

## PROTEOMIX AND ENZYME KINETICS IN NORMAL AND CATARACTOUS HUMAN LENS

Dr. Ajit V. Pandya

HOD, C. U. Shah Science College, Ashram Road, Ahmedabad - 380014, India.

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\*Correspondence for

Author

Dr. Ajit V. Pandya

HOD, C. U. Shah Science

College, Ashram Road,

Ahmedabad - 380014, India

### ABSTRACT

Opacity of lens is called cataract. Blindness due to cataract increases to great extent globally, more than 50 % people are experiencing profound or total loss of vision due to cataract. Oxidative damage plays major role in cataract development and defects are recorded in the antioxidant and related enzymes in lens during this disease. The mean value of GLUTATHION REDUCTASE activity is  $1.463 \pm 0.079$  and  $0.730 \pm 0.062$  n moles/min/mg, GLUTATHION-S-TRANSFERASE activity is  $1.780 \pm 0.069$  and  $0.545 \pm 0.342$  n moles/min/mg and  $\gamma$ -GLUTAMYL TRANSPEPTIDASE activity is  $9.595 \pm 0.094$  and  $3.7 \pm 0.216$  n moles/min/mg respectively for normal and cataractous lenses.

An explanation for fall in GLUTATHION REDUCTASE activity would be the inhibitory effect of oxidants on the activity of reducing enzymes. The activity of GLUTATHION-S-TRANSFERASE is very low compared to GLUTATHION REDUCTASE and  $\gamma$ -GLUTAMYL TRANSPEPTIDASE. The turn over of GSH by  $\gamma$ -GLUTAMYL TRANSPEPTIDASE and GLUTATHION-S-TRANSFERASE is thought to be groups led with several factors including GSH level. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported in mixed type of cataract.

**KEY WORDS:** Glutathion Reductase (GR), Glutathione S- Transferase (GST), Glutamyl Trans Peptidase (GTP), Human Lens

### INTRODUCTION

Cataract is an opacification of the ocular lens that results in diminished vision and eventual blindness (Thylefors et al., 1995). Out of 41 million people blind globally, about 42% people are experiencing profound or total loss of vision due to cataract. The number of blinds in

India is estimated to 18.7 million, out of which 9.5 million, the blindness is due to cataract. A single primary cause of cataract most likely does not exist.

Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours (Hiller et al., 1977, Zigman et al., 1979). Oxidative stress plays major role in cataractogenesis and defects are recorded in the antioxidant and related enzymes in lens and peripheral blood during this disease.

The protecting glutathione system of the lens including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transferase, g-glutamyl transpeptidase, GSH, GSSG, NADPH, etc. have been reported in the lens. Thus, GSH metabolism can be expected to be a significant factor in the defense of lens against cataractogenesis. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported with the progression of cataract (Rathbun et al., 1983).

Quantitatively, at least, the most significant protective system in the lens is that involving the reversible oxidation of glutathione. Normal lenses maintain a steady state of concentration of GSH; however this begins to drop in lenses undergoing cataract formation. This has been found to be true in almost all experimental cataracts and also in human senile cataracts. One of the functions of this high level of reduced Glutathione is probably to maintain protein sulphhydryls in the reduced form (Kinoshita et al., 1964). Being small and mobile molecules, glutathione (GSH) reacts with potential oxidants before they could interact with the lens proteins. Thus, it would react with oxygen and also act as a scavenger for any free radicals generated by ionizing radiation, UV and visible light, or univalent reduction of oxygen. Lens glutathione related enzymes are also capable of clearing mixed disulphide of glutathione and lens proteins. This provides the lens with a possible additional route for the regeneration of protein sulphhydryl. Thus the study related with such protective enzymes help in identifying their key roles in the cataractogenesis and oxidative insult in the lens.

## **MATERIALS AND METHODS**

**Collection of material:** The lenses of the patients who were undergoing cataract surgery at Nagari Eye Hospital, Ahmedabad, by medical Dr. involved in surgery, were collected. The cataract type in these patients was diagnosed with the help of slit-lamp biomicroscope. Several eyes with clear lens were obtained from C. H. Shamaria eye bank, Red Cross society, Ahmedabad, India.

(1) The Y-GLUTAMYL TRANSPEPTIDASE activity was assayed by the method of Tate and Meister [7].

The activity was expressed as units/hr/g fresh lens, where a unit of Y-GLUTAMYL TRANSPEPTIDASE is equal to the  $\mu$  moles of p-nitroaniline released per minute.

(2) The GLUTATHION-S-TRANSFERASE activity was assayed by Worholm et al., method (1985).

