol CO_2 liberated/lens per 4 h, respectively). While HMS activity (as indicated by [1-^{14}C]glucose oxidation) reverted to basal value by 0.08 mM H_2O_2 and was undetectable at higher peroxide concentrations, these data clearly demonstrate that both in terms of basal rate and in the capacity to respond to H_2O_2-induced stress, the guinea pig lens has a much more active HMS than does the rat lens.

From in vitro studies it has been shown that zeta-crystallin functions as an NADPH:quinone oxidoreductase [7]. To determine whether zeta-crystallin in the intact lens could reduce quinones, we added quinone substrates to the medium and measured the effect on the HMS activity in cultured rat and guinea pig lenses. Based on earlier data [7], 1,2-naphthoquinone and juglone were used as representative substrates of zeta, while 9,10-anthraquinone and menadione were used as quinones that were not substrates. Judging from the CO_2 derived from [1-^{14}C]- and [2-^{14}C]glucose (Table II), it was evident that juglone stimulated the oxidation of [1-^{14}C]glucose, as well as reconversion of HMS-derived pentose phosphate to hexose phosphate. Concentrations beyond 20 μM juglone, however, lowered the 1-^{14}C/6-^{14}C and 2-^{14}C/6-^{14}C ratios considerably, as compared to values observed at maximal stimulation of HMS activity. A similar pattern of enhancement in HMS activity was produced by 1,2-naphthoquinone, the maximum stimulation being observed at a concentration of 15 μM (5.8-fold stimulation as compared to control values, data not shown.) Inclusion of even the lowest concentration of juglone (5 μM) in the incubation medium seemed to completely knock out the basal HMS activity in rat lenses (data not shown).

Exposure to 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), a glutathione reductase inhibitor, is known to abolish stimulation of HMS activity that occurs in rabbit lenses exposed to hydrogen peroxide [10]. Experiments employing BCNU were, therefore, conducted to determine whether glutathione metabolism played a role in the stimulatory effect of juglone or 1,2-naphthoquinone on the HMS. As can be inferred from data presented in Table III, pretreatment of guinea pig lenses with 0.5 mM BCNU did not result in inhibition of the juglone-stimulated HMS activity, while hydrogen peroxide-stimulated increases in [1-^{14}C]glucose oxidation were prevented by such treatment. A slight increase in HMS activity was observed when guinea pig lenses were treated with 9,10-anthraquinone or menadione (quinones which are not substrates of zeta); this effect was essentially eliminated by pretreatment with BCNU (Table III). Data obtained on levels of GSH in guinea pig lenses revealed that H_2O_2 (at all levels which stimulated the HMS) mediated substantial decreases in GSH, while in the juglone exposed lenses decreases in GSH were not observed until the higher stimulatory doses of juglone were reached (Fig. 1).

Distribution profiles for oxidized vs. reduced $NADP^+$ in guinea pig lenses incubated with juglone

TABLE II

Juglone induced elevation of the hexose monophosphate shunt activity in guinea pig lenses

Values represent mean ± S.D. of four individual observations in each case. Control lenses were incubated in the presence of 0.5% ethanol (vehicle in which juglone was dissolved), while the other lenses were exposed to the indicated concentrations of juglone.

Concentration of juglone (μM)	μmols CO_2/g lens per 4 h				
	1-^{14}C	6-^{14}C	2-^{14}C	1-^{14}C/6-^{14}C	2-^{14}C/6-^{14}C
0	0.69 ± 0.017	0.06 ± 0.001	0.27 ± 0.008	12.2	4.9
5	0.70 ± 0.025	0.06 ± 0.005	0.29 ± 0.007	11.3	4.7
10	2.50 ± 0.014	0.09 ± 0.003	0.86 ± 0.008	27.1	9.4
15	3.10 ± 0.024	0.10 ± 0.003	1.02 ± 0.050	32.2	10.7
20	4.73 ± 0.208	0.10 ± 0.004	1.67 ± 0.026	49.3	17.1
25	3.24 ± 0.278	0.10 ± 0.010	0.94 ± 0.032	33.4	9.7

