Lens Proteomics: Analysis of Rat Crystallin Sequences and Two-Dimensional Electrophoresis Map

Kirsten J. Lampi, Marjorie Shiib, Yoji Ueda, Thomas R. Shearer, and Larry L. David

PURPOSE. To determine the sequence of four rat β-crystallins, confirm the sequences by mass spectrometry, and produce a two-dimensional electrophoresis (2-DE) map of soluble crystallins in young rat lens.

METHODS. New or additional sequences were determined for βB1, βB3, βA3, and βA4-crystallin cDNAs from Sprague-Dawley rats, and the deduced protein sequences confirmed by mass spectrometry. The identity and relative abundance of each crystallin was then determined by 2-DE of soluble protein from whole lenses of 12-day-old rats, image analysis, and tandem mass spectrometry (MS/MS) spectra of peptides from in-gel digests.

RESULTS. The previously unreported sequence of rat βA4 cDNA encoded a 195-amino-acid protein. Additional cDNA sequencing identified the previously unknown N-terminal sequence of rat βA3, found two differences from the previous amino acid sequences of both rat βB1 and βB3, and detected a polymorphism at residue 54 in rat βB3. These new sequences were then confirmed by whole protein masses and MS/MS spectra of proteolytic digests. 2-DE analysis provided a more detailed map of rat crystallins than previously available and allowed the composition of crystallins in young rat lens to be compared with that in young human lens.

CONCLUSIONS. This report provides baseline data that will facilitate the analysis of posttranslational modifications in rat crystallins during cataract. Detection of a polymorphism in the sequence of rat βB3 suggests that crystallins in humans could also exhibit polymorphisms. The unusual abundance of rat βB3 and low abundance of βB2 may account for the increased susceptibility of rat crystallins to insolubilization during aging and cataract.

Several experimental treatments used to induce cataracts in rats include streptozotocin-induced diabetes, galactose feeding, ionizing radiation, inhibition of cholesterol synthesis, steroid treatment, overdose of selenium, and culture with oxidants or calcium ionophore. In most of these models, covalent modification of crystallins, followed by phase separation of lens cytosol and formation of water-insoluble aggregates, may play important roles in opacification. Some of the modifications detected in rat crystallins that could contribute to insolubilization are mixed disulfide formation; glycation; cross-linking by UV, transglutaminase, or disulfides; phosphorylation; deamidation; and proteolysis.

Two-dimensional electrophoresis (2-DE) and mass spectrometric analysis, tools used in the emerging field of proteomics, hold great promise for determining which crystallin modifications lead to cataract. 2-DE is capable of simultaneously resolving complex mixtures of modified crystallins. These resolved crystallins can then be quantified by image analysis, and posttranslational modifications on excited spots can be determined by mass spectrometry (MS). To perform these studies, it is essential that the complete sequences of crystallins be known so that experimental and calculated masses may be compared. Furthermore, standardized 2-DE maps are required for reference to determine which species represent modified crystallins and which are cataract specific. Therefore, the purpose of this study was to determine the cDNA sequences of several rat β-crystallins that were either unknown or were inconsistent with preliminary mass spectrometric data, to proofread the newly deduced sequences of β-crystallins by measuring masses of whole crystallin subunits and peptide digests, and to produce a standardized 2-DE map of crystallins for young rat lens.

METHODS

Isolation of RNA and Protein from Rat Lens

Lenses for RNA isolation were obtained from 12- to 28-day-old Sprague-Dawley rats (BK International, Fremont, CA). Treatment of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Lens mRNA and total RNA was isolated from dissected tissue, by means of a kit (Fast Track; Invitrogen Life Technologies, Carlsbad, CA) and RNA extraction reagent (TRizol; Invitrogen Life Technologies), respectively. Soluble crystallins were isolated from 12-day-old rats by homogenization, using a ratio of 20 lenses/mL of buffer containing 20 mM phosphate, 1 mM EGTA (pH 7.0), and one freshly added tablet of protease inhibitor/10 mL buffer (Complete Mini Protease Inhibitor Cocktail; Roche Molecular Biochemicals, Indianapolis, IN). The watersoluble and -insoluble fractions were separated by centrifugation at 20,000g for 30 minutes at 4°C. Protein content was measured using the bicinchoninic acid (BCA) assay and bovine serum albumin standard (Pierce, Rockford, IL).

