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Review Article

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Microbial Urate Oxidase as Anti Hyperuricemic Agent: Current Scenario and Future Prospects

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Abstract Urate oxidase catalyzes the degradation of uric acid, a product of purine disintegration pathway, into allantoin, carbondioxide and hydrogen peroxide. Owing to its efficacious uricolytic activity, urate oxidase employed to treat hyperuricemia and gout. Urate oxidase enzyme has been isolated from a wide range of organisms, including plants, animals and microorganisms. This review catalogues major sources of urate oxidase enzyme, its mechanism of action, modification, dosage and administration, past and present applications along with future prospects.

Keywords Urate oxidase, Uric acid, Gout, Applications

Introduction

A wide range of applications of non-invasive therapeutic methods in medical sciences have now been explored. Enzymes are specific biological catalysts and desirable therapeutic agents for the treatment of metabolic diseases. Enzyme therapy plays a critical role in the treatment of rare and deadly diseases. The development of modified enzyme derivatives has expanded the horizon of biopharmaceuticals. The biomedical studies on enzyme drugs *in vivo* in clinical practice have confirmed their effectiveness and prospects. Modified enzyme derivatives have recently been used as an effective agent in medical sciences against detrimental action of oxidative stress *in vivo* [1, 2, 3].

Uric acid is a product of purine metabolism associated with the breakdown of nucleic acids. Uric acid can also be produced from proteins. In most mammals, uric acid is degraded further to allantoin by urate oxidase, which is excreted freely through kidney in the urine. However, the urate oxidase gene became nonfunctional in human beings, great apes, and lesser apes during evolution and as a result they have higher serum uric acid levels [4].

Uric acid is admitted as a potent antioxidant and free radical scavenger in the blood plasma within normal limits 3.3 to 6.9 mg/dL [5]. However, an increase in uric acid concentration above these limits results in hyperuricemia. Sodium urate crystals form secondary to hyperuricemia. Accumulation of monosodium urate crystals symbolizes gout, which is an inflammatory joint ailment. Other ailments associated with this condition are cardiovascular risk, Lesch-Nyhan syndrome, renal manifestation and some organic acidemias [6, 7, 8].

The development of the urate oxidase enzyme as an effective protein drug for the treatment of gout is a remarkable achievement. Urate oxidase isolated from yeast-like fungal endosymbiont of the brown planthopper, *Nilaparvata lugens* was as active as those from plants and animals [9]. Urate oxidase is a clinical enzyme effective for the oxidation of uric acid crystals in gout disease [10].

Uric acid lowering approaches and their limitations

Hyperuricemia has been established as the major etiologic factor in gout. Xanthine oxidoreductase (XOR) becomes an effective target of drugs for the treatment of gout and hyperuricemia-related diseases as it is a critical enzyme to produce uric acid. Accordingly, XOR-inhibitor such as allopurinol and febuxostat are extensively used for the treatment of gout (Table 1). Allopurinol, a hypoxanthine analog, is hydrolyzed by XOR to oxypurinol. Oxypurinol binds tightly to the reduced molybdenum ion present in the enzyme and thus inhibits the synthesis of uric acid. But continuous use of allopurinol has significant adverse effects. The adverse effects of allopurinol include induced-hypersensitivity reactions, peripheral neuritis, interstitial nephritis, renal toxicity and congenital malformations when used during pregnancy [11].

Febuxostat is an oral, non-purine xanthine oxidase inhibitor which is available as the doses of 40 and 80 mg in the USA and 80 and 120 mg in Europe. The maximum doses approved in the USA and Europe is 80 and 120 mg/d, respectively. Rare and early liver or kidney hypersensitivity reactions and benign skin rashes are few side effects associated with administration of febuxostat [12]. Febuxostat is able to inhibit both the oxidized Mo (VI) and reduced Mo (IV) forms of xanthine oxidoreductase, thus resulting in a more effective blockade of uric acid. Febuxostat has greater hypouricemic activity and less toxicity than allopurinol. However, febuxostat can also lead to hypersensitivity reaction, hepatotoxicity and rhabdomyolysis. Therefore, there has been great effort to develop new drugs with less or no toxicity for the long-term prevention of these hyperuricemia-related diseases [11].