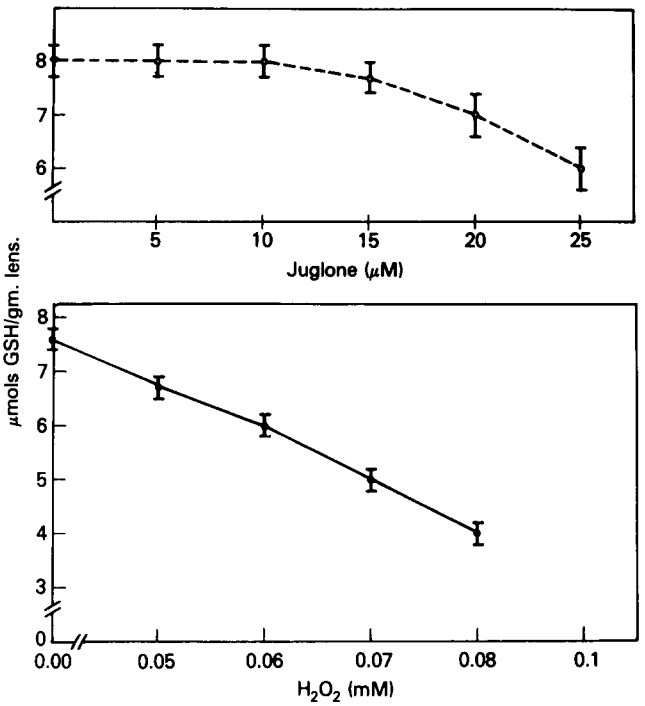

Fig. 1. GSH content of guinea pig lenses incubated in the presence of various concentrations of juglone (upper panel) and hydrogen peroxide (lower panel). GSH was determined as outlined under Materials and Methods. Data given as mean ± S.D. of four independent experiments each.

are represented in Figs. 2 and 3. At the lower concentrations of juglone (0–15 μM), the amount of protein-bound NADPH remained relatively constant; presence of 25 μM juglone was associated with a 25% decrease (Fig. 2A) in this parameter. An opposite effect of smaller magnitude was found, in the amounts of free NADPH in such lenses (the amount of free NADPH at 25 μM juglone being 117 ± 5 nmol/g lens, as opposed to a value of 75 ± 7 nmol/g lens in control lenses, Fig. 2B). About 20% of the lens total $NADP^+$ was found to be protein bound (Fig. 3A) and there was little change observed in this nucleotide pool in juglone-exposed lenses. On the other hand, the amounts of free $NADP^+$ dramatically increased under these conditions – at 25 μM juglone, for instance, the free $NADP^+$ concentration was 573 ± 4.1 nmol/g lens compared to 360 ± 2.2 nmol/g lens in control lenses (Fig. 3B).

In contrast, levels of protein-bound and free NADPH and $NADP^+$ in H_2O_2 treated guinea pig lenses (Table IV) indicated significant changes in contents of free NADPH and $NADP^+$ alone. While 0.05 mM peroxide did not cause any decline in the levels of either free NADPH or $NADP^+$, 0.08 mM peroxide produced a 60% decrease in free NADPH/g lens. In absolute terms this represented a loss of 45 nmol free NADPH which was balanced by an increase of similar

