**Uricase and its clinical applications**

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**ABSTRACT**

Uricase is the therapeutic enzyme used to regulate the concentration of uric acid in the serum. The uric acid is the metabolic product of purine degradation. Normally the concentration of uric acid in the serum is 6.8 mg/dL, beyond this the hyperuricemia condition occurs. These then results into the inflammatory pain in the joints and tissues, the disease is called Gout. Currently 8.3 million people in the US, around 3% population are suffering from Gout. The concentration of uric acid varies according to age and sex. The enzyme uricase is not expressed in the human being, but it is produced by microorganisms, plants and other animals. The uricase not only used to detect uric acid level in serum and urine but also used to control its concentration. The uricase acts on uric acid and convert it into the more soluble compound allantoin. The uric acid is poorly soluble i.e. -11 mg/dL compared to allantoin which is -147 mg/dL. The uricase biosensors are developed to regulate the uric acid level.

**Uric acid + H_2O + O_2 \rightleftharpoons Allantoin + CO_2 + H_2O \ldots \text{Eqn No.1}**

Uric acid is poorly water soluble as -11 mg/dL compared to the product allantoin which has solubility -147 mg/dL. Hence allantoin is easily excreted through the urine.

**Table no.1 Physico-chemical properties of Uricase:**

<table>
<thead>
<tr>
<th>Physico-Chemical Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (Dalton)</td>
<td>145000-150000</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.3</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>9.5</td>
</tr>
<tr>
<td>Optimum Temperature (°C)</td>
<td>30-35</td>
</tr>
<tr>
<td>Denaturation Temp. (°C)</td>
<td>40-60</td>
</tr>
</tbody>
</table>

**Table no.2 Uricase Sources**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>8.5</td>
<td>35</td>
<td>42.77 U/ml</td>
<td>(13)</td>
</tr>
<tr>
<td>Microbacterium spp.</td>
<td>8.5</td>
<td>37</td>
<td>1.0 U/ml</td>
<td>(18)</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>5.5</td>
<td>30</td>
<td>7.1 U/ml</td>
<td>(19)</td>
</tr>
<tr>
<td>Streptomyces exfoliates</td>
<td>8.0</td>
<td>45</td>
<td>0.5 U/ml</td>
<td>(56)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>8.5</td>
<td>30</td>
<td>15.43 U/ml</td>
<td>(57)</td>
</tr>
<tr>
<td>Bacillus thermomutatus</td>
<td>8.5</td>
<td>25-80</td>
<td>1.25 U/ml</td>
<td>(7)</td>
</tr>
<tr>
<td>Giliovitax geng</td>
<td>8.5</td>
<td>25</td>
<td>275.98 U/ml</td>
<td>(58)</td>
</tr>
<tr>
<td>Gloeocystidium viride</td>
<td>7.5</td>
<td>30</td>
<td>63.14 U/ml</td>
<td>(59)</td>
</tr>
</tbody>
</table>

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can be observed that the fungus cultures are the overproducers of uricase. In addition to the microbial source, uricase is also reported from different leguminous plants as cowpea, soybeans, bean, and the leaf of chickepa, broad bean, wheat and tobacco leaves. Also uricase is found in leaves, stems, roots and seeds of flax plant (Linum usitatissimum).

2. Uricase Activity:

The uricase activity is assayed with respect to uric acid concentration either by disappearance of substrate uric acid detected by a decrease in the absorbance at 293 nm or increase in absorbance of product allantoin in presence of uricase.

The activity of purified uricase is inhibited by the metallic complexes and some heavy metals shown in table no 3. The 93% of inhibition of uricase activity is observed with Potassium thiocyanate at concentration of 10 Mm.

Table no. 3: Uricase activity inhibition by Compound:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibitory Conc. (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.02</td>
<td>75</td>
</tr>
<tr>
<td>Fe</td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>Zn</td>
<td>2.0</td>
<td>80</td>
</tr>
<tr>
<td>Co</td>
<td>1.0</td>
<td>60</td>
</tr>
<tr>
<td>Ni</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td>Potassium thiocyanate</td>
<td>10.0</td>
<td>93</td>
</tr>
<tr>
<td>Potassium ferrocyanide</td>
<td>15.0</td>
<td>10</td>
</tr>
</tbody>
</table>

2. Uricase Biosensor:

The biosensor can be developed to detect uric acid qualitatively as well as quantitatively by immobilisation of uricase either in matrix e.g. polypyrrole, or on gold/amino acid nanocomposites, or on chitosangraft polyaniline composite film or is covalently immobilized with the help of glutaraldehyde as a cross linker onto electrochemically synthesized polyaniline films. Due to the covalent attachment the efficiency and half life of the uricase is increased. The affinity of the Uricase towards the substrate uric acid is increased from 3.4 x 10^-1 mM/L to 5.1 x 10^-3 mM/L. The following electrochemical reaction takes place at the time of response measurement: The number of electrons generated is directly proportional to the substrate uric acid conversion by uricase.