The enzyme activity was expressed as units/hr/g fresh lens. One unit of GLUTATHION-S-TRANSFERASE is the amount of the enzyme that catalyses the formation of 1  $\mu$  mol of 1 chloro-2, 4 dinitrophenyl glutathione per minute at 30 °C.

(3) Glutathion Reductase activity was estimated by the method of Carlberg and Mannervij (1985).

The activity of GLUTATHION REDUCTASE was expressed as units/hr/g fresh lens, where a unit of GLUTATHION REDUCTASE activity is defined as the amount of enzyme that catalyzes the reduction of 1  $\mu$  mole of NADPH per minute.

(4) Determination of Proteins: The soluble, insoluble, total proteins of the lenses were determined by the standard method of Lowry et al., (1951).

**Statistical analysis:** All results were expressed in mean  $\pm$  SD. One way analysis of variance (ANOVA) was used to test the significance of difference and Bonferroni test to test the significance of difference between control and different cataract types. The p value less than 0.05 is considered as significant. The results are expressed Glutathion Reductase by considering values of control lens as control as 100%.

## RESULTS

### Glutathion-s-transferase

As shown in Table-1, the enzyme GLUTATHION-S-TRANSFERASE activities in both normal and cataractous lenses. The GLUTATHION-S-TRANSFERASE activity is  $1.780 \pm 0.069$  and  $0.545 \pm 0.342$   $\mu$  moles/min/mg (mean  $\pm$  S.E.) for normal and cataractous lenses. The decreases in activity during cataractous condition are by 69.38% for GLUTATHION-S-TRANSFERASE. This major change in activity of crucial enzymes is highly significant and p-value is less than 0.01.

One of the key enzyme associated with GSH metabolism is GLUTATHION-S-TRANSFERASE. GLUTATHION-S-TRANSFERASE, a family of proteins having multiple detoxification effects have been observed which is responsible for this reason. It was also

observed that the activity of GLUTATHION-S-TRANSFERASE reduces with increase age and severely reduces under cataractous condition i.e. 70% compared to normal human lens. One of them has reported 73% reduced activity of GLUTATHION-S-TRANSFERASE in the brown dense cataracts (Rao et. al., 1983).

### **Y-Glutamyl transpeptidase**

Similarly Table-1 shows Y-GLUTAMYL TRANSPEPTIDASE activities in normal and cataractous human lenses. The Y-GLUTAMYL TRANSPEPTIDASE activity is  $9.595 \pm 0.094$  and  $3.7 \pm 0.216$  n moles/min/mg (mean  $\pm$  S.E.) respectively for normal and cataractous lenses. This reduce activity under cataractous condition is by 61.43% for Y-GLUTAMYL TRANSPEPTIDASE. The difference in activity of this crucial enzyme is highly significant and p-value is less than 0.01.

Since GSH is entirely degraded within the lens (Sippel, 1983), the r-glutamyl cycle seems to play an important role in the lens. Y-GLUTAMYL TRANSPEPTIDASE reacts very effectively with GLUTATHION-S-TRANSFERASE and all the enzymes involved in the GSH cycle. The activity of Y-GLUTAMYL TRANSPEPTIDASE is very low compared to GLUTATHION REDUCTASE and GLUTATHION-S-TRANSFERASE. The oxidation of GSH by Y-GLUTAMYL TRANSPEPTIDASE is thought to be groups led with transport of amino acids across the membrane by the same enzyme. This mechanism is highly effective in the lens, since it has a rapid turnover of GSH and is able to transport amino acids in to the tissue (Reddy, 1979). Any change in such mechanism may alter the Y-GLUTAMYL TRANSPEPTIDASE activity in the lens. The rapid turnover of GSH here would indicate rapid detoxification (oxidation) mechanisms coming into being.

Several epidemiological studies have claimed that antioxidants such as GSH and vitamin C have prevention role against the development of cataract (Bates, et.al ,1999, Bleau, et.al ,1998, Kupfer C, et.al, 1994, Carr,et.al,1999, Diplock,et.al,1998, Bunce, et.al, 1996, Malik A, et.al ,1995 , Ringvold A, et.al ,1996). Eventually the cumulative action of oxidative activities on GSH bringing about its oxidation could hamper the detoxifying mechanism causing reduction in the GSH levels. Due to fall in r – glutamyl cysteine synthetase (r-GCS) activity, the level of GSH decline rapidly in lenses with increase in age. Since GSH is a substrate for Y-GLUTAMYL TRANSPEPTIDASE, its decrease would inhibit the feedback mechanism thus lowering the activity. All together GSH and its metabolizing enzyme activity affect one another which are key factor for cataract development.