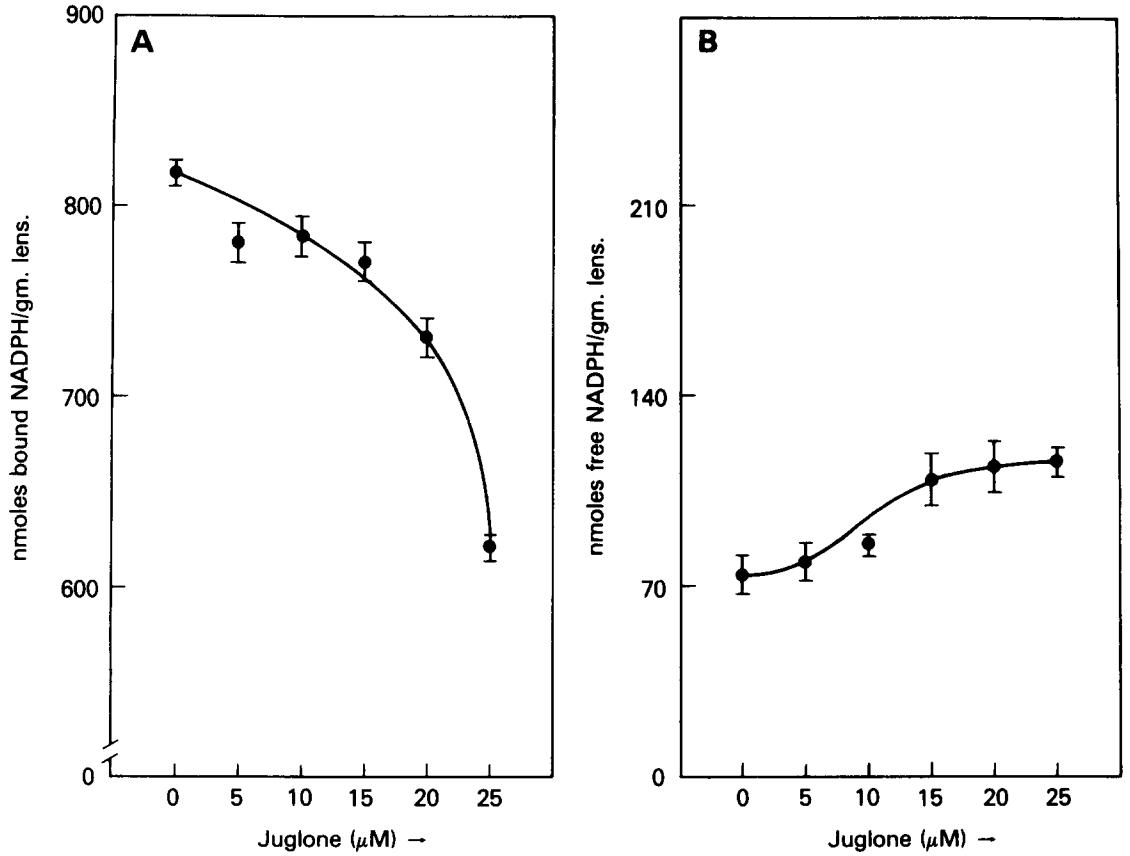

Fig. 2. Values of protein bound (A) vs. free (B) NADPH in guinea pig lenses incubated with juglone. These lenses were processed for estimation of free/protein-bound nucleotides after incubation as described earlier (Materials and Methods); and values represent mean ± S.D. of four individual data points each.

TABLE III

Effect of BCNU on hydrogen peroxide and quinone stimulated increases in [1-^{14}C]glucose oxidation in guinea pig lenses

Lenses were pretreated for 4 h in M-199 in the presence/absence of 0.5 mM BCNU. The lenses were then incubated for another 4 h in fresh medium containing either 0.5% ethanol (vehicle in which quinones were dissolved), hydrogen peroxide, or quinone and [1-^{14}C] or [6-^{14}C]glucose. Results are mean ± S.D. for four lenses each, except in the case of menadione ($n = 2$).