A non-recombinant urate oxidase under trade name Uricozyme ^{TM} was introduced in France since 1975 and in Italy since 1984. Urate oxidase was derived from a fungal culture of *Aspergillus flavus.* It was limited in use because of its slow production and scarce purity. Its proteic nature, poor process of purification and administration made hypersensitivity reactions very probable. The immunogenicity and hypersensitivity were due to high number of impurities in the preparations. The limitation of drug efficacy was due to immunogenicity which leads to the production of antibodies [13].

Table 1: Different urate-lowering drug therapy

S. No.	Type of Drug	Examples
1	Uricostatic drugs	Allopurinol
		Oxipurinol
		Febuxostat
2	Uricosuric drugs	Losartan
		Probenecid
		Sulfinpyrazone
		Benzbromarone
3	Uricolytic drugs	Uricozyme (urate oxidase)
		PEG-modified urate oxidase
		Recombinant urate oxidase

Mechanism of action of urate oxidase

Urate oxidase (urate oxygen oxido-reductase, EC 1.7.3.3) is a vital enzyme associated with the purine disintegration pathway. Urate oxidase is highly specific towards uric acid and catalyzes the oxidative opening of the purine ring of uric acid to produce allantoin, carbondioxide and hydrogen peroxide (Fig. 1). The byproduct allantoin so formed is five to ten times more soluble than uric acid and undergo easy renal elimination. The accumulation of uric acid is a causative factor of gout in humans. Hence, the action of urate oxidase on the urate crystals helps in dissolution and excretion of uric acid [14]. Urate oxidase has its application in the production of low purine food content. It also acts as a mild oxidizing agent in oxidation based hair dyes [15, 16].

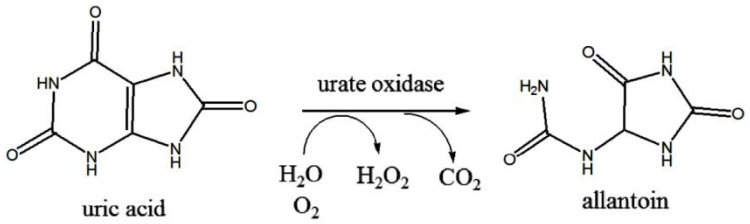

Figure 1: Action of urate oxidase on uric acid

Sources of urate oxidase enzyme

Microorganisms have proved to be very efficient and economical source of urate oxidase enzyme owing to their economic cultivation, stability, flexibility in process modification and optimization [17]. All these characteristics facilitate the large scale microbial production of enzymes. Urate oxidase is widely distributed among the plants and animals. Other sources for the isolation of enzymes include bacteria, fungi, actinomycetes and algae. Different producers of urate oxidase have also been listed (Table 2).

Table 2: Various sources of urate oxidase

Source	Reference	Source	Reference
Bacteria			
Bacillus cereus	[18]	*Mycobacterium intracellulare*	[23]
B. firmus	[19]	*Psuedomonas acidovorans*	[24]
B. thermocatenulatus	[20]	*P. aeruginosa*	[25]
Comamonas sp.	[21]	*P. putida*	[26]
Kleibsella pneumonia	[22]	*Sphingobacterium thalpophilum*	[27]
Animals			
Camelus dromedarius liver	[28]	*Rana catesbeiana*	[31]
Rat hepatic cells	[29]	House musk shrew liver	[30]
Guinea pig liver	[30]	Ox kidney	[32]
Plants			
Lotus japonicas	[33]	*Phaseolus vulgaris*	[36]
Glycine max	[34]	*Medicago sativa*	[37]
Sesbania exaltata	[35]	*Cicer arietimum*	[38]
Blue green algae			
Chlamydomonasreinhardtii	[39]		
Actinomycetes			
Arthrobacter globiformis	[40]	*S. graminofaciens*	[43]
Nocardia farcinica	[41]	*S. alboniger*	[42]
Streptomyces orientalis	[42]	*S. citreus*	[42]
S. albidoflavus	[43]	*S. corchorusii*	[42]
Fungi			
Candida tropicalis	[44]	*Puccinia recondite*	[48]
C. utilis	[45]	*Rhizopus oryzae*	[49]
Aspergillus terreus	[46]	*Gliocladium viride*	[50]
A. flavus	[46]	*Neurospora crassa*	[51]
Mucor hiemalis	[47]	*Trichoderma* sp.	[46]
Recombinant and mutated strains			
Hansenula polymorpha harboring *C. utilis* urate oxidase gene	[52]	*Arxula adeninivorans* recombinant urate oxidase	[16]
E. coli harbouring *A. flavus* urate oxidase gene	[53]	Urate oxidase mutant of *Arabidopsis thaliana*	[54]

