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Ascorbate Free Radical Reductase Activity in Vertebrate Lenses of Certain Species

Shuji Matsukura*, Masayasu Bando*,
Hajime Obazawa*, Mikako Oka† and Makoto Takehana†

*Department of Ophthalmology, Tokai University School of Medicine, Isehara, Japan; †Kyoritsu College of Pharmacy, Tokyo, Japan

Purpose: To clarify the function of ascorbate free radical (AFR) reductase in the antioxidation system of different vertebrate lenses.

Methods: The soluble and insoluble fractions were prepared from bullfrog, guinea pig, rat, rabbit, swine, and bovine lenses, and membrane-bound enzymes in the insoluble fraction were extracted by 0.3% Triton X-100. Ascorbate free radical reductase and diaphorase activities in each fraction were determined.

Results: Ascorbate free radical reductase activity in the lens soluble fraction was the highest in the bullfrog. That in the guinea pig and rabbit was at the next level. There was only a little activity in rat and swine lenses, and none was detected in the bovine lenses. However, a large species difference in AFR reductase activity was not observed in the 0.3% Triton X-100 extracts. Diaphorase activity was three to nine higher than AFR reductase activity in the soluble fractions of bullfrog, guinea pig, and rabbit. In the 0.3% Triton X-100 extracts of all animal species used, it was very high, 108 to 311 times the AFR reductase activity.

Conclusion: These results indicate that the lens soluble and membrane-bound AFR reductase in the different animals may be individual enzyme molecules and have different antioxidative functions. Because the lenses of bullfrog, guinea pig, and rabbit are known to contain a near-ultraviolet (UV) light-absorbing compound, reduced pyridine nucleotide, at a high concentration, the soluble AFR reductase activity is expected to be high in the vertebrate lenses with a near-UV light filter, to enhance the antiphoto-oxidation capacity of ascorbate. **Jpn J Ophthalmol 2001;45:233–239** © 2001 Japanese Ophthalmological Society

Key Words: Antioxidation, ascorbate free radical reductase, lens membrane fraction, lens soluble fraction, near-UV light filter.

Introduction

The lens is maintained in a very high reductive state, and oxidation of the lens constituents is known to be an early event in the development of cataracts.[1] In the lens, there exist such antioxidation systems as ascorbate redox cycle[2] and glutathione redox cycle,[3] and a decrease in the activity of such antioxidation systems appears to be closely associated with lens oxidation. However, the lens antioxidation systems are not yet fully understood.

Bando and Obazawa[2,4] have reported previously that ascorbate free radical (AFR) reductase is a key enzyme in ascorbate regeneration in the human lens, and that the reductase also exhibits diaphorase activity (reduced nicotinamide adenine dinucleotide [NADH]-dependent reduction activity of oxidants such as ferricyanide and dichlorophenolindophenol). A decline in AFR reductase and diaphorase activity is closely correlated with lens protein aggregation in age-related cataractogenesis and aging.[5,6] In the human lens, about 80% of AFR reductase activity[2,7] and about 60% of diaphorase (ferricyanide reductase) activity[6,7] are extracted in the soluble fraction. We have recently found that in the bovine lens, little AFR reductase activity and only 30% of diaphorase

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Correspondence and reprint requests to: Shuji MATSUKURA, MD, Department of Ophthalmology, Tokai University School of Medicine, Isehara-shi, Kanagawa-ken, 259-1193, Japan

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activity are detected in the soluble fraction,[8] although we have succeeded in extracting membrane-bound AFR reductase activity from the bovine lens insoluble fraction by 0.3% Triton X-100.[8] Ascorbate in the bovine lens soluble fraction may be maintained predominantly in the reduced state by sulphydryl groups, such as glutathione. Thus, it is presumed that there may be some species difference in AFR reductase activity between different animal lenses.

The present paper reports soluble and membrane-bound AFR reductase activities in bullfrog, guinea pig, rat, rabbit, swine, and bovine lenses, and shows that the soluble AFR reductase activity tends to be higher in lenses with the function of near-ultraviolet (UV) light filter.

