The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/48381

Please be advised that this information was generated on 2020-01-10 and may be subject to change.
Blue native gel electrophoresis and mass spectrometric identification of multi-protein complexes in retinal pigment epithelial cells

K.J.A. VANHOUTTE1,2, B.P.M. JANSSEN1, M.A. GERRITS1, R.O. VOGEL2, W.J.G. PLUK3,4 and J.J.M. JANSSEN1

Introduction
Vision starts with the photoactivation of visual pigments in the rod and cone photoreceptor cells. It critically depends on a continuous supply of the ligand 11-cis retinal to the bleached or newly synthesized visual pigment apo-protein opsin. Responsible for this supply is the visual cycle also designated retinoid cycle (1). The visual cycle is best described as the sequential action of proteins in the photoreceptor cell outer segments and in the adjacent retinal pigment epithelium (RPE). In the RPE, enzymes and retinoid transporters control the conversion of the all-trans-retinol (vitamin A) into 11-cis-retinal. Human hereditary retinal degenerations have been found associated with mutations in genes encoding these retinoid processing proteins (e.g. RPE65, cRALBP, RDH5, LRAT, RGR, etc.). Our hypothesis is that the biochemical and enzymatic reactions of the visual cycle, including potentially toxic oxido-reductase reactions in the RPE cell, are tightly organized in subcellular Multi-Protein Complexes. Interestingly, in a recent interaction study of cRALBP, Nawrot et al. (2) also proposed a retinoid processing complex in the apical region of the RPE, adjacent to the rod outer segments, dedicated to regeneration of the chromophore (3). As a first step towards further dissection of retinoid processing complexes, we report here on preliminary proteomics experiments that focus on the analysis of the interactome of visual cycle proteins in the bovine RPE. The experimental approach is depicted in figure 1. In summary, to investigate the native configuration, we combined Blue Native (BN) gel electrophoresis with high-accuracy mass spectrometry (4, 5).

Materials and methods
Blue Native Gel Electrophoresis
Multi-protein complexes in a bovine RPE cells were resolved by non-denaturing Blue Native (BN) gel electrophoresis (6). First, a RPE membrane pellet was extracted from 70 bovine eyes, obtained from a local slaughterhouse. The pellet was solubulized with 1.6% dodecyl maltoside in 1.5M AminoCaproic Acid and 75 mM BisTris (pH 7.0). The solubulized RPE samples were run on 5-15% gradient gels cast on a BioRad protean II minigel system, together with a native high molecular weight (HMW) marker (Amersham Biosciences). Gels were run at 35 V for 30 min and at 300-450 V (10 mA) for 3 hours. After BN gel electrophoresis, the protein complexes were visible as sharp Coomassie blue stained bands. First dimension BN strips could be further analyzed by Western blot analysis on second dimension 10% SDS-PAGE gels after a denaturating incubation in 1% SDS / 1% β-mercaptoethanol solution.

LTQ ICR Fourier Transform Mass Spectrometry
In this study we excised visible BN complexes with a scalpel for further mass spectrometric sequence analysis (7) at the Nijmegen Proteomics Facility. In short, after in-gel trypsin digestion, reduction and alkylation, the peptides were extracted from the gel, desalted and concentrated on C18-StageTips and injected with an automated sample robot in the high throughput nano-LC system (Agilent 1100). The effluent, ionized by nano Electro Spray Ionization was sprayed into a LTQ ICR-FTMS (Finnigan LTQ FT™, Bremen, Germany) mass spectrometer set to acquire data in data dependent mode, i.e. tandem MS spectra are taken by the linear ion trap from 4 precursor ions with highest intensity in the MS-spectrum, acquired in the ICR-FTMS detector. Peptides were searched against the mammalian sequences of NCBI non redundant protein databases using the MASCOT search engine (http://www.matrixscience.com).

Results
Visual inspection of the BN gel revealed prominent BN protein complexes with an apparent mass ranging from 200 to 1000 kDa. This pattern was reproducible in different BN runs (different gradients tested) and in independent RPE isolations. Preliminary mass spectrometric analysis of digested slices revealed on average 24 proteins per complex, identified with more than two unique rank 1 peptides. We found mainly hydrophobic membrane-associated proteins. Surprisingly, in almost all analyzed samples three visual cycle retinoid processing proteins were identified with a high peptide coverage. Other retinoid processing proteins seem less abundant: RDH11, LRAT, IRBP do not appear in all complexes. We argue therefore that 11-cis-retinol-dehydrogenase (RDH5) may co-localize with a RPE-specific 65 kDa protein (RPE65) and Retinal G-protein-coupled Receptor (RGR). This result was further investigated by...
Western analysis of RGR, RDH5 and RPE65 on second dimension SDS PAGE gels. We found that these proteins comigrate in high molecular weight complexes. Interestingly, the MS analysis disclosed proteins expressed in the metabolic active RPE which may associate with lipid / redox metabolism. Several proteins were identified as ‘unnamed’ and may represent interesting unidentified proteins. Alternative subcellular compartment fractionations with sucrose gradient centrifugation are now performed to purify the putative retinoid processing complexes.

Conclusion

The proteomics approach “Blue Native Gel electrophoresis- nanoLC – MS/MS”, here for the first time applied in a preliminary study on isolated retinal pigment epithelial cells, provides fast high-accuracy analysis of Multi-Protein Complexes in the RPE. We mainly identified hydrophobic membrane-associated proteins that are difficult to resolve by standard 2D IEF gel electrophoresis. Our data provide insight into interacting proteins in the retina and more specifically in the RPE. It confirms previous co-immunoprecipitation studies (8) pointing towards a retinoid processing interactome of which three visual cycle proteins RPE65, RGR and RDH5 form a central core.

Outlook. This type of analysis helps to identify new and yet unknown players in ocular (retinoid) metabolism, potentially associated with human hereditary retinal conditions. Alternative sample sources like for example RPE fractions (9), human post-mortem retinal tissue (10), cell lines with an active retinoid processing machinery or isolated retinas of gene-KO animals, may also provide valuable starting material for proteomics studies in the retina, with the final aim to gain insight into the molecular (patho-)physiology.

Acknowledgements

This project was financially supported by “De Landelijke Stichting voor Blinden en Slechtzienden”. Mass spectrometry experiments were performed at the Nijmegen Proteomics Facility (www.proteomicsnijmegen.nl)

References