Bacterial sources

Urate oxidase production in *B. cereus* strain DL3 has been described by [18]. The production of urate oxidase of *B. cereus* has also been reported by [10]. They characterized surfactants mediated urate oxidase production. A thermostable urate oxidase has been further characterized from the bacteria *B. thermocatenulatus* by [20]. Screening, enrichment and optimization for urate oxidase production by isolating *B. firmus* DWD-33 from soil was reported by [19]. *Comamonas* sp. has found to be a good source of urate oxidase [21]. A FAD-dependent urate oxidase enzyme has been characterized from *K. pneumonia* [55]. Urate oxidase production was also recorded from *Micrococcus*, *P. otitidis* and *Brevibacterium* [6, 56, 57].

Fungal sources

A pioneer analysis in the regulation of enzymes from the purine catabolic pathway in member of the zygomycetes was done by [49]. They demonstrated that the control of *R. oryzae* urate oxidase enzyme differs from that found in *N. crassa* and *A. nidulans*. The minimal medium containing NH4Cl as sole nitrogen source fail to exhibit urate oxidase activity in *R. oryzae* but was found to induce by exogenous uric acid. The two chromium uric acid complexes were found superior to free uric acid as sole source of nitrogen and act as inducer for urate oxidase production form *N. crassa* [58]. Urate oxidase production was reported in *R. oligosporus, Agaricus bisporus*, *A. carbonarius*, *A. sydowii*, *Botrytis fabae*, *Alternaria altrnata* and *Phytophthora palmivora*, *Curninghamella echinulata* and *Mortierella* sp [59]. Similar urate oxidase production from *R. nigricans*, *Cunninghamella* sp. and *Mucor* sp. was recorded by [60]. The production of constitutive urate oxidase by isolated *P. chrysogenum* was reported by [61]. Further, *B. cinerea*, *A. porri*, *Gliocladium* sp., *Neurospora* sp., *Cladosporum herbarium* and *T. koningi* were also reported for urate oxidase production [60, 62].

Plant sources

Urate oxidase from nodules of a model legume, *L. japonicas* was reported by [34]. They also detected urate oxidase in nonsymbiotic tissues particularly in roots. The study conducted by [63] demonstrated the presence of a single copy of the urate oxidase producing gene in the chickpea genome and its expression not only in nodules, but also in leaves and roots. Urate oxidase isolated from *P. vulgaris* revealed 88.8% identity with soybean urate oxidase [37]. The activity of urate oxidase obtained from soybean root nodules was not inhibited by amino acids, ammonium, adenine and allopurinol which were in contrast with results reported for the cowpea enzyme [64]. The urate oxidase from non-symbiotic tissue of soybean cotyledons was first reported by [65] and demonstrated that the urate oxidase in soybean cotyledons is identical to that in soybean nodules. The cofactor less urate oxidase has been reported from soybean root nodules [66]. The roots of corn and tobacco had a very low urate oxidase activity [67], while only a small amount of urate oxidase was later detected in pea and soybean leaf extracts [68].

Animal sources

The crystalline urate oxidase from hepatic microbodies of rat was prepared by [29]. The distribution of urate oxidase was reported in liver of five mammals, namely dog, bovine, horse, house musk shrew and guinea pig. The evidence availed by immune-electrophoresis indicates that the urate oxidase purified from the house musk shrew is considerably different from the other five mammals tested. The results obtained from immune-diffusion indicate that hog urate oxidase has four sites of partial antigens while the enzyme of other mammals has only part of them [30]. The characterization of urate oxidase from ox kidney was carried out by [32]. They suggested that the enzyme is very similar to pig liver urate oxidase.

