BIOCHEMISTRY OF EYE TISSUE

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METABOLIC PATHWAYS IN EYE TISSUE

• Glycolysis (aerobic & anaerobic)
• HMP shunt
• Poliol pathway
• TCA cycle
GLYCOLYSIS

• Is the major pathway for glucose metabolism – to obtain energy

• Occurs in the cytosol of all cells

• Can function either aerobically or anaerobically

• In aerobic condition – glucose was converted into pyruvate in cytosol – then pyruvate enter the mitochondria and will be oxidized to acetyl CoA – the C atom will be oxidized into CO$_2$ through the TCA cycle.

• The electron from the substrate will be oxidized in electron transport chain – to produced H$_2$O + ATP
REACTIONS OF GLYCOLYTIC PATHWAY

Glucose + ATP $\rightarrow$ glucose 6-P + ADP
Glucose 6-P $\leftrightarrow$ fructose 6-P
Fructose 6-P + ATP $\rightarrow$ fructose 1,6-BP + ADP
Fructose 1,6-BP $\leftrightarrow$ di-OHacetone-P + glyceraldehyde 3-P
Glyceraldehyde 3-P + Pi + NAD $\leftrightarrow$ 1,3-bisphosphoglycerate + NADH + H+
1,3-bisphosphoglycerate + ADP $\leftrightarrow$ 3-P glycerate + ATP
3-P glycerate $\leftrightarrow$ 2-P glycerate
2-P glycerate $\leftrightarrow$ P-enolpyruvate + H2O
P-enol pyruvate + ADP $\rightarrow$ pyruvate + ATP
Aerobic glycolysis
Anaerobic glycolysis
HMP shunt (Pentose phosphate pathway)

- Alternative pathway for glucose oxidation

- Function – **NOT** to produce energy – to produce **NADPH**

- NADPH – important **reductor** intracell
HMP SHUNT

Part I: Oxidative phase

NADP  NADPH

Glucose $\rightarrow$ G 6-P $\rightarrow$ Ribulose 5-P

G6P DH

Part II: Non oxidative phase

Transketolase, transaldolase

Ribulose 5-P $\rightarrow$ Glucose 6-P

TPP
POLYOL PATHWAY

• Present in many tissues

• Function – not fully understood

• Activity of polyol pathway – can lead to major problems in DM – cataract diabetica, neuropathy, nephropathy
POLYOL PATHWAY

Aldose reductase

Glucose \[\rightarrow\] Sorbitol

NADPH + H^+ \[\rightarrow\] NADP^+

Sorbitol dehydrogenase

Sorbitol \[\rightarrow\] Fructose

NAD^+ \[\rightarrow\] NADH + H^+
TCA CYCLE

• Common metabolic pathway for CH, lipid, protein

• C atom – oxidized to CO$_2$

• Electron & H – transferred into electron transport chain through oxidative phosphorylation produce H$_2$O + ATP
Problems of the eye

- Exposed to the $\uparrow$ pO2 atmosphere (21 % $\text{O}_2$), pollutants & irritant (~ lung)

- Very long turn-over protein (~ erithrocyte)

- Risk of damage by too much light exposure (~ chloroplast in plant)
CORNEA(1)

- Eye – is an extension of nervous system – the metabolism ~ nervous system – major fuel is glucose

- Glucose uptake by cornea – were used
  - 30 % - for aerobic glycolysis
  - 65 % - HMP shunt ( to produced NADPH)

- Activity of HMP shunt in the cornea – highest of any other mammalian tissue
CORNEA (2)

• Cornea has ↑ activity of GSH reductase

• Corneal epithelium – permeable to atmospheric O$_2$ – needed for aerobic metabolism – cornea was prone to the formation of reactive oxygen species (ROS)

• Activity of the HMP shunt and GSH reductase – maintain the reduced state of the cornea – neutralize the ROS that harmful to the cornea
Protein disulfide were reduced by glutathione reductase.
LENS (1)

• The outward-facing side of the lens – covered by the lens fibers – which synthesize **crystallin** – the major protein of the lens

• The new lens fibers push the older ones toward the center of the lens – formed the lens nucleus

• The nucleus, mitochondria & other organelles are lost during fibre maturation

• The lens increase in weight and thickness with age and become less elastic
LENS (2)

- Lens is bathed on outer side by aqueous humor and on inner side by vitreous humor
- Lens has **no blood supply**, but metabolically active

Protein of the lens
∀ \( \alpha, \beta, \gamma \)-crystallin
- Albuminoids
- Enzymes
- Membrane proteins
Crystallin

- 90 % of the lens soluble proteins
- Is a *long-lived protein* – damaged of crystallin – were accumulated
- The organization of crystallin – were important to maintain the transparency of the lens
- Denaturation, oxidation and aggregation of crystallin – lead to loss of transparency
Lens proteins must be maintained in native aggregated state

Lens are sensitive to changes of

• redox state - maintained by GSH reductase
• osmolarity – maintained by Na\(^+\)/K\(^+\)-ATPase
• UV irradiation
• ↑ of metabolite
Glucose used by the lens

- 85% - for energy through glycolysis
- 10% - HMP shunt
- 3% - by TCA cycle – presumably by the cells at the periphery
CATARACT

• Opacity of the lens caused by changes of solubility and aggregation of the lens protein

• Cataract senilis – changes of crystallin – related with age – due to deamidation, racemisation of aspartyl residue