Sequencing of Rat βA4, βA3, βB1, and βB3 cDNAs

After reverse transcription, the cDNA for rat βA4 was amplified by PCR, using both the 3′ and 5′ rapid amplification of cDNA ends (RACE) systems (Invitrogen Life Technologies), as previously described.
Gene specific RACE PCR primer sequences were designed using the previously published sequence of bovine bA4 (GenBank accession no. M60328; GenBank is provided in the public domain by the National Center for Biotechnology, Bethesda, MD, and is available at http://www.ncbi.nlm.nih.gov/genbank) so that the sequence of the 5' and 3' RACE PCR products overlapped. PCR products were then cloned and plasmid DNA isolated as previously described. 18,19 To amplify the unknown 5' end of rat bA3 cDNA, the same procedure described for the 5' RACE of rat bA4 cDNA was used, except a gene-specific primer targeted to nucleotides 134-153 and a nested primer targeted to nucleotides 43-62 of the published 3' sequence of rat bA3 were used (GenBank accession no. X15143).

Preliminary mass spectrometric analysis of rat bB3 and bB1 indicated that the calculated and experimentally measured masses did not match. Gene specific RACE PCR primer sequences were designed using the previously published sequence of bovine bA4 (GenBank accession no. M60328; GenBank is provided in the public domain by the National Center for Biotechnology, Bethesda, MD, and is available at http://www.ncbi.nlm.nih.gov/genbank) so that the sequence of the 5' and 3' RACE PCR products overlapped. PCR products were then cloned and plasmid DNA isolated as previously described. 18,19

To amplify the unknown 5' end of rat bA3 cDNA, the same procedure described for the 5' RACE of rat bA4 cDNA was used, except a gene-specific primer targeted to nucleotides 134-153 and a nested primer targeted to nucleotides 43-62 of the published 3' sequence of rat bA3 were used (GenBank accession no. X15143).

Preliminary mass spectrometric analysis of rat bB3 and bB1 indicated that the calculated and experimentally measured masses did not match.
match. Therefore, cDNAs coding for each protein were sequenced. Standard PCR was performed with primers corresponding to nucleotides 3-22 and 692-709 of βB3 (GenBank accession no. X05899) or with primers corresponding to nucleotides 76-97 (GenBank accession no. M13527) and nucleotides 603-624 of βB1 (GenBank accession no. X05900). The PCR product of rat βB1 cDNA was cloned (Original TA cloning kit; Invitrogen Life Technologies). The PCR product of rat βB3 cDNA was purified (QIAquick PCR Purification kit, Qiagen, Valencia, CA) and directly sequenced, using the same primers as were used for the original PCR. All DNA sequencing was performed by the Oregon Health and Science University Molecular Microbiology and Immunology (OHSU-MMI) Research Core Facility (http://www.ohsu.edu/core).

Isolation of β-Crystallin Subunits

Soluble crystallins were fractionated using a 2.5 x 95-cm column (Sephacryl S-300 HR; Amersham Pharmacia Biotech, Piscataway, NJ) maintained at 4°C. The mobile phase buffer contained 20 mM Tris (pH 7.5), 1.0 mM EGTA, and 100 mM NaCl and flowed at 25 mL/h. Collected peaks of α-, βH-, βL-, and γ-crystallins were then concentrated and desalted by ultrafiltration (YM10 membranes; Millipore, Bedford, MA) and dried by vacuum centrifugation. Individual βH-crystallin subunits were isolated by anion-exchange HPLC using a 7.5 x 75-mm diethylaminoethyl (DEAE) column (5-PW; TosoHaas, Montgomeryville, PA). Before chromatography, βH-crystallin aggregates were denatured and reduced by dissolving in 6 M urea, 10 mM Tris (pH 8.5), 50 mM dithiothreitol (DTT), and incubation at 37°C for 30 minutes. The DEAE column mobile phase contained 6 M urea, 10 mM Tris (pH 8.5), and 2 mM DTT at a 1-mL/min flow rate. Three to 10 mg βH was injected, and after a 15-minute wash, β-subunits were eluted with a 0- to 80-mM NaCl gradient over 100 minutes.