Materials and Methods

Lenses

Lenses from six vertebrate species were used in this study; bullfrog (24 lenses), Hartley guinea pig (10 lenses), Wistar rat (28 lenses), Japanese albino rabbit (10 lenses), swine (4 lenses), and bovine (4 lenses). Ages of the animals used, except rats (about 8 weeks old) and rabbits (about 6 months old), were uncertain, but all of them seemed to be young, based on their soluble lens protein content (Table 1). Swine and bovine eyes were obtained from a local slaughterhouse, and other vertebrate eyes were freshly enucleated from laboratory animals euthanized with overdoses of anesthetics. Each lens was excised from the enucleated eye by a posterior approach, and kept frozen in a closed container at −80°C until used. Wet weights of swine and bovine lenses were measured at the lens extraction, and those of other animal lenses done immediately after thawing when used in experiments. All animal procedures were in accordance with the ARVO resolution on animals and ophthalmic research.

Preparation of the Lens Soluble and Insoluble Fractions

Preparation of the lens soluble and insoluble fractions, and of 0.3% Triton X-100 extracts in the insoluble fraction were carried out at 0–4°C. Each lens was homogenized with about 10 times its weight of 0.1 M KCl, 10 mM K-phosphate (pH 7.2) in a glass homogenizer, and then, soluble and insoluble fractions were separated from the homogenate by centrifugation at 15,000 × *g* for 1 hour. In the preparation of 0.3% Triton X-100 extracts, the insoluble fraction was washed once with about three times lens weight of the above phosphate buffer, and the suspension was centrifuged at 20,000 × *g* for 30 minutes. The resulting precipitate was suspended with about two times lens weight of 0.3% Triton X-100 (in 0.1 M KCl, 10 mM K-phosphate, pH 7.2). After standing for 10 minutes, the suspension was centrifuged at 20,000 × *g* for 30 minutes, and the supernatant thus obtained was designated as membrane-bound enzyme extracts. In this membrane-bound enzyme extraction, Triton X-100 was used at an adequate concentration of 0.3%, because at a higher concentration this detergent inactivated appreciably (more than 20%) the ascorbate oxidase added in the AFR reductase activity assay.

Assays of AFR Reductase and Diaphorase Activities

Ascorbate free radical reductase and diaphorase (ferricyanide reductase) activity were spectrophotometrically determined by measuring the oxidation rates of 100 μM NADH at 340 nm in the presence of 4.5 μM AFR (generated by 1 mM ascorbate plus 0.12 unit/mL ascorbate oxidase) and of 150 μM ferricyanide, respectively, as described previously.[4,6] The 1% Triton X-100 was added in the measurement of diaphorase activity. A final concentration of Triton X-100 was 0.075 or 0.15% in the AFR reductase assay for 0.3% Triton X-100 extracts in the insoluble fraction, but no difference in activity was observed between these low concentrations of the detergent. The time between slaughter and removal of swine and bovine lenses was 2 to 5 hours, longer than that of other animals, but it was proved using the rabbit lens that AFR reductase and diaphorase activities in the lens soluble and insoluble fractions did not change during this time period. Protein was assayed by the bicinchoninic acid method[9] using bovine serum albumin as the standard.

Results

AFR Reductase Activity in Different Vertebrate lenses

Ascorbate free radical reductase activity per milligram soluble protein in the lens soluble fraction was highest in the bullfrog (Table 1). Activity in guinea pig and rabbit was at the next level, there was only a little activity in rat and swine, and none was detected in the bovine lens soluble fraction. The AFR reductase activity was also detected in 0.3% Triton X-100 extracts (membrane-bound enzyme extracts) of the lens insoluble fraction of every species used (Table 2), although the lens insoluble fraction itself could not be assayed for reductase activity because of its

Table 1. Ascorbate Free Radical (AFR) Reductase and Diaphorase Activities in Soluble and Insoluble Fractions of Different Vertebrate Lenses

Species	Proportion of Soluble Protein (%)	Soluble Fraction		Insoluble Fraction
		AFR Reductase Activity*	Diaphorase Activity*	Diaphorase Activity†
Bullfrog	92.5 ± 0.4 (n = 4)	2.56 ± 0.41 (n = 6)	7.95 ± 0.28 (n = 6)	45.2 ± 4.4 (n = 6)
Guinea pig	94.4 ± 0.2 (n = 4)	0.90 ± 0.02 (n = 6)	8.53 ± 0.64 (n = 6)	261.9 ± 23.7 (n = 6)
Rabbit	93.6 ± 0.9 (n = 8)	0.85 ± 0.22 (n = 12)	3.79 ± 0.69 (n = 12)	40.4 ± 19.0 (n = 8)
Rat	78.0 ± 0.9 (n = 4)	0.05 ± 0.01 (n = 6)	1.47 ± 0.17 (n = 8)	15.1 ± 0.8 (n = 6)
Swine	92.8 ± 0.3 (n = 8)	0.02 ± 0.01 (n = 8)	6.07 ± 1.28 (n = 8)	168.3 ± 9.3 (n = 8)
Bovine	88.4 ± 0.5 (n = 8)	null	2.78 ± 0.44 (n = 8)	53.0 ± 3.4 (n = 8)