Actinomycetes as a source

Actinomycetes considered as an important source for production of medically important enzymes and bioactive metabolites. The strains of *S. alboniger*, *S. sclerotialus*, S. *orientalis*, *S. citreus* and *S. corchorusii* were known urate oxidase producers. The effect of gamma radiation on the urate oxidase activity was conducted and it was found that low gamma radiation doses stimulate the enzyme production by *S. sclerotialus* and *S. corchorusii* [42]. In addition to *Streptomyces* sp., *A. globiformis* was also reported to produce urate oxidase [41].

Engineered microorganism as a source

The commercially available urate oxidase preparations purified from *B. fastidiosus*, *A. globiformis* and *C. utilis* were expensive. It is used for construction of urate-selective amperometric biosensors. The construction of the urate oxidase-overproducing recombinant microorganisms helps to reduce the production cost of urate-selective amperometric biosensors which are used in clinical diagnosis of uric acid levels. The construction of engineered microorganisms has made possible the fabrication of cheap and reliable urate-selective amperometric biosensors. Recombinant *H. polymorpha* overproducing urate oxidase was constructed by genomic integration of urate oxidase gene. The urate oxidase activity enhanced 40-fold compared to the parental strain [69]. Successful expression of urate oxidase gene of *C. utilis* in *H. polymorpha* strain was carried out by [52]. The production of recombinant urate oxidase has reached 52.3 U/ml extracellularly and 60.3 U/ml intracellularly in fed-batch fermentation after 58 h of induction period which are much higher than the earlier reported expression systems. This was the first report about the heterologous expression of urate oxidase in *H. polymorpha*. *E. coli* harboring *A. globiformis* urate oxidase gene produced 20-fold higher urate oxidase than the original *Arthrobacter* strain, even in the absence of an inducer [53]. Urate oxidase from *A. flavus* was expressed in the yeast *S. cerevisiae* in a soluble and active form [70]. The researchers cloned and expressed the *B. subtilis* urate oxidase gene in *E. coli.* The purified enzyme showed an optimal reaction temperature and pH of 37^{o}C and 8.0, respectively and retained 90% of its activity even after 72 h of incubation at -20^{o}C and 4^{o}C [71].

Mode of production and optimization process

An attempt was made to optimize the production of urate oxidase by *B. subtilis* using submerged fermentation. The culture conditions found to be ideal for maximum urate oxidase production were pH 7.0, temperature 30°C, sucrose 1% (w/v) and peptone 0.2% (w/v) and incubation time 24 h. Sucrose and peptone were proved to be the best carbon and nitrogen source for maximum urate oxidase activity [72].

The nutritional requirement for the urate oxidase production from *C. testosteroni* was analysed by [7]. They performed the production in shake flasks. The maximum urate oxidase activity obtained at pH 7 and 250 rpm. Cellulose as carbon and peptone as nitrogen sources were proved to be best for urate oxidase production. The researchers [73] demonstrated that dextrose acts as compromising carbon source for urate oxidase production by *P. aeruginosa.* Sucrose was found the best carbon source for urate oxidase production in *A. flavus* [74]. Lactose obtained as an ideal carbon source for urate oxidase production [75]. The maximum urate oxidase production from *A. niger* was obtained with peptone as nitrogen source [76]. Peptone was also observed to enhance urate oxidase activity of *G. viridae* [50].

The optimization of production level of urate oxidase from *S. rochei* was performed by [77]. Plackett-Burman experimental design and response surface methodology was used to determine the combined effect of different process parameters. Plackett-Burman experimental design was employed to screen fifteen variables. Incubation time, medium volume and uric acid concentration were identified as most significant positive independent variables affecting urate oxidase production. The predicted urate oxidase production by *S. rochei* after central composite design was 47.49 U/mL. There was three fold hike in enzyme production as compared to the unoptimized medium (16.1 U/mL). The Plackett-Burman design was also employed to enhance the production of urate oxidase by *P. aeruginosa*. Among fifteen tested variables; pH, $CuSO_4$ and $FeSO_4$ were identified as most effective based on their high significant effect on urate oxidase production. Using this method, a near optimum medium formulation was selected with 15-folds hike in urate oxidase yield. Response surface methodology was used to adopt the best process conditions. The optimal combination of media constituents obtained for urate oxidase production was as follows: pH 5.5, $CuSO_4$ (10^{-3} M) and $FeSO_4$ (10^{-2} M). The maximum predicted urate oxidase activity was 7.1 U/ml/min, which was 16.5 times higher than the unoptimized medium [78].