Measurement of Crystallin Subunit Masses

Approximate 5-μg samples of whole α-crystallin aggregate or isolated β-crystallin subunits were injected onto a 0.5 x 150-mm column (C18 Targa; Higgins Analytical, Mountain View, CA), and masses were determined by on-line analysis of eluents by electrospray ionization mass spectrometry (ESIMS) on an iontrap system (model LCQ; ThermoFinnigan, San Jose, CA). The column used a 10-μL/min flow rate and linear gradient of 10% to 75% acetonitrile over 40 minutes in a mobile phase containing 0.1% acetic acid. Mass spectra of proteins eluting from the C18 column were deconvoluted by computer (Xcalibur software with BioWorks; ThermoFinnigan). Mass accuracy of better than 0.02% was confirmed, using horse myoglobin.

Confirmation of β-Crystallin Sequences

DEAE-purified βB3, βA3, βA4, and βB1 were incubated with 10 mM DTT at 37°C for 30 minutes in 6 M urea and then alkylated by addition of 20 mM iodoacetamide at room temperature for 15 minutes. Proteins were then dialyzed to remove urea, and each protein was digested overnight with trypsin, gluc, or aspN proteases, under the conditions recommended by the manufacturer (Roche Molecular Biochemicals). Peptide digests were then analyzed by ESI-MS, using the same column and instrument described earlier, except with a linear 7.5% to 37% acetonitrile gradient over 50 minutes. Mass spectra were collected during the liquid chromatography run using a data-dependent "triple-play" strategy. This consisted of a full mass scan (m/z 300-2000), zoom scan on the most abundant ion to determine charge state, and a tandem mass spectrometry (MS/MS) scan to collect collision-induced dissociation (CID) spectra on peptides. Automated analysis of CID spectra to determine the amino acid sequence of peptides was performed on computer (SEQUEST software; ThermoFinnigan) as described by Yates.
**TABLE 1. Comparison of Calculated and Measured Masses of Isolated Rat α and β-Crystallins**

<table>
<thead>
<tr>
<th>Crystallin</th>
<th>Calculated Mass</th>
<th>Measured Mass</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>αA</td>
<td>19,834.1</td>
<td>19,833.9</td>
<td>−0.2</td>
</tr>
<tr>
<td>αA insert</td>
<td>22,489.2</td>
<td>22,489.5</td>
<td>+0.3</td>
</tr>
<tr>
<td>αB</td>
<td>20,130.8</td>
<td>20,131.4</td>
<td>+0.6</td>
</tr>
<tr>
<td>βB1</td>
<td>28,003.4</td>
<td>28,002.0</td>
<td>−1.4</td>
</tr>
<tr>
<td>βB2</td>
<td>23,291.8</td>
<td>23,292.0</td>
<td>+0.2</td>
</tr>
<tr>
<td>βS (L 41)</td>
<td>24,271.1</td>
<td>24,271.5</td>
<td>+0.4</td>
</tr>
<tr>
<td>βS (S 41)</td>
<td>24,245.0</td>
<td>24,247.5</td>
<td>+0.5</td>
</tr>
<tr>
<td>βA1</td>
<td>25,193.9</td>
<td>25,193.1</td>
<td>−0.8</td>
</tr>
<tr>
<td>βA2</td>
<td>Unknown</td>
<td>221,152.2</td>
<td></td>
</tr>
<tr>
<td>βA3</td>
<td>25,312.3</td>
<td>25,308.9</td>
<td>−3.4</td>
</tr>
<tr>
<td>βA3 12-215</td>
<td>22,292.5</td>
<td>22,290.9</td>
<td>−1.6</td>
</tr>
<tr>
<td>βA4</td>
<td>23,924.8</td>
<td>23,922.0</td>
<td>−2.8</td>
</tr>
</tbody>
</table>

* Masses were calculated without N-terminal methionines, except for αA, αA insert, αB, and βA3, which retained N-terminal methionines. All calculated masses included 42 mass units for the N-terminal acetyl group, except βA3 12-215 containing a free N-terminus.

