Betaine-homocysteine Methyltransferase Is a Developmentally Regulated Enzyme Crystallin in Rhesus Monkey Lens*

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We describe herein the characterization of a major 45-kDa protein from the soluble βH-crystallin fraction of rhesus monkey (Macaca mulatta) lens. Based on partial peptide sequence, immunoreactivity, and enzymatic activity, this protein has been identified as betaine-homocysteine S-methyltransferase (BHMT; EC 2.1.1.5), an enzyme that catalyzes the methylation of homocysteine using either betaine or thetins as methyl donors. This protein was found to be expressed abundantly in the nuclear region of the monkey lens, reaching ~10% of the total nuclear protein, but was barely detectable in the epithelium and cortex regions of the lens. Because the nucleus represents the early embryonic and fetal stages of lens development, we infer that BHMT expression in the lens of the eye is developmentally regulated. By virtue of its high abundance, BHMT can be considered an enzyme crystallin (ψ-crystallin). This is the first enzyme crystallin to be found in primate lenses.

Crystallins, the major structural proteins of the eye lens, are the primary determinants of the refractive properties of this tissue. There are two major classes, the ubiquitous crystallins (α and β/γ) present in all vertebrates and the so-called enzyme crystallins, which occur in phylogenetically restricted groups (1, 2). The enzyme crystallins are major soluble lens proteins that are either identical or closely related to metabolic enzymes. More than 10 such proteins have been identified and characterized from a variety of vertebrate species (3, 4). Although it seems that the recruitment of metabolic enzymes as lens crystallins generally occurs through the modification of gene expression without previous gene duplication, very little is known regarding the molecular basis of their high expression in the lens or their selective recruitment to serve as crystallins (2–5).

In this study we have identified and characterized a prominent protein from Rhesus monkey (Macaca mulatta) lens and found it to be identical to betaine-homocysteine S-methyltransferase (BHMT): EC 2.1.1.5). Although expression of this protein was previously reported to be confined to the liver and kidney (6–9), we report here that it is expressed at very high levels in the central (nuclear) region of the monkey lens, tissue laid down during embryonic and fetal development. Because of its high abundance in the monkey lens nucleus, we consider BHMT to be a developmentally regulated enzyme crystallin, which we have named ψ-crystallin.

EXPERIMENTAL PROCEDURES

Fresh rhesus monkey lenses were obtained from 2–3-year-old animals that were used by the vaccine testing service at the Center for Biologics Evaluation and Research, Food and Drug Administration (Bethesda, MD). Human lenses were obtained from the National Disease Research Interchange (Philadelphia, PA), and Sprague-Dawley rats were from Taconic Farms (Germantown, NY). Bovine eyes were from a local slaughterhouse. All lenses were stored at ~70°C until processed.

Tissue Processing—Both monkey and human lenses were microdissected into different layers as described earlier (10). Lenses from different species were homogenized in 50 mM Tris buffer, pH 7.5, containing 1.0 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 M NaCl, and 5 mM 2-mercaptoethanol, and the soluble tissue extracts were obtained by centrifugation (25,000 × g) at 4°C. Protein concentrations were determined by the Bradford assay (11) using bovine serum albumin as standard.

Separation of βH-Crystallin Fraction—The βH-crystallin fraction from monkey lens was prepared by separating the lens soluble homogenate (25,000 × g) on a Superose 12 HR gel filtration column (Amersham Pharmacia Biotech) attached to an Amersham Pharmacia Biotech high performance liquid chromatography system. Crystallin fractions were eluted from the column with 50 mM Tris buffer, pH 7.5, containing 1.0 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 M NaCl, and 5 mM 2-mercaptoethanol at a flow rate of 0.4 ml/min. Fractions (0.8 ml) containing βH-crystallin were pooled and subjected to electrophoretic analysis.

Electrophoresis and Western Blotting—SDS-polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Laemmli (12) using a Hoefer Mighty Small apparatus. Gels contained either 12.5 or 10% acrylamide and were stained with either Coomassie Blue R-250 or Sypro Red. Gels stained with Sypro Red were scanned using a Molecular Dynamics Storm 860 PhosphorImager. To obtain a partial peptide sequence of the major 45-kDa protein from the βH-crystallin fraction of monkey lens, proteins were separated by SDS-PAGE, transferred onto a polyvinylidene difluoride membrane (Applied Biosystems, Foster City, CA), and visualized with Ponceau S. The 45-kDa protein was cut from the blot and sequenced by Harvard Microchemistry Laboratories (Boston, MA). Immunoblots were developed according to the Bio-Rad protocol using an antibody (polyclonal) raised against recombinant human liver BHMT. Transfer of proteins from SDS-PAGE gels to a nitrocellulose membrane was accomplished using a Bio-Rad transfer unit, and protein bands were visualized using a peroxidase-linked second antibody and 4-chloro-1-napthol substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD).