*nmol reduced nicotinamide adenine dinucleotide (NADH) oxidized·min^{-1}·mg^{-1} soluble protein.
†nmol NADH oxidized·min^{-1}·mg^{-1} insoluble protein.

strong turbidity. Specific activity per milligram extracted protein of the membrane-bound AFR reductase was comparable to the soluble reductase activity in that bullfrog, and was almost at the same level in bullfrog, rat, rabbit, and bovine lens insoluble fractions, but a little higher in guinea pig and swine.

In this study, 78.0–94.4% of total lens protein was soluble in the vertebrate lenses used (Table 1). The percentage of insoluble protein was small (5.6% in guinea pig to 22.0% in rat), and moreover, protein from the insoluble fraction extracted by 0.3% Triton X-100 was only 3.6% (rat) to 13.9% (guinea pig) of total insoluble protein (Table 2). Accordingly, AFR reductase activity per milligram lens protein in the soluble fraction and in 0.3% Triton X-100 extracts of each animal lens was calculated from the results of Tables 1 and 2 based on total protein content. The activity in the soluble fraction changed little after the calculation, but the activity of the membrane-bound reductase was greatly diminished in every species (Figure 1). The lens membrane-bound AFR reductase is thought to reduce only AFR generated locally around the lens cell membrane.

Diaphorase Activity in Different Vertebrate Lenses

High diaphorase activity was detected in the lens soluble and insoluble fractions of every species (Table 1). Particularly, 0.3% Triton X-100 extracts in

Table 2. Ascorbate Free Radical (AFR) Reductase and Diaphorase Activities in 0.3% Triton X-100 Extracts of Insoluble Fractions of Different Vertebrate Lenses

Species	Rate of Protein Extraction (%)	AFR Reductase Activity*	Diaphorase Activity*
Bullfrog	11.1 ± 0.2 (n = 8)	2.53 ± 0.05 (n = 6)	273.1 ± 3.7 (n = 6)
Guinea pig	13.9 ± 0.2 (n = 8)	8.61 ± 0.56 (n = 6)	1953.4 ± 65.4 (n = 6)
Rabbit	9.6 ± 1.9 (n = 16)	2.64 ± 0.30 (n = 8)	362.7 ± 91.3 (n = 8)
Rat	3.6 ± 0.3 (n = 8)	1.05 ± 0.25 (n = 6)	326.1 ± 24.7 (n = 6)
Swine	10.3 ± 0.9 (n = 16)	6.57 ± 0.55 (n = 8)	1427.2 ± 93.7 (n = 8)
Bovine	9.8 ± 0.3 (n = 16)	1.71 ± 0.05 (n = 8)	463.4 ± 8.6 (n = 8)

*nmol reduced nicotinamide adenine dinucleotide (NADH) oxidized·min^{-1}·mg^{-1} extracted protein.

Jpn J Ophthalmol
Vol 45: 233–239, 2001

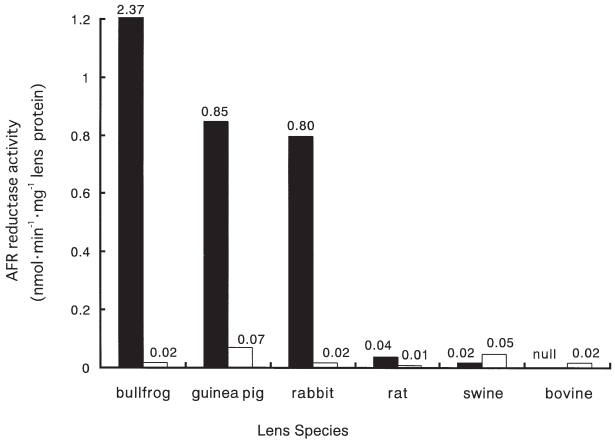

Figure 1. Ascorbate free radical (AFR) reductase activity (nmol NADH oxidized·min^{-1}·mg^{-1} lens protein), calculated from data of Tables 1 and 2 based on total protein content, in soluble fraction (■) and 0.3% Triton X-100 extracts (□) of different vertebrate lenses.

