Porphyryins & Porphobilinogen

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**Porphyryns**

- Heterocyclic macrocycle organic compounds

- Composed of four modified pyrrole subunits (tetrapyrrole) by methene bridges

- It has 26 (delocalized) pi electrons, It has highly conjugated double bonds

- The 4 nitrogen atoms at the center of the ring are excellent at conjugating metals because of their lone pairs

- It has very intense absorption bands in the visible region at Soret peak (band) in blue region of visible spectrum near 400-410 nm wavelength

- It formed deep violet/blue color due to an electron dipole movement (π-π) trans.

- The name "porphyrin" comes from the Greek word *porphyros*, meaning *purple*

- Porphyrins are the conjugate acids of ligands that bind metals to form complexes
Physical properties

• **Color:**
  – Coloration around 405 nm
  – Usually red

• **Fluorescence:**
  – around 620 nm
  – Reddish-pink color

• **Chelation:**
  – Arrangement of nitrogen atoms allows chelation of metal atoms such as iron, that participate in oxidative metabolism
Porphyrin types

• Uroporphyrin: **URO**
  – Water soluble
  – Heme precursor
  – Found in **urine**

• Coproporphyrin: **COPRO**
  – Water soluble
  – Heme precursor
  – Found in **urine and feces**

• Protoporphyrin: **PROTO**
  – Water insoluble
  – Heme precursor
  – Found in **feces**
Total porphyrins screening

• One of the first indication of porphyria is production of dark colored urine sample

• Porphyrins have characteristic UV/VIS absorption peak (Soret peak) in the region 400-410 nm

• Coproporphyrin has peak absorption between 402-403 nm

• Uroporphyrin has peak absorption between 406-407 nm
Substances which interfere in test

- Aminosalicylic acid
- Birth control pills
- Barbiturates
- Ethyl alcohol
- Morphine
Porphyrens sample preparation

• Urine sample: (Prefer sample is DU-Urine sample)

1. Sample must be covered with aluminum foil
2. Sample is centrifuge and filtered out from solid particles
3. Sample (0.5 mL) is acidified with 2.7M of HCL (2 mL)
4. Incubation in dark for an hour
5. Sample is scanned from 300 to 500 nm -> spectrum position
6. Construction of baseline peaks from either side of main peak (Around 390 to 420 nm)
UV/VIS spectroscopy (190-700 nm)
Total-porphyrins peak analysis

Total porphyrins in ug/L = 2A (λmax) – (A390 + A420)e ; where e=4740 ug/L
Reporting porphyrin

• If the urine result < 300 nmol/L, report as **negative**

• Results of urine > 300 nmol/L; its **(positive)** should be expressed as (nmol porphyrins/mmol creatinine)

• Urine porphyrin/creatinine ratio: <35 nmol/mmol creatinine

• The total porphyrin concentration per mmol of creatinine is the best indicator of total porphyrin excretion.
Sample collection

• It can also be measured on blood, urine, biological tissues and fecal samples

• Venous blood sample should be collected and transport covered in ice

• Patient should be 12-14 hours fasting

• Three types of porphyrins can be measured:
  1. Coproporphyrin
  2. Protoporphyrin (little difficult)
  3. Uroporphyrin
Other techniques & methods

1. UV/VIS spectrophotometer offering narrow slits with aid of skilled BMS

2. Fluorescence spectroscopy; excitation and emission wavelengths = better selectivity e.g. good for fecal porphyrins analysis

3. HPLC for urine, plasma, & tissue extracts fecal (*false negative*)
Chromsystem kit for porphyrins in urine

Lots of fractionation & extraction methods available;
Urine -> Johnson et al., & Feces -> Lockwood et al., & Pudek et al., & Plasma -> Kennedy & James
Porphyryns in urine/plasma

• Its fast and reliable method for differential diagnosis

• Preparation requires only one mixing & one centrifugation step. (Sample + stabilization + Priming + ISTD)

• C-18 column

• Gradient system with two mobile phase

• Fluorescence detector at 405-620 nm
Resulted peaks for urine porphyrins

In plasma, Uroporphyrin curve is split into (I & III)
Porphobilinogen (PBG)
Porphyobilinogen (PBG)

- Its pyrrole with molar mass of 226.229 g/L

- It is generated from (ALA) by the enzyme ALA dehydratase
PBG Test procedures in urine sample:

1. Urine pH is adjusted to 6 – 8

2. Syringe containing with organic resins is filled with sample

3. Expel the urine sample wash-up with distilled water X2

4. Elution reagent is filled in syringe and mixed

5. Expel the solution into reaction tube containing DMAB
Watson-Schwartz test

- PBG + dimethylaminobenzaldehyde (DMAB) + acid solution → Magenta color
Watson-Schwartz test: interferences

- Specificity: Urobilinogen in urine also produces magenta color in similar reaction condition

- Thus, Organic extraction is carried to remove these interfering substance

- Syringe contain an anion exchange resin which binds to PBG and other interference are wash-out
Watson-Schwartz test: interferences
The End

• Questions?