• Cataract diabetica – caused by ↑ osmolarity of the lens – due to ↑ activity of aldose reductase and poliol dehydrogenase of polyol pathway
CATARACT DIABETICA

• DM – hyperglycemia - glucose level in the lens ↑ - convert into sorbitol by aldose reductase – and then by sorbitol / poliol dehydrogenase were converted into fructose

• Acummulation of sorbitol & fructose – ↑ osmolarity of the lens and denaturation of protein – opacity of the lens – cataract diabetica

• N – activity of aldose reductase in the lens was not significant – because Km for glucose was ↑
Lens contain tryptophane metabolite – can absorbed UV light, but if exposure to UV light >> - act as photosensitisizer – produced singlet O2 → damaged and cross-linking lens protein

Long exposure to UV light – risk factor for cataract formation

Isolate protein from cataract lens – contain product of oxidative protein damage

Cataractous lens – ↑ level of H$_2$O$_2$, ↓ level of GSH
Glucose $\rightarrow$ Glucose 6-P

G 6-P dehydrogenase

NADP$^+$

NADPH + H$^+$

GSSG

GSH

Sorbitol

Polyol dehydrogenase

NAD$^+$

NADH + H$^+$

Fructose

Interrelation of metabolism in the lens
VITREOUS HUMOR

• Contain hyaluronic acid

• ROS attack – cause depolymerization of hyaluronic acid – loss of viscosity
RETINA

• Retina was vascular tissue, but contain no blood vessel in the fovea centralis

• Mitochondria were present in the retina cells, rod & cone, but no mitochondria at the outer segment of rod & cone (at the location of visual pigment)
RETINA

• *Uptake* $O_2$ of retina $\uparrow$ - because of $\uparrow$ energy need for neurotransmission, synthesis and recycle of molecule important for visual function

• Brief ischemia – lead to irreversible visual damage

• *Uptake* $O_2 \uparrow$ - consequences for $\uparrow$ ROS formation

• Lipid in rod and cone – contain $\gg$ PUFA (particularly DHA) – susceptible to lipid peroxidation
• Rhodopsin can sensitisized formation of singlet $O_2$

• Outer segment of photoreceptor - contain highest DHA compared to any other tissue - exposure to light – can induced lipid peroxydation - lipid peroxide – can damage the protein of the retina

• Deficiency of Se and vitamin E – could caused ↓ level of PUFA and accumulation of fluorescent product of retinal pigment epithelium (RPE)
Enzymatic antioxidant was found in all parts of the eye

- Glutathione peroxydase
- Catalase
- Glutathione S-transferase
- Superoxyde dismutase (SOD)
  - CuZnSOD – in all parts of the eye – very susceptible to glycation (~ DM) and reaction with $\text{H}_2\text{O}_2$
  - MnSOD - >> in the RPE
Epithel of cornea contain

- Ascorbic acid
- GSH
- SOD (superoxyde dismutase)
- Catalase & GSH peroxydase
- Ferritin
  - chelate Fe ion
  - may be important for protection of DNA from UV light
    - exposure of UV light – translocation of ferritin from cytosol to the nucleus
Outer side of cornea – bath by tears - contain
• >> ascorbic acid
• uric acid
→ protect cornea from pollutant such as O₃, NO₂• and SO₂

• lactoferrin – chelate Fe
→ eye irritation – tears production >> → protect the Fe-dependent free radical reaction
Antioxidant in the lens

• Lens contain $\uparrow$ **GSH** level $\sim$ liver

• GSH level highest in lens epithelium, lowest in the nucleus of the lens

• GSH protect the $-\text{SH}$ group of crystallin – protect crystallin from aggregation – protect the opacity of the lens

• Capability to synthesize GSH in the lens $\downarrow$ in aging – predisposing factor for cataract formation

• Ratio of GSH/GSSG in the lens – maintain in $\uparrow$ level by glutathione reductase which need NADPH from HMP shunt
O$_2^-$ → H$_2$O$_2$ → Fe$^{2+}$ → Fe$^{3+}$ → OH·

Fenton reaction

2GSH → GSSG → NADP$^+$ → NADPH + H$^+$

SOD

H$_2$O$_2$ → H$_2$O

catalase

2H$_2$O + O$_2$

GSH Px

GSH Rx

ENDOGENOUS ANTIOXIDANT
Outer segment of rod and RPE - >> contain α-tocopherol

• Deficiency of α-tocopherol in animal – damage of cell and accumulation of lipofuscine in the RPE

 ∀ α-tocopherol – ↓ severity of cataract

• Premature babies (predisposing factor for retinopathy of prematurity, ROP) – α-tocopherol level was ↓
Ascorbic acid was found in tears, cornea, aqueous humor, lens, vitreous humor and RPE

- Cornea contain ascorbic acid in highest concentration – important to protect from UV light

- Nocturnal animal – ascorbic acid level in the eye ↓

Ascorbic acid could
- Recycle the α-tocopherol radical
- Interaction with GSH
- Scavange singlet O₂, O₂•-, OH• and other ROS
• Deficiency of ascorbic acid in the diet of animal for months – ↓ level of ascorbic acid, GSH and α-tocopherol

• Degradation of ascorbic acid induced by light – ↑ the H₂O₂ level

• In oxidative state – ascorbic acid could cause glycation reaction with protein, including crystallin

• Epidemiological studies – indicate ascorbic acid has beneficial effect for the eye