The sensor is capable of detecting uric acid from serum at the rate of 100 serum samples in 94 min.-

Gout is one of the most common autoinflammatory arthritis disorder caused due to hyper accumulation of uric acid crystals (especially monosodium urate crystals (MSU)) in the joints and serum. This results into the development of Lesch Nyan syndrome. Recently it has been estimated that above 8.3 million people in United States at around 3% of the populations are suffering from gout. Along with MSU crystals two other types of crystals are also contribute to gout as calcium pyrophosphate dehydrate leads to pseudogout and basic calcium phosphate (BCP/hydroxyapatite). Bone erosion is commonly observed in the chronic gout disorder due to the accumulation of MSU which results into the joint damage and deformity. Uric acid is the end product of purine metabolism and the hyperuricemia condition arises after the deposition of uric acid beyond its solubility point.
6.8 mg/dL. However, the low concentration of uric acid acts as an antioxidant which scavenges serum free radicals and protects against cancer. The concentration of uric acid is regulated by the endogenous metabolism, reabsorption and excretion rate by the kidney. The most common cause of hyperuric acid accumulation is the reduced renal clearance. This results in the hypertension and increase in the kidney-related disorders. Along with these, alcohol consumption, obesity, hypertension, use of diuretic drugs and low dose of aspirin also contribute to the gout. Hypertriglyceridemia is being observed in 80% gout populations. The disease is most probably common in the middle-aged men and is increasing in the elder populations along with postmenopausal women. The current research on animals and humans conclude that the accumulation of monosodium urate crystals results into the stimulation of inflammatory response which indeed activates the nitric oxide, prostaglandins and proinflammatory cytokines as IL-1, IL-1β, which are produced by macrophages, dendritic cells, monocytes and inflammasome complex. Anti-inflammatory therapies including colchicines, nonsteroidal anti-inflammatory drugs, and glucocorticoids are commonly used decades ago for the treatment.

4. Classification of Gout:
The gout has been classified as
4.1 Primary gout:
It is being classified as an abnormal uric acid metabolism without any known symptoms.
4.2 Secondary gout:
It is caused by an acquired disorder.

5. Diagnosis of Gout:
The physical identification of gout can be done by observation to patient’s big toe. The interphalangeal joint of the big toe of skeletal remains of patient possesses cavitations, erosions and osteophytic margins. The abnormal metabolite concentrations in serum and urine are principle indicators of many pathogenic conditions including xanthinuria, hyperurecemia and gout.

The various diagnosing techniques are available as,
5.1 Conventional Radiography (CR)
The CR involves the passing of X-rays through the body part onto the flat detector which then produces the projectional image.
5.2 Ultrasonography (US)
Now a days, US is prominently used for the detection of gout which directly shows MSU crystal deposition in early gout. US of bones and joints is non-invasive, nonirradiating, and cheap comparatively to the other techniques.
5.3 Magnetic Resonance Imaging (MRI)
MRI have efficient sensitivity to detect bone erosion rather than US which detects MSU deposition.

Besides these, several other methods are there to determine the concentration of uric acid such as high performance liquid chromatography (HPLC), chemiluminescence, electrochemical method and UV-Visible spectroscopy.

5.4 Diagnosis and Quantification of Uric acid by Uricase
Based on the uricase enzymatic reaction, the concentration of uric acid in serum and urine is determined by measuring the absorption spectra at 293 nm by UV-Visible spectroscopy. The absorption spectra measures the decrease in concentration of uric acid after reacting with uricase.

6. Purine Metabolism Pathway (SS)

Table No. 6: Concentration of uric acid with age and sex
Table no. 6 shows the variability of concentration of uric acid with respect to age and sex. After attainment of menopause, the female uric acid concentration becomes nearly equal to the matured male. Hence the chances of MSU crystal formation are more in the males exceeding age 50.

7. Conclusion:
Hence it seems from this review article that the concentration of MSU crystals can be reduced into the soluble form i.e. allantoin with the help of uricase enzyme. The uricase can be produced on large scale by using above mentioned microbes. The enzyme can be used without any side effects in contrast to the drugs. The uricase biosensors are prominently used to measure uric acid level qualitatively as well as quantitatively. Hence finally it is concluded that the uric acid level in serum can be regulated by the use of uricase as a therapeutic drug.

REFERENCES


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