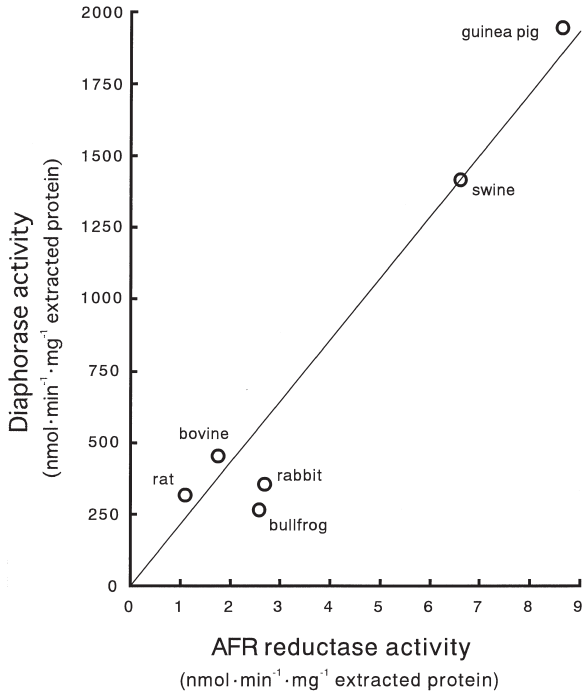

Figure 2. Linear correlation between ascorbate free radical (AFR) reductase and diaphorase activities in 0.3% Triton X-100 extracts of insoluble fractions of different vertebrate lenses. Y = 215.145X (R^2 = 0.977, $P < .0001$).

the insoluble fraction had much higher localized diaphorase activity (Table 2). It was estimated from the protein extraction rate of Table 2 that a large portion (66.4% in bullfrog to 104.6% in guinea pig) of diaphorase activity in the insoluble fraction was extracted by 0.3% Triton X-100.

In the Triton X-100 extracts of every animal used, the activity ratio of diaphorase to AFR reductase was very high, 108 to 311 (Table 3), and there was a significantly linear correlation in enzyme activity (Figure 2); whereas, such correlation was not observed in the lens soluble fraction. However, in the soluble fractions of bullfrog, guinea pig, and rabbit lenses with higher AFR reductase activity, the enzyme activity ratio was 3 to 9 (Table 3). Our previous paper[4] reported that the activity ratio was 3 to 6 also in the soluble AFR reductase partially purified from the human lens soluble fraction by DEAE-cellulose ion-exchange and 5′AMP-Sepharose 4B affinity column chromatography. These results suggest that diaphorase activity in the lens soluble AFR reductase is much lower compared to that in the membrane-bound enzyme fraction, and that the lens soluble reductase and the membrane-bound AFR reductase may be individual enzyme molecules.

Table 3. Activity Ratio of Diaphorase to Ascorbate Free Radical (AFR) Reductase in Different Vertebrate Lenses

	Activity Ratio (Diaphorase/AFR Reductase)	
Species	Soluble Fraction	0.3% Triton X-100 Extracts
Bullfrog	3	108
Guinea pig	9	227
Rabbit	4	137
Rat	29	311
Swine	304	217
Bovine	–	271

Discussion

Ascorbate free radical reductase is extensively present in various tissues of animals and plants,[10,11] and in the liver and adrenal gland, it is distributed mainly in the membrane part of mitochondria and microsomes.[11] Recently, it has been reported that AFR reductase activity is also found in the liver plasma membrane.[12] Our present study (Table 2) indicates that in every animal lens examined here, membrane-bound AFR reductase can be extracted from the insoluble fraction by 0.3% Triton X-100. The lens, except for the epithelial cells, loses a large part of intracellular organelles such as nucleus and mitochondria, and a number of fragments of the plasma membrane are primarily contained as membranous components, in the lens insoluble fraction.[7,13,14] As mentioned in Results, 70–100% of diaphorase ac-

tivity in the lens insoluble fraction is extracted by 0.3% Triton X-100. Therefore, it is supposed that most AFR reductase activity in the lens insoluble fraction is extracted from the fragments of plasma membrane by the detergent action of the 0.3% Triton.