Two-dimensional Electrophoresis—Tissue samples obtained from monkey lenses were subjected to two-dimensional electrophoresis as described earlier (10).

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1 The abbreviations used are: BHMT, betaine-homocysteine S-methyltransferase; PAGE, polyacrylamide gel electrophoresis.

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described earlier (10). Briefly, samples were prepared in 9 m urea containing 2% Nonidet P-40, 2% ampholytes (pH range, 3–10), and 10 mM dithiothreitol. The first dimension, isoelectric focusing, was carried out using Amersham Pharmacia Biotech immobilized nonlinear pH gradients (pH 3–10) and was run for 32,000 V-h. The second dimension was run on 18 × 18-cm, 15–18% acrylamide gradient SDS slab gels using the ISO-DALT system (Hoefer Scientific Instruments). Gels were stained with colloidal Coomassie Blue G.

BHMT Enzyme Assay—BHMT activity was determined as described by Garrow (13). In brief, the assay contained 5 mM N-homocysteine, 2 mM dimethylacetothione (0.8 μCi), a sulfonium analog of betaine, and 50 mM Tris-HCl (pH 7.5). The final reaction volume was 0.5 ml. Reactions were initiated with the addition of tissue extract and carried out for 1–2 h at 37 °C. Reactions were stopped by chilling the tubes in ice water, and reactants were applied to a Dowex 1-X 4 (OH, 200–400 mesh) column (0.6 × 3.75 cm). The unreacted dimethylacetothione was washed from the column with water, and the reaction products, methylyhomocysteine and methionine, were eluted with 1.5 n HCl and quantified by scintillation counting. All samples were assayed in duplicate. BHMT activity is expressed as units per mg of protein. A unit is defined as 1 nmol of methionine produced/h.

Metabolite Analysis—The levels of lens homocysteine, cystathionine, methionine, cysteine, and dimethylglycine were determined by Metabolite Laboratories, Inc. (Denver, CO). These assays were performed using the stable isotope dilution methods described by Stabler et al. (14) and Allen et al. (15). Samples were prepared by homogenizing freshly excised lenses in 10 mM potassium phosphate buffer, pH 7.5, containing 0.14 M NaCl at a concentration of 100 mg of tissue/ml of buffer. The homogenates were quickly frozen and stored at −20 °C until shipped (frozen) to Denver for analysis. Betaine analysis was performed as described by Mar et al. (16) at the Department of Nutrition, University of North Carolina (Chapel Hill, NC). Lens betaine was extracted with MeOH/CHCl₃, derivatized with 4-bromo-phenacyl triflate, and quantified with high-performance liquid chromatography using UV detection.

RESULTS

By chance observation it was noted that rhesus monkey lens contained a water-soluble protein that migrated on SDS-PAGE as a ~45-kDa band. This polypeptide, which was not apparent in samples from lenses of various other species, was found to elute from gel filtration columns with the β-crystallin fraction, indicating a native molecular weight of ~150,000. In an earlier report, Sato et al. (17) had observed the same band in the β-crystallin fraction of rhesus monkey lens and had noted that it did not appear to be a β-crystallin polypeptide; they did not attempt to identify the band.

To identify the polypeptide, the monkey lens β-crystallin fraction was subjected to 10% SDS-PAGE analysis and blotted onto a polyvinylidene difluoride membrane. After visualization by staining with Ponceau S, the 45-kDa band was cut from the blot and sent to the Harvard Microchemistry Laboratories facility for microsequencing. After tryptic digestion, the sequences of two peptides totaling 33 residues were obtained, and these sequences were subjected to a BLAST search against the NCBI protein data bank to detect sequence homology with known proteins. Both peptide sequences were found to be 100% identical to the sequence of human liver BHMT.