Pretreatment condition	Incubation conditions	μmols CO_2/g lens per 4 h		
		1-^{14}C	6-^{14}C	1-^{14}C/6-^{14}C
Normal	normal	0.68 ± 0.006	0.06 ± 0.002	10.8
+ BCNU	normal	0.69 ± 0.007	0.06 ± 0.006	11.8
Normal	50 μM H_2O_2	1.25 ± 0.185	0.07 ± 0.003	16.7
+ BCNU	50 μM H_2O_2	0.67 ± 0.043	0.06 ± 0.003	11.2
Normal	+ vehicle	0.68 ± 0.006	0.06 ± 0.003	11.3
Normal	+ 15 μM NQ	4.04 ± 0.073	0.17 ± 0.007	23.8
+ BCNU	+ 15 μM NQ	3.70 ± 0.188	0.16 ± 0.004	23.5
Normal	+ 20 μM juglone	4.70 ± 0.210	0.15 ± 0.011	30.8
+ BCNU	+ 20 μM juglone	4.80 ± 0.251	0.16 ± 0.008	30.1
Normal	+ 20 μM ANQ	0.82 ± 0.015	N.D.	-
+ BCNU	+ 20 μM ANQ	0.72 ± 0.008	N.D.	-
Normal	+ 20 μM MEN	0.83, 0.85	N.D.	-
+ BCNU	+ 20 μM MEN	0.74, 0.71	N.D.	-

N.D., not determined; NQ, 1-2 naphthoquinone; ANQ, 9,10-anthraquinone; MEN, menadione.

magnitude in the amount of free $NADP^+$ (Table IV). Finally, the overall content of total NADPH + $NADP^+$ remained virtually constant in juglone-treated lenses as compared to control values, however the NADPH/$NADP^+$ ratio decreased from 1.98 in untreated (control) lenses to 1.12 in lenses exposed to 25 μM juglone (Table V). As was the case with glutathione (Fig. 1), a change in the redox ratio of the pyridine nucleotides was observed only when the juglone concentration was 20 μM or higher.

Discussion

In order to investigate the significance of the markedly high levels of NADPH and $NADP^+$ and activities of glucose 6-phosphate dehydrogenase and