### Glutathion reductase

Also Table-1 shows GLUTATHION REDUCTASE activities in normal and cataractous human lenses. The age Glutathion Reductase groups are matched and are compared for both normal and cataractous eye lenses. The mean value of Glutathion Reductase activity is  $1.463 \pm 0.079$  and  $0.730 \pm 0.062$  n moles/min/mg (mean  $\pm$  S.E.) in normal and cataractous lenses respectively. It decreases under cataractous condition by 50.1%.

Reported results show activity of GLUTATHION REDUCTASE decreases during aging and cataractogenesis. Other Glutathion Reductase groups of scientist has also reported decrease activity of GLUTATHION REDUCTASE (Rao, et. al, 1983). The fall in Glutathion Reductase activity would result in decrease in GSH concentration, since, the system loses its capacity to regenerate GSH from G-S-S-G. The decrease in Glutathion Reductase activity will also affect the proteins, since the fall in GSH levels would result in disturbances in the maintenance of protein-SH Glutathion Reductase groups in reduced form giving rise to protein – protein disulfide bonds or protein – GSH disulfide bonding leading to Glutathion Reductase aggregation of these proteins.

An explanation for fall in Glutathion Reductase activity would be the inhibitory effect of oxidants on the activity of reducing enzymes (Zigman, 1980). For the same reason GLUTATHION REDUCTASE is an –SH dependent enzyme. Since photo or chemical oxidation of specific amino acids (i.e. Tryptophane) can react with proteins and GSH – SH Glutathion Reductase groups, it may be postulated that the oxidative loss of Glutathion Reductase activity is due to tying – up of essential –SH Glutathion Reductase groups in the enzyme (Zigman, 1980). Photo oxidation is more in India due to sun hours and tropical regions. Nevertheless protein disulphide bonds still accumulate slowly. Perhaps, this indicates that the GLUTATHION REDUCTASE system cannot quite cope with the rate of oxidation in the cataractous lens (Pandya A.V, 2007). Decreases activity of GLUTATHION REDUCTASE could affect two major constituent of the lens – proteins and GSH, leading to the accumulation of GSSG, and H<sub>2</sub>O<sub>2</sub> which is toxic to the lens as they are strong oxidative substance.

### Protein profiles of lens

The average values for total protein (TP), soluble protein (SP) and insoluble protein (ISP) in normal and cataractous human lenses are shown in table-2. It shows significant differences between normal and cataractous lens. There is significant increase in the level of ISP in

cataractous condition where as significant decrease in the level of SP in cataractous lens compared to normal lens.

The change in amount of TP is negligible. With reference to TP there is insignificant difference between normal and cataractous lenses. The percentage of SP in normal and cataractous lenses are 79.19% and 35.56% respectively, whereas that of ISP are 20.8 and 64.30 respectively compared to total protein. It shows increase in the level of ISP during cataractous condition. Significant difference exists between these two parameters in normal and cataractous lenses. All values are expressed as mean  $\pm$  s.e . and p-value is less than 0.01.

The changes leading to the production of these modified proteins isolated from the cataractous and normal human lens and the relationship between these is discussed here.

Many of the changes observed during all types of nuclear cataract. Opecification and aggregate formation of proteins during cataractous condition could be due to oxidation (Pandya A.V.,2010). Examples of such changes would be methionine sulphoxide and disulphide bond formation in the proteins. This will lead young scientist to consider are due to the oxidative modification of proteins in the nucleus of the lens. It has long been believed that oxidation is involved in many types of cataract.

	<b>TABLE-1</b>	
<b>Type of Lens</b>	<b>Normal</b>	<b>Catractous</b>
<b>Enzymes</b>	54 $\pm$ 11 Yrs.	58 $\pm$ 14 Yrs.
<b>GST</b> (n moles/min/mg)	1.780 $\pm$ 0.069 (n = 20)	0.545 $\pm$ 0.342 (n = 45)
<b>Y-GTP</b> (n moles/min/mg)	9.595 $\pm$ 0.094 (n = 22)	3.70 $\pm$ 0.216 (n = 39)
<b>GR</b> (n moles/min/mg)	1.463 $\pm$ 0.079 (n = 22)	0.730 $\pm$ 0.062 (n = 42)

- -----All values are expressed as mean  $\pm$  S.E.
- ▶ Numbers in the parenthesis are sample sizes (n).
- ▶ For all p-value < 0.01

**Table-2.Total protein , soluble protein and insoluble protein content in normal and cataractous human lenses**

Type	Total protein (ug/mg)	Soluble protein (ug/mg)	Insoluble protein (ug/mg)
Normal (n = 28) 60 ± 10 Yrs.	382.09 ± 29	302.60 ± 26	79.48 ± 7
Cattractous (n = 48) 58 ± 12 Yrs.	402.15 ± 31	143.03 ± 14	258.59 ± 21

-----► All values are expressed as mean ± S.E.

-----► n = sample sizes.

-----► For all p-value < 0.01

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