NADH-cytochrome b_5 reductase has been reported to be involved in membrane-bound AFR reductase activity of either mitochondria,[15] microsomes,[16] or plasma membrane.[17] Bando et al[18] have also reported that the kinetic properties of diaphorase activity in the human lens insoluble fraction are similar to those of NADH-cytochrome b_5 reductase. Isolated NADH-cytochrome b_5 reductase itself has a very high activity of diaphorase (ferricyanide reductase),[19] and activity to reduce AFR as well.[20] Recently, however, Villalba et al[17] have described that an essential electron transport requiring NADH-cytochrome b_5 reductase and coenzyme Q increases AFR reductase activity in the liver plasma membrane. They[17] have further suggested that this plasma membrane electron transport system maintains coenzyme Q and vitamin E in reduced states to protect against membrane lipid peroxidation. From our present study, it can be hypothesized that the lens plasma membrane also possesses such an antioxidation electron transport system including NADH-cytochrome b_5 reductase. Because membrane-bound AFR reductase activity in the lens is a little higher in guinea pig and swine than in bullfrog, rabbit, rat, and bovine (Table 2), the lens plasma membrane electron transport may be more active in the former species, but it is unknown why the activity is significantly different among the species. The reason remains to be investigated in conjunction with the elucidation of the functions and components of the antioxidation electron transport system in the lens plasma membrane.

When ascorbate is oxidized, AFR is formed first, and then dehydroascorbate is generated spontaneously by dismutation of AFR. Ascorbate in animal tissues can be regenerated from AFR by AFR reductase, and/or from dehydroascorbate by the nonenzymatic reaction with sulphydryl groups.[2] So far, soluble AFR reductase is seldom detected in animal tissues other than the lens. In the lens, the membrane-bound AFR reductase seems to be active only locally around the cell membrane. Therefore, most of the ascorbate in the lens soluble fraction has to be maintained in the reduced state by soluble AFR reductase and/or sulphydryl compounds, such as glutathione. Our present study shows that there is a large species difference in soluble AFR reductase activity among vertebrate lenses. The soluble enzyme activity is high in the lenses of bullfrog, guinea pig, and rabbit, low in rat and swine, and not detected in bovine lenses (Table 1 and Figure 1). Judging from the activity ratio of diaphorase to AFR reductase (Table 3), AFR reductase activity in the lens soluble fraction of the former three species may be mainly due to an enzyme different from NADH-cytochrome b_5 reductase (with a very high diaphorase activity), which may be involved in the membrane-bound AFR reductase activity and can be more or less responsible for the slight activity in the lens soluble fraction of the latter species. Bando et al[4,21] have reported previously that a major part of the AFR reductase is partially purified, about 50-fold, from the human lens soluble fraction by DEAE-cellulose ion-exchange, 5′AMP-Sepharose 4B affinity and Sephacryl S-200 HR gel filtration column chromatography. Enzymological properties of the soluble enzyme clearly differ from those of NADH-cytochrome b_5 reductase itself. The analysis of a partial amino acid sequence of the purified soluble AFR reductase is currently under investigation.

Ages of animals should be investigated as one of the factors involved in the species difference in lens AFR reductase activity. Rats about 8 weeks old, and rabbits about 6 months old were used in the present study. Other animals used in this study also seemed to be young, judging from their soluble lens protein content (Table 1). As a preliminary investigation of the aging factor, we have determined AFR reductase and diaphorase activity in the lens of a 20-month-old rabbit (data not shown). Ascorbate free radical reductase and diaphorase activity (per milligram extracted protein) in 0.3% Triton X-100 extracts of the lens insoluble fraction of a 20-month-old rabbit were reduced to about one half the activity of the lens of a 6-month-old rabbit. However, there was little difference in the enzyme activity (per milligram soluble protein) of the lens soluble fraction between the rabbits in these age groups. At least in the rabbit lens soluble fraction, therefore, age-related changes in enzyme activity are negligible during the 6- to 20-month period. The effect in other animal lenses and more details about the rabbit lens are still unknown.