† βB3 crystallin contained a polymorphic residue 41 as either leucine (L) or serine (S).

‡ βA3 12-215 is an N-terminal truncated form of βA3 missing 11 residues from the N-terminus, because of cleavage by endogenous calpains.

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**2-DE and Identification of Rat Lens Crystallins**

Immobilized pH gradient (IPG) gel strips (18 cm, pH 5–9) were produced using a composition recommended by the manufacturer (Immobiline Hydride; Amersham Pharmacia Biotech). A detailed protocol for pouring IPG gels can be found at a Web site maintained by Angelika Gorg at the Technical University of Munich (http://www.edv.agrar.tumuenchen.de/blm/deg/manual/manfrm.htm). Dried 3-mm wide IPG strips were rehydrated overnight in a reswelling tray (Immobiline DryStrip; Amersham Pharmacia Biotech), as recommended by the manufacturer. The 0.4-μL reswelling solution for each gel contained 8.1 M deionized urea, 2% 3-(3-cholamidopropyl)dimethylammonio)-2-hydroxy-1-propanesulfonate (CHAPS) detergent, 50 mM DTT, 2% IPG buffer (pH 6–11), a trace of bromophenol blue tracking dye, and 400 μg soluble lens proteins from 12-day-old rats. Isoelectric focusing was then performed on 24 cm 12% SDS-PAGE gels

Pharmacia Biotech) using a program of 500 to 3500 V over 1.5 hours, at a temperature of 35°C.

2-DE gels were prepared for the second dimension by two sequential 15-minute incubations in 6 M urea, 50 mM Tris (pH 8.8), 50% glycerol, 2% SDS, and 0.001% bromophenol blue containing alternately, 2% DTT and 2.5% iodoacetamide. The second-dimension separation was then performed on 24 × 18.5 cm 12% SDS-PAGE gels (IsoDalt; Amersham Pharmacia Biotech). Gels used for image analysis were stained using Coomassie blue G-250, and gels used for in-gel digestion of proteins were negatively stained with amidazole-zinc.

Gel images were analyzed by computer (Melanie 3 software; GeneBio, Geneva, Switzerland). The grid showing the pH of the first-dimension IPG gels and relative molecular weight of the second-dimension gels were determined by computer (Melanie 3) using the calculated pIs of αA (5.52), βB3 (6.83), and γC (7.52) and position of low-molecular-weight SDS-PAGE standards (Bio-Rad, Hercules, CA). The calculation of protein pIs was performed on computer (GeneWorks 2.5 software; Oxford Molecular, Campbell, CA), taking into account whether the protein was N acetylated. The assignment of pH on the IPG gels was validated by comparison with an internal standard of carbamylated rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH; G-5262; Sigma, St. Louis, MO) produced using the method of Link. The pIs of the various carbamylated species were determined by sequentially removing lysines from the sequence of GAPDH before calculation of pIs. The estimated pIs of crystallins differed by less than 0.15 pH units between the two methods.

Crystallin subunits were identified on 2-DE gels by MS. Negatively stained spots from one to three 2-DE gels of soluble protein from 12-day-old lenses were manually excised, washed, and dried and proteins within gel slices digested using sequencing grade-modified trypsin (Promega, Madison, WI), as previously described. Crystallins were then identified by on-line ESI-MS analysis of peptides as described earlier (SEQUEST software; ThermoFinnigan) for interpretation of MS/MS spectra.