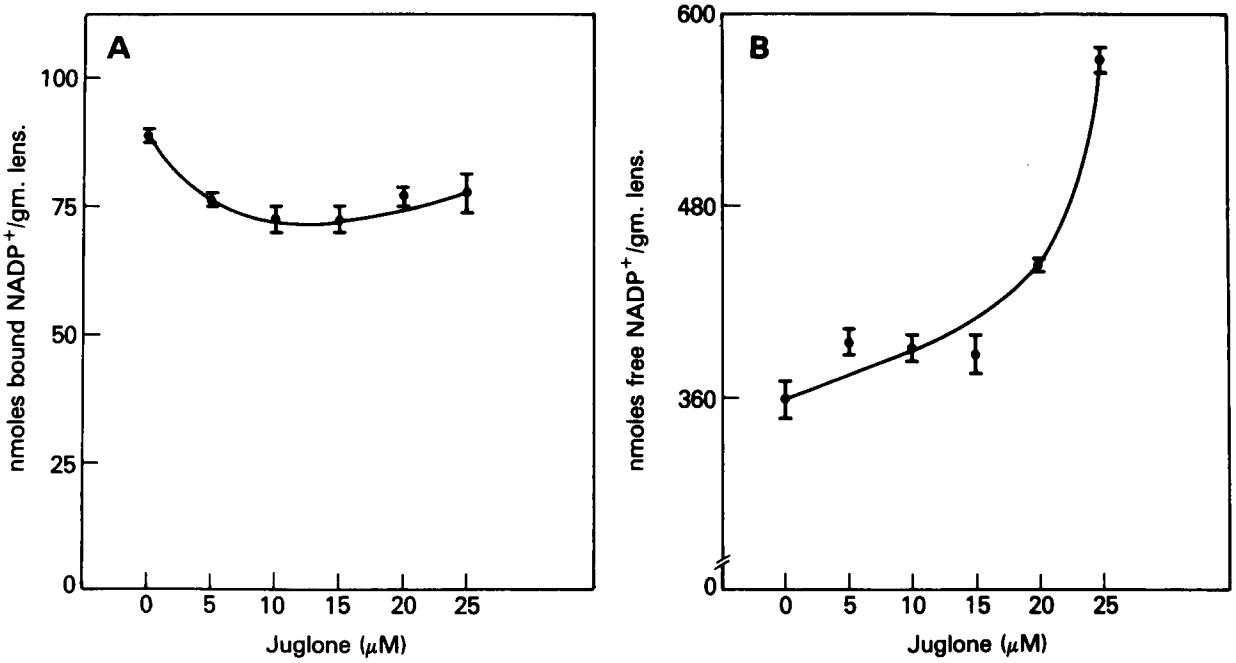

Fig. 3. Levels of protein-bound (A) vs. free (B) $NADP^+$ in juglone exposed guinea pig lenses. Control lenses were incubated with 0.5% ethanol (vehicle in which juglone was dissolved). Values indicate mean ± S.D. of four independent experiments each.

TABLE IV

Changes in levels of protein-bound vs. free NADPH and $NADP^+$ in hydrogen peroxide treated guinea pig lenses

Guinea pig lenses were incubated in the concentrations of hydrogen peroxide indicated; following which each lens was used for estimating total and bound NADPH or $NADP^+$. Free NADPH was estimated as the difference: total NADPH–protein bound NADPH, while bound $NADP^+$ is represented as the difference: total $NADP^+$–free $NADP^+$. Data given as mean ± S.D. for four independent values each.

Concentration of H_2O_2 (μM)	NADPH (nmols/g lens)		$NADP^+$ (nmols/g lens)	
	free	bound	free	bound
0	75 ± 2.8	810 ± 11.2	366 ± 11.2	80 ± 2.8
50	76 ± 3.1	806 ± 14.4	348 ± 11.9	82 ± 6.4
60	52 ± 3.6	802 ± 12.6	384 ± 11.0	84 ± 2.4
70	41 ± 2.9	806 ± 13.4	400 ± 8.4	87 ± 2.6
80	30 ± 2.8	804 ± 11.9	421 ± 8.4	85 ± 3.2

6-phosphogluconate dehydrogenase in the guinea pig lens, we undertook a detailed study of the HMS in these lenses. The HMS (or more specifically the two dehydrogenases cited above) is the principal source of NADPH in cells. In addition, the glucose 6-phosphate dehydrogenase reaction is believed to be the initial, committed, rate-limiting step of the HMS.

A comparative set of experiments was initially utilized for this purpose, in which the ability of H_2O_2 to stimulate HMS activity, was monitored in both guinea pig and rat lenses (Table I). The objective of this approach was to enable us to determine whether the existence of a large amount of an NADPH-specific binding protein in the guinea pig lens correlated functionally, with an increased basal activity of the HMS. The results depicted in Table I in addition to supporting the contention that basal rate of HMS activity (represented by [1-^{14}C]glucose oxidation) in the guinea pig lens is substantially greater than in the rat lens, also provide evidence that the large pool of $NADP^+$ does undergo redox cycling. Furthermore, it appears that the guinea pig lens has greater capacity for detoxifying hydrogen peroxide than do rat or rabbit lenses, a property that may be related to the higher amounts of NADPH present in the former lens. These differences in capacity of lenses in organ culture to defend themselves against damage caused by H_2O_2 probably also reflect differences in the concentration of H_2O_2 to which the lenses are normally exposed in vivo; the aqueous humor H_2O_2 concentrations are 0.047 ± 0.010 vs. 0.009 ± 0.005 mM, in the guinea pig and rat, respectively [11]. The decline in stimulation of guinea pig lens HMS activity beyond 0.06 mM H_2O_2 concentrations appears to result from a general increase in oxidative damage to the lens as is indicated by decreased GSH (Fig. 1) and NADPH (Table IV). This conclusion is supported by the work of Giblin and McCready [12] who have demonstrated a significant increase in HMS activity induced in rabbit lenses by hydrogen peroxide. Increased production of NADPH under these circumstances was also shown to be required for the reduction of GSSG formed under oxidative stress.