Fig. 2A depicts the lens soluble protein profiles from different species as seen on SDS-PAGE. The monkey lens homogenate showed a distinct and prominent band in the range of 45 kDa, which was not apparent in the other species with the exception of two monkey β-crystallin bands in the middle of the diagram. The Coomassie Blue staining. Densitometric analysis indicated that the 45-kDa band accounted for 0.5–1% of total monkey lens protein. Western blot analysis using polyclonal antibody raised against human recombinant BHMT demonstrated strong and specific reactivity with the monkey sample only (Fig. 2B).

BHMT enzyme activity was determined on whole lens extracts from several species using an assay described by Garrow (13). The BHMT activity of monkey lens was extremely high relative to that of all other species tested. In fact, guinea pig, bovine, monkey, and human, respectively. Positive immunoreactivity to BHMT was seen only in the monkey lens homogenate. The lens grows by adding layers of cells at the periphery, in a manner analogous to the growth rings of a tree; thus the oldest cells are at the center, or nucleus, and the tissue becomes progressively younger moving from the center to the lens periphery (cortex). It is thus possible to analyze tissue from different lens layers representing different stages of lens development and different tissue age. In contrast to similar dissection of human lenses, clear demarcation of cortex and the various nucleus zones was not evident in the monkey lenses; thus the layers dissected are simply concentric layers of progressively older cells as one moves from the epithelium to the central nucleus. The distribution of BHMT protein and activity was determined for different layers of the monkey lens. Freshly obtained monkey lenses were microdissected into epithelium/capsule and six or seven other layers of lens fibers in a concentric fashion from the lens periphery to its center (10). Fig. 3 illustrates the SDS-PAGE analysis of soluble homogenates prepared from one set of such samples. The 45-kDa protein was found to be strikingly concentrated in the most central regions of the lens. It was not detectable by Coomassie Blue staining in

![Fig. 1. Alignment of partial amino acid sequences of two peptides from 45-kDa protein of monkey lens. Both sequences were 100% identical to the sequence of human liver BHMT.](image)

![Fig. 2. SDS-polyacrylamide gel electrophoresis of lens soluble proteins from different species (A) and a Western blot of the same samples probed with antiserum to recombinant human liver BHMT (B). Lanes 1–5 represent lens soluble proteins from rat, guinea pig, bovine, monkey, and human, respectively. Positive immunoreactivity to BHMT was seen only in the monkey lens homogenate.](image)
the epithelium or in peripheral layers of cortex (layers 1–3), whereas in layer 4, one can see a prominent 45-kDa band, which increases dramatically in intensity in layers 5 and 6. In layers 5 and 6, this protein band was found to comprise 5 and 10% of total protein, respectively, as determined by densitometric analysis.

To determine whether this 45-kDa band represents a single protein, we also subjected these same fractions to two-dimensional electrophoresis. Fig. 4, A–C, illustrates the two-dimensional patterns of monkey lens proteins taken from outer cortical, outer nuclear, and central nuclear layers, respectively. The proteins present in the bottom half of all three panels are the crystallin polypeptides and are similar in all three samples. Enzymes and other housekeeping proteins are found primarily at higher molecular weights. The location of BHMT is indicated in Fig. 4B. It is clear that the staining at ~45 kDa seen in one-dimensional SDS-PAGE results largely from three closely spaced basic polypeptides. These three spots represent charge variants of BHMT, as confirmed by running purified recombinant human BHMT as a control (results not shown). The relative intensity of these spots is increased in the central nucleus (Fig. 4C), whereas in the outer cortex (Fig. 4A) they are absent, or virtually so.

To correlate BHMT protein distribution with its enzymatic activity, we have also assayed activity in the different layers of another monkey lens. As shown in Fig. 5, activity was distributed in a pattern similar to that found for the protein by electrophoretic analysis, with very high activity in the center of the lens compared with peripheral layers of fibers or with the epithelium. It was of obvious interest to determine the level of BHMT expression in human lens. Initial investigation with two pairs of lenses from adult donor eyes revealed no obvious band on SDS-PAGE at the expected position for BHMT. Analysis for BHMT activity in the same lenses indicated a low but measurable activity. In contrast to the non-primate lenses, in which no activity was detected, the human lenses had activity equal to ~1% that of the monkey lens. After discovery of the developmental regulation of BHMT expression in monkey lens, lenses from four fresh human donor eyes were dissected into nucleus and cortex and assayed for BHMT activity. As shown in Table I, it is apparent that as in the monkey lens, activity is concentrated in the older tissue of the interior region of the lens. This is further demonstrated by the higher activity present in a human fetal lens (~15 weeks of gestation) compared with the adult human lenses assayed.