Our previously reported AFR reductase activity[21,22] in the soluble fraction of the aged and cataractous human lens cortex is nearly at the same level as or at a little higher level than that (Table 1) of the bullfrog lens, which had the highest activity in this study. van Heyningen[23] has reported that the human lens contains a particular soluble glucoside of 3-hydroxykynurenine at a high concentration of 1–2 mM (roughly equal to the ascorbate content[24,25]), and that this compound absorbs near-UV light (300–400 nm), maximally at

Jpn J Ophthalmol
Vol 45: 233–239, 2001

around 365 nm, and is thought to have the function of near-UV light filter. In addition, it has been reported that almost 100% of UV light below about 300 nm is absorbed by the cornea and aqueous humor,[26,27] and that a high concentration of ascorbate in the anterior eye segment such as in the corneal epithelium and aqueous humor appears to act as UV light filter in the region of about 200–300 nm.[28] In the bullfrog, guinea pig, and rabbit lenses, 3-hydroxykynurenine glucoside is absent. However, these animal lenses contain another near-UV light absorbing compound, NADH or NADPH (with an absorption maximum at around 340 nm) at a high concentration of 0.4–1 mM,[29–31] and among the three animal lenses, the reduced pyridine nucleotide concentration is reported to be the highest in the bullfrog lens with the highest activity of soluble AFR reductase.[31] In the lenses of such animals and including the human lens, which contains a near-UV light absorbing compound at a high concentration, the light absorption may be accompanied by formation of various free radicals; ascorbate scavenges the free radicals, and the AFR generated is reduced back efficiently and rapidly to ascorbate by the lens soluble AFR reductase. Thus, it is suggested that these animal lenses can play the role of near-UV light filter without suffering light damage by the efficient ascorbate regeneration system.

The level of soluble AFR reductase activity in the rat, swine and bovine lenses is very low, as shown in Table 1 and Figure 1 of this study. Because the corneas of swine and bovine are thick, wavelengths of near-UV light reaching the animal lenses are assumed to be significantly longer than 300 nm, and the lenses may not be required to contain a near-UV light-absorbing compound at a high concentration. The possibility should also be considered that in the swine and bovine lenses, the antioxidation system of SH compounds such as glutathione may be active enough to protect them from photooxidation. We will investigate these possibilities in the near future. On the other hand, the cornea of the rat is thin, and UV light below 300 nm can reach the lens. However, the rat is nocturnal, not exposed to much UV light, and even the lens ascorbate content is low.[24,25] Therefore, it is thought that the anti-photooxidation system in the rat eye, including the lens, does not need to be active.

In conclusion, our investigation suggests that the vertebrate lens has two types of soluble and membrane-bound AFR reductase, which are individual enzyme molecules and have different antioxidative functions. Particularly, the soluble AFR reductase seems to be closely associated with the lens, and its activity may be high in the lenses with the function of near-UV light filter, so that it enhances the antiphotooxidation capacity of ascorbate.

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References

1. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J 1995;9:1173–82.
2. Bando M, Obazawa H. Ascorbate free radical reductase and ascorbate redox cycle in the human lens. Jpn J Ophthalmol 1988;32:176–86.
3. Reddy VN, Giblin FJ. Metabolism and function of glutathione in the lens. In: Nugent J, Whelan J, eds. Human cataract formation (Ciba Foundation Symposium 106). London: Pitman, 1984:65–87.
4. Bando M, Obazawa H. Ascorbate free radical reductases and diaphorases in soluble fractions of the human lens. Tokai J Exp Clin Med 1995;20:215–22.
5. Bando M, Obazawa H. Activities of ascorbate free radical reductase and H_2O_2-dependent NADH oxidation in senile cataractous human lenses. Exp Eye Res 1990;50:779–84.
6. Matsukura S, Bando M, Obazawa H. Ferricyanide reductase activity in cataractous human lens. Ophthalmic Res 1996; 28(Suppl 2):11–5.
7. Bando M, Obazawa H, Ishii Y. Reduced nicotinamide adenine dinucleotide-dependent reductase in the human lens. Atarashii Ganka (J Eye) 1993;10:483–7.
8. Matsukura S, Bando M, Obazawa H. Ascorbate free radical reductase activities in soluble and plasma membrane fractions of the lens. Atarashii Ganka (J Eye) 1999;16:383–6.
9. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85.
10. Arrigoni O, Dipierro S, Borraccino G. Ascorbate free radical reductase, a key enzyme of the ascorbic acid system. FEBS Lett 1981;125:242–4.
11. Diliberto EJ Jr, Dean G, Carter C, Allen PL. Tissue, subcellular, and submitochondrial distributions of semidehydroascorbate reductase: possible role of semidehydroascorbate reductase in cofactor regeneration. J Neurochem 1982;39:563–8.
12. Villalba JM, Navarro F, Córdoba F, et al. Coenzyme Q reductase from liver plasma membrane: purification and role in trans-plasma-membrane electron transport. Proc Natl Acad Sci USA 1995;92:4887–91.
13. Alcala J, Maisel H. Biochemistry of lens plasma membranes and cytoskeleton. In: Maisel H, ed. The ocular lens: Structure, function, and pathology. New York: Marcell Dekker, 1985: 169–222.
14. Furukawa K, Iwata S. Maku tanpakushitsu (Membrane proteins). In: Iwata S, ed. Suishoutai—Sono Seikagakuteki Kikou (The lens—Its biochemical mechanism). Tokyo: Medikaru Aoi Shuppan, 1986:104–11.
15. Ito A, Hayashi S, Yoshida T. Participation of a cytochrome b_5-like hemoprotein of outer mitochondrial membrane (OM

