Mapping Ocular Surface Enzymes in Healthy Subjects

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Introduction

Enzymes are

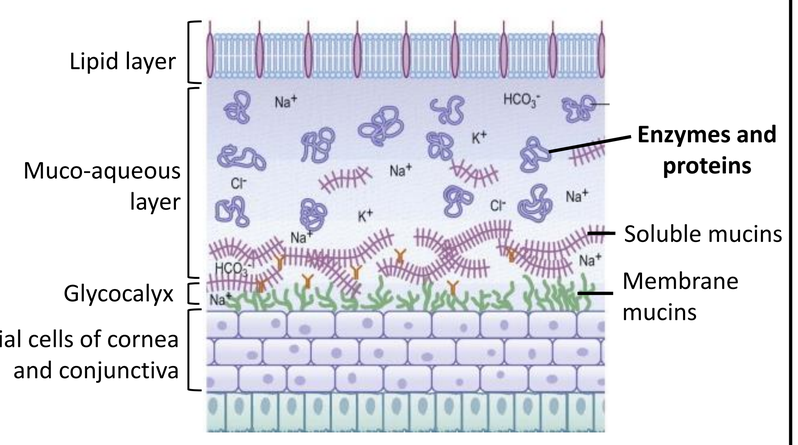
- biocatalysts with the critical task of accelerating biochemical reactions in living cells and organisms [1]
- the largest class of proteins in tear film [2]
- involved in metabolism, cell division, gene expression, and immune system [2]

Knowledge about ocular surface (OS) enzymes helps us to

- reveal involved biological processes and links with signaling pathways
- predict metabolism of ocular drugs [3]
- develop novel prodrug strategies and drug delivery systems [3]

Aim: To determine and classify the enzymes of tear fluid collected with capillary tubes and Schirmer strips based on nine comprehensive tear proteomics studies

Structure of tear film



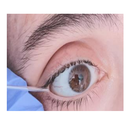
Modified from R. R. Hodges and D. A. Dartt, *Int. Rev. Cytol.*, 2003

Methodology

1. Pooling proteins

5 studies - Capillary tube

De Souza *et al*., Genome Biol., 2006
Hua *et al.,* BMC Ophthalmol., 2020
Kandhavelu *et al*., J. Proteomics, 2016
Nättinen *et al.,* Trans. Vis. Sci. Tech., 2020
Ponzini *et al.,* Int. J. Mol. Sci., 2021



5 studies - Schirmer strip

Akkurt Arslan *et al*. Metabolites, 2021
Aass *et al*., Anal. Biochem., 2015
Dor *et al*., Exp. Eye Res., 2019
Nättinen *et al*., Trans. Vis. Sci. Tech., 2020
Zhou *et al*., J. Proteomics, 2012

2. Profiling and classification of enzymes

(a) Identification

Tear enzymes

ExplorEnz - The Enzyme Database
Approved International Union of Biochemistry (IUBMB) Enzyme nomenclature

SEP* enzymes

SEP*, Schirmer strip-extracted proteome

(b) Classification

EC numbers
Enzyme Commission

main class
1. oxidoreductases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases
7. translocases

subclass
often tells you often what type of compound or bond it acts on

sub-subclass
further classification

serial number
"just" makes it so each enzyme has its own unique EC number

Classification of enzymes according to substrates and reaction they catalyze

UniProt

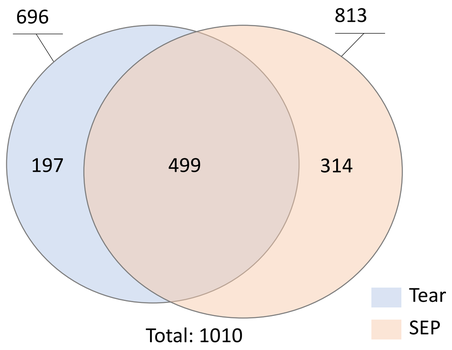
(c) Functional annotation and enrichment analysis

Bioinformatics databases to reveal biological processes and signaling pathways and enriched terms

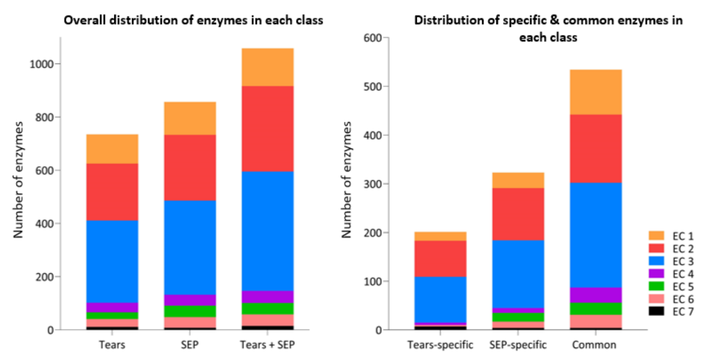
reactome
Metascape A Gene Annotation & Analysis Resource
KEGG
DAVID Bioinformatics Database