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**RESULTS**

Before this study, the entire sequence of rat βA4 and N-terminal sequence of rat βA3 were unknown. The complete cDNA sequence of rat βA4 was determined by a combination of 3’ and 5’ RACE PCR. The resultant 800-bp sequence (GenBank accession no. AF013247; Fig. 1A) encoded a protein 195 amino acids in length, because of the removal of the N-terminal methionine in the mature protein. The cDNA for rat βA3 was similarly amplified by 5’ RACE PCR to yield a 161-bp sequence (GenBank accession no. AF013248) encoding the N-terminal 52-amino-acid residues of the protein, 38 of which were previously unreported (Fig. 1B). βA3 is the only rat β-crystallin that retains its N-terminal methionine in the mature protein. This 5’ sequence also contained the alternate start codon that yields βA1, a protein identical with βA3, except there are 18 fewer amino acids at its N terminus.

Preliminary mass spectrometric measurements of rat βB1 and βB3 did not match masses calculated from the previously reported sequences (SwissProt P02525 and P02524, respectively; provided by the Swiss Institute of Bioinformatics, Geneva, Switzerland, and available at no charge to academics at

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**FIGURE 4.** ESI-MS measurement of isolated rat βB3 crystallin. The peak at 24,245.7 corresponds to βB3 with serine at residue 41, whereas the mass at 24,271.5 corresponds to βB3 with leucine at residue 41. The alteration producing the species at 24,314.2 is unknown.
http://www.expasy.org). Therefore, cDNAs for rat βB1 and βB3 were amplified by PCR and sequenced. The resultant 741-bp sequence for rat βB1 cDNA (GenBank accession no. AF286652) was similar to the previous genomic sequence of rat βB1, except three additional nucleotides were found (Fig. 1C, underscore) that were missing in the previous sequence (GenBank accession no. M13527). These additional nucleotides resulted in a change in the deduced amino acid sequence of rat βB1 at residue 16 from Y to P and an additional amino acid D at residue 17.

![Confirmation of rat βB1 (A), βB3 (B), βA3 (C), and βA4 (D) sequences by MS/MS analysis of protein digests using trypsin, GluC, and AspN proteases. Peptides covering the entire sequence of each protein were confirmed by MS/MS, including the underscored sequences that differed from previously reported sequences in βB1 and βB3. Numbers above each bracketed sequence are the calculated monoisotopic mass for each recovered peptide confirmed by MS/MS. The mass of each cysteine residue was increased by 57.1 because of the alkylation with iodoacetamide. All proteins were N acetylated, and, with the exception of βB3, all N-terminal methionines were removed. The polymorphic residue 41 of βB3 containing either serine or leucine was also confirmed by MS/MS (Fig. 6).](image)
An amplified 660-bp cDNA coding for rat βB3 was also sequenced (GenBank accession no. AF287304) and compared with the previous cDNA sequence (GenBank accession no. X05899). A difference in nucleotide 54 in the new sequence resulted in a change in amino acid 13 from N to S (Fig. 1D). This difference was also previously detected by Edman sequencing.20 Direct sequencing of the ββB3 PCR product also resulted in the detection of a polymorphism at nucleotide 138 (Fig. 2). Both nucleotides T and C were detected at this position, leading to either S or L at amino acid 41 (Fig. 1D). An additional difference was also found at nucleotides 275-277, resulting in the alteration of amino acid 87 from A to R.

To further examine the sequences of β-crystallins, individual subunits were isolated from the βH-crystallin aggregate by anion exchange in 6 M urea. Because of their relatively distinct pIs, rat β-crystallins are more easily separated during anion-exchange chromatography than are bovine β-crystallins.27 The separation resulted in the appearance of seven peaks, which on analysis by SDS-PAGE yielded nearly homogenous βB3, βB2, βB1, βA312-215, βA1, and βA4 and a mixture of βA2 and βA3 (Fig. 3). The masses of the isolated rat β-crystallin subunits were measured by ESIMS (Table 1). Values calculated from the new sequences of rat βA4, βA3, βB1, and βB3 and the previous sequence of rat βB2 (Swiss Prot P26775) differed less than 3.4 mass units from the measured values, which was within the expected error of the instrument. The measured mass of rat βA2 (22,153.2) could not be confirmed, because the sequence is unknown. The deconvoluted spectrum of polymorphic rat βB3 is shown in Figure 4. The major species of rat βB3 containing L at residue 41 had a mass of 24,245.7, whereas the origin of the minor higher molecular weight form at 24,314.2 is unknown. The deconvoluted spectrum of peptides 37-54 of rat βB3 is shown in Figure 6B. The MS/MS spectra of tryptic peptide 37-54 of rat βB3 containing the polymorphic residue 41 as S is shown in Figure 6A, and the same peptide with residue 41 as L is shown in Figure 6B.