Urate oxidase in recombinant form

Rasburicase® is a recombinant urate oxidase form of *A. flavus* which was expressed in *S. cerevisiae*. It has been evaluated in children suffering malignancy. It was found to be more effective than the non-recombinant product. The capability of recombinant urate oxidase to reduce uric acid levels in serum and check development of urate

crystals has been confirmed by some surveys [13, 79, 80]. The researchers showed that Rasburicase® is effective in lowering urate oxidase without the need for dialysis in patients undergoing treatment [81].

Table 3: Various modes of urate oxidase purification

Sources	Techniques used	Purification fold	Recovery (%)	Specific activity (U/mg protein)	Reference
Enterobacter cloacae	Sephadex G-200, DEAE-cellulose, DEAE-Sephadex	13	- - -	6.6	[82]
Aspergillus niger	Sephadex G-200, Sephadex G-100, DEAE-Sephadex	17.8	11.11	105.9	[83]
Psuedomonas aeruginosa	DEAE-Cellulose ion exchange, Sepharose 6B	22.21	16.40	2337.5	[84]
Microbacterium sp.	DEAE-cellulose ion exchange, Toyopearl HW-65, Sephdax G-75	19.70	31	5.32	[85]
Sphingobacterium thalpophilum	CIM-CM monolithic disk	14	80	- - -	[28]
Puccinia recondite	DEAE-cellulose, xanthine-agarose	- - -	103	8.4	[49]
Caprine kidney	Ion-exchange, gel filtration chromatography	10	- - -	700	[86]
Bacillus subtilis	pMAL-c2 system with cross linked amylase, DEAE-Sephacel column	- - -	- - -	13.9	[87]
Gliomastix gueg	DEAE-cellulose column	33.20	17.60	428.70	[88]

Purification of urate oxidase

Urate oxidase is used in the form of injectable drug for the treatment of hyperuricemia and gout in humans. The sensitivity of the application demands high degree of purity of this enzyme. As the desired enzyme generally found associated with undesirable macromolecules, different purification techniques were used for the purification of urate oxidase from various microorganisms. Different purification techniques adopted were as follow:

- Organic solvent precipitation,
- Ammonium sulphate precipitation,
- Gel filteration chromatography and
- Affinity chromatography

The different strategies of urate oxidase purification have been summerised in Table 3. The Ni-NTA column was used to purify the recombinant urate oxidase obtained from *B. Subtilis.* The specific activity of purified enzyme (60 kDa) increased to 2.1-fold with 56% recovery. MALDI-TOF analysis unveiled molecular mass of 58 kDa which was in agreement with the expected mass of the recombinant enzyme [71]. The Sephadex G200 and DEAE-cellulose DE52 chromatography were used by [89] to purify urate oxidase from *Candida* sp. The molecular weight determined by the gel filtration and SDS-PAGE was found to be 70-76 kDa. The recovery ratios were from 96 to 101%. The purification of urate oxidase from *C. utilis* was also performed by using aqueous two-phase system consisting of PEG/$(NH_4)_2SO_4$. The 25% (v/v) PEG2000, 9% (w/w) $(NH_4)_2SO_4$, 2% (w/w) NaCl at pH 7.5 were used to form aqueous two-phase system and addition of 5% (v/v) crude enzyme showed the best separation capability with recovery rate of 93% and 10-fold purification [90].

In case enzyme to be purified is intracellular, cell disruption was necessary to purify enzyme. The intracellular urate oxidase of *S. exfoliatus* UR10 was purified using DEAE cellulose followed by carboxymethyl-cellulose and Sephadex G-75 chromatography. The purified urate oxidase showed a single protein band of 44 kDa in SDS-PAGE [91]. However, the urate oxidase purified from *Microbacterium* sp. strain ZZJ4-1 applying DEAE-cellulose ion

exchange, hydrophobic and molecular sieve chromatography found to exhibit molecular mass of 34 kDa on SDS-PAGE [85]. Ox kidney urate oxidase was purified to 3800-fold by n-butanol extraction followed by gel filtration and ion-exchange chromatography [32]. Urate oxidase was purified from *N. crassa* to nearly 1000-fold to homogeneity. The urate oxidase obtained was a tetramer composed of four identical subunits. The molecular weight of native urate oxidase was 123 kDa [92]. Salting in crystalisation approach was provided by [93] as an alternative to chromatography for purification of urate oxidase. The study revealed that crystallization approach to purification offers advantage in reducing cost and for developing effective industrial process. Due to its strong salting-in effect, urate oxidase can be purified using crystallization as effectively as purified by current chromatography techniques.