To determine whether high BHMT expression in animal lenses might result in altered levels of BHMT substrates and products, or other sulfur amino acids, freshly enucleated lenses from rat, bovine, and monkey were extracted and assayed for these metabolites as outlined under “Experimental Procedures.” Among the various metabolites measured (Table II), the level of cystathionine was remarkably different, i.e. 10–20-fold higher, in monkey lens compared with the other species. The levels of homocysteine and dimethylglycine were marginally higher in the monkey.

**DISCUSSION**

This study demonstrates that catalytically active BHMT is present at very high levels in the central region of rhesus monkey lens. At the very center of the lens it represents ~10% of total protein, a value 5-fold higher than that previously reported for pig liver (13). In contrast, in the cortex and epithelium BHMT was not detected on SDS-PAGE analysis, and activity was negligible. Because of its mode of growth, the oldest cells in the lens are at the center, and the youngest are at the periphery. All the cells in the lens are preserved from the embryonic to adult stages, and the central nucleus of the lens contains cells produced during embryonic and fetal development (10). Furthermore, cells in the lens nucleus have terminally differentiated with loss of cell nucleus and other or-
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TABLE I

BHMT activity in human lens

| Age       | Cortex | Nucleus |
|-----------|--------|---------|
| Human lens|        |         |
| 18        | 0.6    | 10.1    |
| 24        | 0.1    | 11.5    |
| 35        | 0.3    | 6.0     |
| 50        | 0.2    | 4.0     |
| Fetal (whole, ~15 weeks) |        | 64.8    |
| Monkey lens| 2–3   | 0.1,024 |

Activity reported as units per mg of protein where 1 unit is defined as 1 nmol of methionine formed/h using dimethylacetothetin as methyl donor substrate.

TABLE II

Lens sulfur metabolite levels

|                | Monkey | Cow  | Rat  |
|----------------|--------|------|------|
| Methionine     | 11.6   | 1.20 | 29.2 |
| Cysteine       | 15.8   | 18.8 | 49.7 |
| Homocysteine   | 4.62   | 2.22 | 1.23 |
| Dimethylglycine| 2.76   | 0.87 | 1.22 |
| Cystathionine  | 21.6   | 1.11 | 2.11 |

Values are expressed as μmol/100-mg lens wet weight.

The vertebrate eye lens is a transparent, avascular structure, the function of which is to refract incident light onto the retina. Its refractive properties are determined by a small number of very abundant proteins called crystallins (1, 18). A protein is generally designated a crystallin if it constitutes ≧5% of the total protein of the lens (1–3). It is now believed that during vertebrate evolution, the composition and properties of eye lenses have been modified by the direct gene recruitment of enzymes and stress proteins as crystallins (1–5). Even the ubiquitously expressed α- and β/γ-crystallins are now known to have arisen from families of stress proteins, presumably quite early in vertebrate evolution (1–5). More than 10 enzyme-crystallins have been identified to date; all seem to be restricted to certain evolutionary lineages (1–5); and it has been presumed that these proteins have been recruited as crystallins more recently during evolution relative to the α- and β/γ-crystallins. Although it has been argued that gene recruitment by the lens is largely a random function of neutral evolution (3, 5), current evidence suggests that it is primarily an adaptive process (2, 4, 19–22).