Additional mass measurements of αA, αAinsert, and αB were performed by separation of α aggregate by reversed-phase chromatography and online ESIMS. The masses of the three rat α-crystallin subunits matched closely with masses calculated from their reported sequences (Table 1). Separation and measurement of the whole masses of rat γ-crystallins was also performed. However, the data suggest that at least two of the six previously reported sequences of rat γ-crystallins contained discrepancies or that there also may be polymorphisms in their sequences (data not shown).

To further analyze the crystallin proteome of rat, soluble proteins from the lenses of 16-day-old animals were separated by 2-DE (Fig. 7A). The identities of the various crystallin subunits on the 2-DE gels were then determined by analysis of between 30% and 90% of each protein’s sequence by MS/MS of peptides (Fig. 7B). The analysis identified all known rat crystallin subunits, including previously unreported βA2 and for the first time resolved γS from a truncated form of βA3 missing 11 residues from its N terminus (βA312-215). The combination of βA312-215 and γS on 2-DE gels may have led to the overestimation of the abundance of these two species in earlier work.26,28 Because the sequences of rat βA2 and γS remain unknown, the identities of these proteins were determined based on identification of several peptides with sequences that are identical with the published sequences of bovine βA2 and γS (Swiss Prot P26444 and P06504).

Analysis of digests from gels also allowed assignment of several crystallin modifications. For example, MS/MS spectra confirmed that the spot labeled βA312-215 was missing 11 residues from its N terminus. The MS/MS spectrum of the...
tryptic peptide TLPTTK belonging to the new N terminus of βA3 (residues 12-17) was found in the digest of this spot, as well as the tryptic peptide from the intact C terminus (data not shown). The MS/MS spectrum of the N-acetylated peptide AQTNPMPGSMGPWK from the N terminus of βA1 also confirmed that the spot labeled βA1/αAinsert contained βA1 and not an N-terminally truncated form of βA3. Unlike mouse βA1 and αAinsert,1 rat βA1 and αAinsert did not resolve from one another during 2-DE. αAinsert (Swiss Prot P24623) is identical with αA, except for an insertion of 23 extra amino acids because of differential splicing. The identification of αAinsert was based on analysis of peptides common to both αAinsert and αA. Peptides from the unique 23-amino-acid region of αAinsert were not recovered from the gel digest. However, because of its much higher relative molecular weight on the gel compared with αA, the protein was most likely αAinsert, rather than a modified form of αA.

A faint spot just below βB1 was identified as an acidic form of βB3. The alteration causing this acidification of βB3 remains unknown. The acidification was not due to proteolysis, because MS analysis indicated that the N and C termini of the protein remained intact (data not shown). This minor species could be the result of an as yet unidentified additional polymorphism in rat βB3, possibly corresponding to the unknown form of βB3 with a mass of 24,314 in Figure 4. By coincidence, this acidic form of rat βB3 migrated to an identical position after 2-DE as the major form of mouse βB3.1

Because of their high sequence homology, the six γA–F crystallins were difficult to fully resolve and identify by 2-DE. For example, there are only four amino acid differences between the reported sequences of γE and γF (Swiss Prot P02528 and P10068, respectively). The proximity of the γ-crystallin spots also caused some contamination of one protein with another during MS/MS analysis of peptides. This required confirmation that the peptide unique to each protein caused a major ion peak during the reversed-phase separation of the digest. The identification of the γ-crystallins in this study confirmed the earlier assignments made by Voorter et al.28 The additional species marked with numbers 1 to 4 adjacent to γA-F-crystallins were composed of poorly focused γA-F-crystallins that migrated to more acidic positions than the major forms of γA-F. The cause of these additional γ species on the 2-DE gels is unknown. Similar acidic forms of γ-crystallins were also observed on 2-DE gels of mouse lens crystallins.3