TABLE V

Total $NADP^+$ + NADPH and NADPH / $NADP^+$ ratios for guinea pig lenses following incubation with juglone

Results expressed as mean ± S.D. of four individual observations in each case. Ratios are calculated for total (protein-bound + free nucleotide) NADPH/$NADP^+$. Assays of protein-bound vs. free nucleotide were performed as outlined in Materials and Methods.

Concentration of juglone (μM)	Total $NADP^+$ + NADPH (nmols/g lens)	NADPH/ $NADP^+$
0	1339.6 ± 10.8	1.98
5	1330.4 ± 13.2	1.82
10	1335.0 ± 8.15	1.88
15	1341.2 ± 9.18	1.91
20	1366.7 ± 13.6	1.62
25	1385.7 ± 9.21	1.12

The much higher stimulation of HMS activity in guinea pig lenses by juglone (Table II) is distinctly different in nature from that caused by H_2O_2, since it does not involve GSH metabolism as evidenced by the absence of an effect of the GSH-reductase inhibitor BCNU on this phenomenon. A similar situation has been observed in rabbit lenses exposed to methylene blue [11] where the shunt stimulation is also not affected by BCNU. The lack of significant decrease in GSH levels (Fig. 1) in lenses incubated in concentrations of juglone which strongly stimulated the shunt justifies the conclusion that juglone mediated HMS stimulation in the guinea pig lens is attributable to zeta-crystallin's NADPH:quinone oxidoreductase activity. The fact that 9,10-anthraquinone and menadione, which are not zeta-crystallin substrates, cause only very small increases in oxidation of [1-^{14}C]glucose also strongly supports this conclusion (Table III). The ability of BCNU to negate these small elevations in shunt activity suggests that quinones also exert oxidative stress which activates the GSH redox cycle and hence stimulates shunt activity. It is well known that in many systems redox cycling by quinones produces oxidative damage as a result of free radical generation [14].

An interesting feature of the juglone induced increases in HMP shunt activity relates to the ability of this compound to cause an elevation in $^{14}CO_2$ derived from both [1-^{14}C]- and [2-^{14}C] glucose. The increase in [2-^{14}C]glucose oxidation supports a role for enhanced recycling of HMS derived pentose phosphate to hexose phosphate [13] in the presence of juglone. While [2-^{14}C]glucose could conceivably be oxidized to $^{14}CO_2$ through the citric acid cycle as well, the low 6-^{14}C to 2-^{14}C yields makes this unlikely. Additionally, the lens is known to metabolize most of its glucose to lactate via the Embden-Meyerhof pathway, while the tricarboxylic acid pathway has very low activity in this tissue [15]. This pattern of metabolism is likely the primary reason that we did not detect significant oxidation of [2-^{14}C]glucose in the cultured rat lenses.

Finally, characterization of changes in protein bound versus free NADPH and $NADP^+$ (Figs. 2 and 3) in juglone-exposed lenses provided clear evidence that the overall change in the redox ratio of the phosphorylated pyridine nucleotides, NADPH/$NADP^+$ (Table V), was traceable to a specific decrease in protein-bound NADPH. Taken together with the increased level of free $NADP^+$, this strongly supports the conclusion that the catalytic activity of zeta-crystallin, which converts bound NADPH to $NADP^+$, underlies the stimulation of HMS activity in guinea pig lenses by quinones. Our findings indicate that zeta-crystallin in the intact guinea pig lens is capable of functioning catalytically. The high levels of free $NADP^+$ in guinea pig lens and the high basal HMS activity present strongly suggest that the NADPH and $NADP^+$ do not exist as static pools, but undergo redox cycling. Since it is uncertain that significant concentrations of quinone substrates of zeta-crystallin would be present in guinea pig lens in vivo, another mechanism(s) of NADPH oxidation may need to be invoked to account for this redox cycling. In any case, the present data demonstrate that the zeta-crystallin/NADPH:quinone oxidoreductase in the intact lens is catalytically functional and has the capacity to mediate the HMS linked redox cycling of NADPH when an appropriate substrate is present.

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