At present one can only speculate on the selective advantage provided by BHMT. It could be related to its role in methionine and sulfur amino acid metabolism (23–27). The metabolism of sulfur-containing compounds has been shown to be very important in maintaining lens transparency (18, 28, 29). Deficiencies of methionine or glutathione have been shown to be associated with cataract development (28, 30), and the BHMT-catalyzed conversion of homocysteine to methionine is one of the key reactions at a major regulatory locus for methionine metabolism in liver (23–27). Methionine has particular biochemical significance among amino acids because of its function as a constituent of S-adenosylmethionine (23–27). This latter molecule is a key methyl group donor in many biological transmethylation reactions (23, 31). Lens tissue has been shown to have S-adenosylmethionine and its synthesizing enzyme, methionine adenosyltransferase, perhaps indicating the importance of the transmethylation pathway for maintaining lens function (32). The methylolation of homocysteine to methionine has been shown to be catalyzed by two different enzymes in mammalian tissues, namely, methionine synthase (cobalamin-dependent: EC 2.1.1.13) and BHMT (23, 27). The former is distributed throughout most tissues, whereas the latter is largely confined to the liver and kidney (6–9). It has been reported (33) that two distinct enzymes exist in liver, which catalyze methyl transfer to thetins and/or betaine to homocysteine; however, more recent studies have concluded that there is only a single such enzyme (13, 34, 35). Homocysteine is also converted to cysteine through transulfuration catalyzed by...
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Crystallins are of necessity highly stable proteins. BHMT is highly stable to heat, and this property has been exploited by several laboratories as an effective purification step (13, 34, 35, 38). Furthermore, BHMT can be induced by dietary manipulations or by administration of hormones (6, 7, 39, 40). Inducibility has been proposed as an important factor in enzyme crystallin recruitment (3, 5). Whether either of these characteristics is a primary factor in the high expression of BHMT in the developing rhesus monkey lens remains to be determined.

The lens has an exceptionally high protein content, which is critical for maintaining its transparency and for generating its refractive power (1, 18). Furthermore, lens proteins are extremely long-lived and are chronically exposed to oxidative stress (18, 29, 41). Because structural integrity must be maintained throughout life, protection from oxidative damage to the crystallins and other lens constituents is of paramount importance (18, 29, 41). Sulfur-containing reducing compounds such as glutathione and cysteine have been reported to protect the lens from oxidative damage (18, 28, 29). Interestingly, cystathionine, a transsulfuration intermediate, has been reported to be a superoxide radical quencher (42, 43), and under hyperoxia its levels have been shown to be increased significantly in lung tissue (44). In this study, our analysis of metabolites revealed that when compared with cow and rat lenses, monkey lenses contained 10–20-fold higher cystathionine. High cystathionine could be a factor in the resistance of monkey lenses to oxidative damage (45). Cystathionase activity has not been measured in monkey lenses, but in human lens the activity is very low (46). Because cystathionase activity would be expected to influence the level of cystathionine (47), it would be of interest to determine its activity in lenses from other species, in particular rhesus monkey.

In humans, BHMT was localized to chromosome 5q 13.1–15 (9). If there is only one gene in monkey, recruitment by the lens must have resulted from modification of gene expression rather than gene duplication. Analysis of the gene or genes for BHMT in the rhesus monkey, particularly the promoter region, would be very enlightening. The BHMT protein has some characteristics typical of other enzyme crystallins, such as structural stability and inducibility (13). On the other hand, unlike most enzyme crystallins it is not a ubiquitous housekeeping protein, and it is not expressed at detectable levels in lenses of various species. Furthermore, BHMT is not a pyridine nucleotide binding enzyme as are most enzyme crystallins. It should be noted however that earlier workers (48) suggested possible redox regulation of BHMT structure via aggregation through disulfide formation. The presence of numerous redox active sulfhydryl groups might be a factor in lens recruitment. Interestingly, in a series of papers studying the purified horde glucon-homocysteine methylpherase, Cantonis’s laboratory showed that the enzyme undergoes polymerization, which can be reversed by mercaptans (48, 49). The polymerization appeared to be attributable to intermolecular disulfide bonds, because reagents that react with sulfhydryl groups blocked polymerization (48, 49). Furthermore, evidence was presented suggesting that there are three cysteine residues on the surface of the protein that allow it to polymerize in a three-dimensional, versus linear, aggregate form. It is tempting to speculate that perhaps the enzyme we refer to here as BHMT, and Cantonis and colleagues referred to as thetin-homocysteine methylpherase (48, 49), was recruited as an enzyme crystallin because of its ability to form a three-dimensional gel-forming lattice. In fact, Cantonis’s group also reported that concentrated solutions of highly purified thetin-homocysteine methylpherase would form a gel if stored for several days in the absence of a reducing agent (38).

In conclusion, this study reports that BHMT is a catalytically active enzyme crystallin in rhesus monkey lenses, which is developmentally regulated. This is the first enzyme crystallin to be identified in a primate. Interestingly, although BHMT is not present at “crystallin” levels in the human lens, there are measurable levels in contrast to the non-primate species tested, and human lens BHMT expression is developmentally
regulated as in monkey lens. Further studies are required before the functional role of BHMT in primate lens can be elucidated.