Urate oxidase applications

Application of urate oxidase in clinical determination of uric acid

Urate oxidase has been widely used to clinically diagnose uric acid levels in blood serum and urine samples. Immobilised urate oxidase used to construct uric acid biosensor having good stability, considerable shelf-life and response similar to those obtained by a commercial colorimetric assay kit [94]. Uric acid biosensor was fabricated by [95], consists of urate-immobilized eggshell membrane and an oxygen electrode. The biosensor detected depletion of dissolved oxygen level in uric acid solution on exposure of urate oxidase. The decrease in oxygen level was monitored to determine the concentration of uric acid. The uric acid biosensor has a linear response range of 4.0-640 μM, detection limit of 2.0 μM (S/N = 3) and response time of less than 100 s. The biosensor exhibits extremely good stability with a shelf-life of at least 3 months. Glucose, urea, ascorbic acid, lactic acid, glycine, DL-α-alanine, DL-cysteine, KCl, NaCl, $CaCl_2$, $MgSO_4$, and NH_4Cl are common potential interferents in the samples showed no interferences on the response of the uric acid biosensor. An amperometric biosensor was constructed by covalently immobilizing urate oxidase to the carboxyl groups of the mixed self-assembled monolayer of 11-mercaptoundecanoic acid and 3-mercapto-propionic acid through carbodiimide mediated coupling reaction. This immobilized urate oxidase assembled on 1 cm^2 area of indium-tin-oxide-glass plate. The chrono-amperometric response was measured in order to determine the uric acid concentration in aqueous solution (pH 7.4). This biosensor exhibits a linear response over a concentration range of 0.07 to 0.63 mM with a sensitivity 19.27 μAmM^{-1} and a response time of 25 s with excellent reproducibility. Interfering reagents such as ascorbic acid, urea and glucose did not influence these results [96].

Application of urate oxidase in treatment of gout and tumour lysis syndrome

Gout characterized by dysmetabolism which results in the formation and deposition of monosodium urate crystals. This condition is connected with acute joint pain due to the deposition of monosodium urate crystals in the synovial fluid. Due to this condition, skin and kidney may also be affected by cellulitis, tophaceous deposits, urate nephropathy or kidney stones [97, 98]. A stern consequence of progressive gout is treatment-failure gout (TFG) which results from refractoriness to practicable urate-lowering therapy used to check the deposition of urate crystals by regulating uric acid levels of serum in a sub-saturating range. Clinical characterization of TFG includes painful arthritis, chronic arthropathy, destructive tophi, impaired quality of life and chronic disability. Hyperuricemia and gout related disorders are frequent among patients with TFG. Polypharmacy often applied to treat severe gout and a high burden of cardiovascular and metabolic comorbidities which impart strains in the treatment of TFG [99]. Pegloticase® a PEG-conjugated mammalian (porcine-like) urate oxidase demonstrated marked lowering of serum uric acid and a rapid decrease in the size of tophi [100]. Tumour lysis syndrome (TLS) is a life-threatening complication which arises from tumour cell breakdown. This condition is characterized by hyperuricaemia, hyperkalaemia, hyperphosphataemia, and hypocalcaemia. These associated complications result in cardiac arrhythmias, neurological dysfunction, renal insufficiency or failure and even death [101, 102]. Rasburicase® a recombinant urate oxidase effectively improved outcomes in children and adolescents who were at high risk for tumour lysis syndrome [103].