cytochrome b) in NADH-semidehydroascorbic acid reductase activity of rat liver. Biochem Biophys Res Commun 1981; 101:591–8.

16. Hara T, Minakami S. On functional role of cytochrome b_5. II. NADH-linked ascorbate radical reductase activity in microsomes. J Biochem 1971;69:325–30.
17. Villalba JM, Navarro F, Gómez-Díaz C, Arroyo A, Bello RI, Navas P. Role of cytochrome b_5 reductase on the antioxidant function of coenzyme Q in the plasma membrane. Mol Aspects Med 1997;18(Suppl):s7–13.
18. Bando M, Matsukura S, Obazawa H. Ferricyanide reductase activity in the soluble and plasma membrane fractions of the human lens. Atarashii Ganka (J Eye) 1994;11:1124–6.
19. Kitajima S, Yasukochi Y, Minakami S. Purification and properties of human erythrocyte membrane NADH-cytochrome b_5 reductase. Arch Biochem Biophys 1981;210:330–9.
20. Iyanagi T, Yamazaki I, Anan KF. One-electron oxidation-reduction properties of ascorbic acid. Biochim Biophys Acta 1985;806:255–61.
21. Bando M, Obazawa H. Soluble ascorbate free radical reductase in the human lens. Jpn J Ophthalmol 1994;38:1–9.
22. Bando M, Obazawa H. Regional and subcellular distribution of ascorbate free radical reductase activity in the human lens. Tokai J Exp Clin Med 1991;16:217–22.
23. van Heyningen R. The glucoside of 3-hydroxykynurenine and other fluorescent compounds in the human lens. In: Elliott K, Fitzsimons DW, eds. The human lens—In relation to cataract (Ciba Foundation Symposium 19). Amsterdam: Elsevier, Excerpta Medica, North-Holland, 1973:151–71.
24. Heath H. The distribution and possible functions of ascorbic acid in the eye. Exp Eye Res 1962;1:362–7.
25. Varma SD, Chand D, Sharma YR, Kuck JF Jr, Richards RD. Oxidative stress on lens and cataract formation: role of light and oxygen. Curr Eye Res 1984;3:35–57.
26. Kinsey VE. Spectral transmission of the eye to ultraviolet radiations. Arch Ophthalmol 1948;39:508–13.
27. Boettner EA, Wolter JR. Transmission of the ocular media. Invest Ophthalmol 1962;1:776–83.
28. Brubaker RF, Bourne WM, Bachman LA, McLaren JW. Ascorbic acid content of human corneal epithelium. Invest Ophthalmol Vis Sci 2000;41:1681–3.
29. Giblin FJ, Reddy VN. Pyridine nucleotides in ocular tissues as determined by the cycling assay. Exp Eye Res 1980;31:601–9.
30. Stewart A, Augusteyn RC. Pyridine nucleotides in normal and cataractous human lenses. Exp Eye Res 1984;39:307–15.
31. Zigler JS Jr, Rao PV. Enzyme/crystallins and extremely high pyridine nucleotide levels in the eye lens. FASEB J 1991; 5:223–5.