Image analysis was used to estimate the pI and percentage of each crystallin subunit in the soluble proteins from 12-day-old rats. When the calculated pIs of αA, βB3, and γC were used to calibrate the pH of the IPG strips, the measured pIs of the other crystallins fell within 0.2 pH units of their calculated values. The reproducibility of the IPG gels resulted in an SD of pl estimation of less than 0.02 pH units. Calculation of mean percentage volumes of spots from multiple 2-DE gels prepared from different rats of identical age allowed simple estimation of the abundance of each crystallin (Fig. 8). Monomeric γ-crystallins comprised approximately 60% of the soluble protein of young rat lens, β-crystallins 25%, and α-crystallins 15% (Fig. 8).

### Discussion

These experiments provided the complete amino acid sequence for all rat β-crystallins, except newly detected βA2; detected a polymorphism in rat βB3 crystallin; described methodology to purify rat β-crystallins; confirmed the sequences of rat α- and β-crystallins by MS; produced a standardized 2-DE-based proteome map of rat lens crystallins; and determined the relative abundance of each soluble crystallin subunit in young rats. The results are important, because they will facilitate the precise localization and quantification of posttranslational changes in rat crystallins during aging and cataract.

These data extend earlier work in our laboratory, in which the age-related alterations of rat crystallins were examined in both the water-soluble and -insoluble fractions of the lens nucleus and cortex by 2-DE.26 The previous studies documented extensive partial proteolysis of α- and β-crystallins in the water-insoluble fraction of rat lens nucleus during maturation and cataract. The confirmed sequences of the crystallins determined in this study will now allow a more detailed analysis of these proteolyzed crystallins by MS.

The IPG-based 2-DE map of rat crystallins shown in Figure 7 is more detailed than previously available maps.26,27 This map...
polymorphisms may exist in the crystallins of other species. Rat lenses contain all seven β-crystallins. whereas human lens contains only βB3-crystallin, which comprised nearly 10% of the rat lens soluble protein, is largely undetectable in human lens after birth. In contrast, βB2 was more than three times as abundant in young human lens than in young rat lens. Crystallins in human lens remain water soluble far into adulthood, whereas more than 50% of crystallins of the rat lens nucleus are insoluble by 4 months of age. We hypothesize that the high βB2-to-βB3 ratio is partially responsible for this increased solubility of crystallins in mature human lens. The high percentage of βB3 in rat lens may drive protein insolubilization, because it more readily precipitates after partial proteolysis than do other β-crystallin subunits. In contrast, βB2 is resistant to precipitation, both after partial proteolysis in vitro and during aging in human lens.

Detection of a polymorphism in rat βB3 suggests that polymorphisms may exist in the crystallins of other species as well. A similar study of bovine crystallins detected a polymorphism in bovine βA3 crystallin (David L, unpublished results, 2001). Previously unknown polymorphisms may also be present in human lens crystallins. These polymorphisms may remain undetected, because of the limited amount of redundant sequencing of human crystallin genes and rapid posttranslational modification of human crystallins with age. Certain polymorphisms in human crystallins may have the potential of increasing the risk of cataract. The ability of mutations in major lens proteins to cause congenital cataracts in humans has been well documented.

In conclusion, the results in this study provide baseline data on the composition and primary structure of crystallins in normal rat lens. The information will be useful in future studies to more thoroughly examine both age-related and cataract-specific modifications in rat crystallins. This analysis is important, because similar modifications may be a cause of human cataract.

Acknowledgments

The authors thank Jean Smith and David Smith, University of Nebraska, and Margaret Sheil, Greg Kirby, and John Carver, University of Wollongong, New South Wales, Australia, for performing the initial measurements of rat crystallin masses that began this study.

References

3. Worgul BV, Medvedovsky C, Huang Y, Marino SA, Randers-Pehrson G, Brenner DJ. Quantitative assessment of the cataractogenic po-

![Figure 8](image-url)