REFERENCES

1. Wistow, G., and Piatigorsky, J. (1988) Annu. Rev. Biochem. 57, 479–504
2. Wistow, G. (1993) Trends Biochem. Sci. 18, 301–306
3. De Jong, W. W., Hendriks, W., Mulders, J. W. M., and Bloemendal, H. (1989) Trends Biochem. Sci. 14, 365–368
4. Wistow, G. (1995) Molecular Biology and Evolution of Crystallins: Gene Recruitment and Multifunctional Proteins in the Eye (Molecular Biology Intelligence Service), R. G. Lands, Austin, TX
5. Piatigorsky, J. (1992) J. Biol. Chem. 267, 4277–4280
6. Eriksen, L. E. (1960) Acta Chem. Scand. 14, 2102–2112
7. Finkelstein, J. D., Kyle, W. E., and Harris, B. J. (1971) Arch. Biochem. Biophys. 146, 84–92
8. McKeever, M. P., Wein, D. G., Molloy, A., and Scott, J. M. (1991) Clin. Sci. 81, 551–556
9. Sunden, S. L. F., Renduchintala, M. S., Park, E. I., Mikasz, S. D., and Garrow, T. A. (1997) Arch. Biochem. Biophys. 345, 171–174
10. Garland, D. L., Duglas-Tabor, Y., Jimenez-Asensio, J., Datiles, M. B., and Magno, B. (1996) Exp. Eye Res. 62, 285–291
11. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
12. Loew, U. K. (1970) Nature 227, 680–685
13. Garrow, T. A. (1996) Acta Chem. Scand. A 50, 917–919
14. McKeever, M. P., Weir, D. G., Molloy, A., and Scott, J. M. (1991) Clin. Sci. 81, 1043–1046
15. Allen, R. H., Stabler, S. P., and Lindenbaum, J. (1993) J. Biol. Chem. 268, 14944–14948
16. Mar, M. H. H., Ridky, T. W., Garner, S. C., and Zeisel, S. H. (1995) J. Nutr. 125, 989–997
17. Harding, J. J., and Crabbe, M. J. C. (1984) in The Eye (Davson, H., ed) 3rd Ed. Vol. 1B, pp. 207–492, Academic Press, New York
18. Finkelstein, J. D., and Martin, J. J. (1984) J. Biol. Chem. 259, 9508–9513
19. Reed, V. N., Varma, S. D., and Chakrapani, B. (1973) Exp. Eye Res. 16, 105–114
20. Spector, A. (1985) in The Ocular Lens (Maisel, H., ed) pp 405–438, Marcel Dekker, New York
21. Garland, D. L., Duglas-Tabor, Y., Jimenez-Asensio, J., Datiles, M. B., and Magno, B. (1996) Exp. Eye Res. 62, 285–291
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
23. McKeever, M. P., Wein, D. G., Molloy, A., and Scott, J. M. (1991) Clin. Sci. 81, 551–556
24. Sunden, S. L. F., Renduchintala, M. S., Park, E. I., Mikasz, S. D., and Garrow, T. A. (1997) Arch. Biochem. Biophys. 345, 171–174
25. Garland, D. L., Duglas-Tabor, Y., Jimenez-Asensio, J., Datiles, M. B., and Magno, B. (1996) Exp. Eye Res. 62, 285–291
26. Mar, M. H. H., Ridky, T. W., Garner, S. C., and Zeisel, S. H. (1995) J. Nutr. 125, 989–997
27. Harding, J. J., and Crabbe, M. J. C. (1984) in The Eye (Davson, H., ed) 3rd Ed. Vol. 1B, pp. 207–492, Academic Press, New York
28. Finkelstein, J. D., and Martin, J. J. (1984) J. Biol. Chem. 259, 9508–9513
29. Reed, V. N., Varma, S. D., and Chakrapani, B. (1973) Exp. Eye Res. 16, 105–114
30. Spector, A. (1985) in The Ocular Lens (Maisel, H., ed) pp 405–438, Marcel Dekker, New York
31. Garland, D. L., Duglas-Tabor, Y., Jimenez-Asensio, J., Datiles, M. B., and Magno, B. (1996) Exp. Eye Res. 62, 285–291
32. Bradfords, M. M. (1976) Anal. Biochem. 72, 248–254
33. McKeever, M. P., Wein, D. G., Molloy, A., and Scott, J. M. (1991) Clin. Sci. 81, 551–556