Results

Numbers of identified enzymes

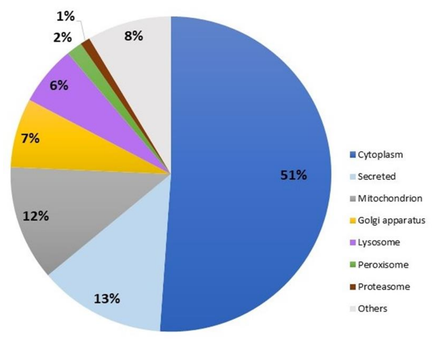


Comparison of enzyme classes in tear and SEP

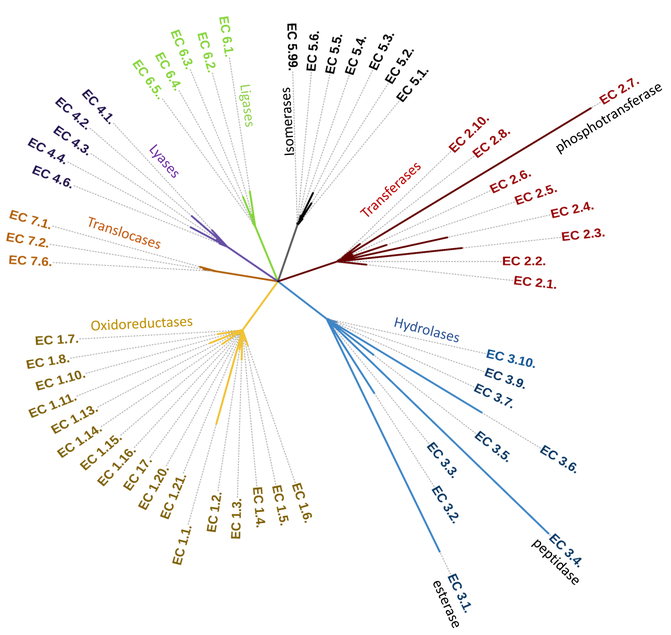


Enrichment analysis

I- Cellular component

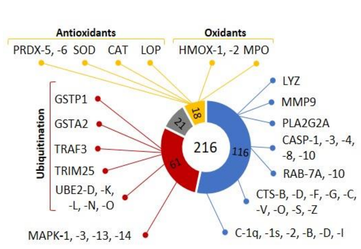


Subclassification of all identified enzymes

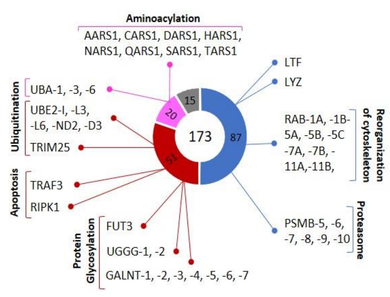


Major signaling pathways

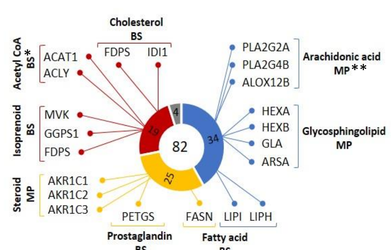
a. Immune system



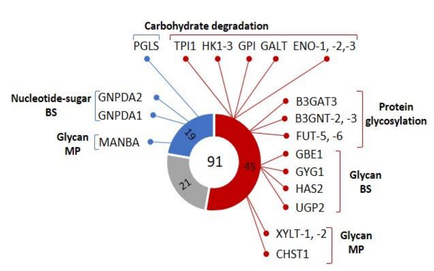
b. Metabolism of proteins



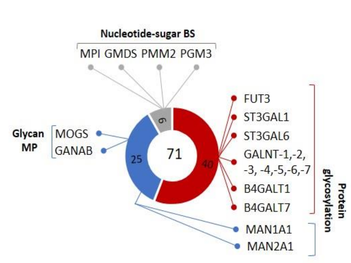
c. Metabolism of lipids



d. Metabolism of carbohydrates



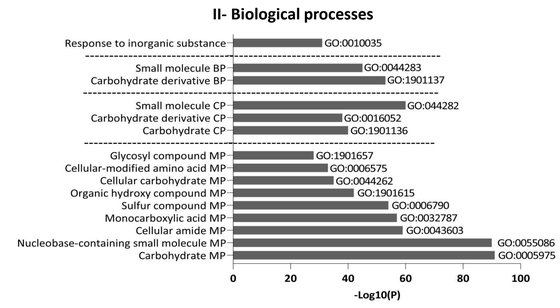
e. Glycosylation



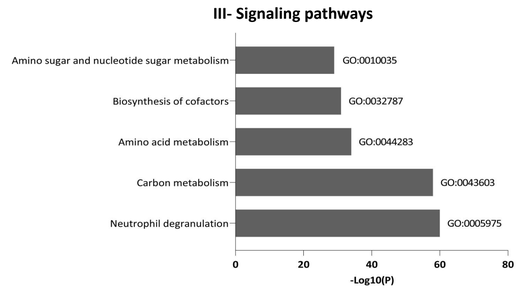
EC 1 - Oxidoreductase
EC 2 - Transferase
EC 3 - Hydrolase
EC 6 - Ligase
Others

*BS, Biosynthesis, **MP, Metabolic process

II- Biological processes



III- Signaling pathways



BP, biosynthetic process, CP, catabolic process, MP, metabolic process,

Conclusions

- ✓ More enzymes, particularly isomerases, can be collected using Schirmer strips.
- ✓ Phosphotransferases, peptidases and esterases are the most represented enzyme subclasses on the OS.
- ✓ Compiled enzymes are mainly involved in immune system, metabolism and glycosylation pathways.
- ✓ This generated dataset can help develop novel ocular prodrug synthesis and drug delivery systems.
- ✓ Combining knowledge of OS enzymes with new approaches and techniques might open up new avenues for the development of novel drugs.

References

[1] P. K. Robinson, Enzymes: principles and biotechnological applications, Essays Biochem., 2015.

[2] L. Porto de Souza Vandenberghe *et al.,* Classification of enzymes and catalytic properties, Biomass, Biofuels, Biochem., 2020.

[3] S. Duvvuri *et al.,* Role of Metabolism in Ocular Drug Delivery, Curr. Drug Metab., 2005.

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