Role of urate oxidase in production of low purine food content

Hyperuricemia and its symptoms are becoming common worldwide. Increased uric acid synthesis from food constituents and reduced renal excretion are the primary causes for elevated serum uric acid levels [11]. During the

production process in the factory the food could be depleted of purines; however a better way would be an addition of urate oxidase enzyme to spice mixtures which are applied to food during preparation. Another convenient way is to produce encapsulated enzymes to be consumed before a meal which then degrade uric acid during digestion in the intestines [104]. Another approach is to degrade uric acid in human gut using a common inhabitant of human intestine or probiotic bacteria. The gene of *C. utilis encoding urate oxidase was cloned. It contained* 303 amino acid residues with molecular mass of 34 kDa. *This c*loned urate oxidase gene from *C. utilis* was recombined in the plasmid of a common inhabitant of human intestine, *Lactobacillus Bulgaria*. *The L. bulgaria* colonize human gut with urate oxidase, which degrade uric acid before it reaches the blood stream. The recombinant plasmid from *L. bulgaria* contained urate oxidase gene with molecular weight of 34 KDa and has enzyme activity up to 0.33 μ/mL [105].

Role of urate oxidase in oxidation based hair dye

Earlier in oxidation based hair dyes, mixture of an oxidation dye and an oxidizing agent was used for colour development where oxidation process helps to polymerize the oxidation dye. The oxidizing agent used in the oxidation hair dyes damaged hair to a great extent because of its strong oxidizing force. To solve this limitation urate oxidase enzyme was utilized in oxidation hair dyes so that hair dyes have mild oxidizing action. They utilized the enzymatic oxidation reaction for hair dyeing. This one-package oxidation hair dye utilizing urate oxidase enzyme has one difficulty. The substrate for urate oxidase enzyme that is uric acid was not easy to dissolve. In order to overcome this problem, solubilized uric acid was used. This step enhanced the excellence of hair dyeing properties. The oxidation hair dyes accomplished with urate oxidase were found effective even with the lowered dye concentration [15].

Modification of urate oxidase

Higher activity, negligible immunogenicity and a long circulation half-life *in vivo* are few salient features that protein drugs must possess. To achieve these characteristic features, various modifications and approaches such as PEGylation, site-specific PEGylation, molecular engineering have been employed. These often used to reduce immunogenicity and improve the pharmacokinetics of protein drugs [106, 107]. Activated PEG(2) synthesized using monomethoxypolyethylene glycol and cyanuric chloride. Activated PEG(2) used to modify urate oxidase purified from *C. utilis*. It modified approximately 36 out of the total 98 amino groups in the urate oxidase molecule. Modification enhanced the retention of high enzymatic activity (45% of native urate oxidase) and results in complete loss of the urate oxidase binding ability towards antiurate oxidase serum from rabbit. Hence, results in much slower clearance of modified urate oxidase from plasma than native urate oxidase [108]. Small number of strands of PEG may be conjugated with urate oxidase by a stable, nontoxic, covalent linkage. There exists different type of linkages such as amide linkages, urethane linkages and secondary amine linkages. Urethane linkages to urate oxidase obtained by incubating urate oxidase in the presence of the succinimidyl carbonate or 4-nitrophenyl carbonate derivative of PEG. Amide linkages to urate oxidase formed by using an N-hydroxysuccinimide ester of a carboxylic acid derivative of PEG. Secondary amine linkages formed by reductive alkylation using PEG aldehyde and sodium cyanoborohydride or using 2, 2, 2-trifluoroethanesulfonyl PEG. Each different form of urate oxidase may have a different optimum with respect to both the size and number of strands of PEG [109]. Study conducted by [110] made use of molecular dynamics simulation approach to improve the structure of a resurrected mammalian urate oxidase having higher enzymatic activity compared to other mammalian counterparts. To overcome the growing concerns of relatively low catalytic activity and therapeutic effect of urate oxidase under physical conditions, a superior *in vivo* urate oxidase delivery system with enhanced stabilities and catalytic activities was developed using a lipid enzymosomal membrane [111, 112, 113].

Dosage and mode of administration

The PEG-urate oxidase conjugates may be administered through injection by intradermal, intravenous, subcutaneous, intraperitoneal or intramuscular routes or inhalation of an aerosolized preparation. The level of uric acid and the size of the individual, determine the effective dose of PEG-urate oxidase [109]. Research conducted by [114] demonstrated the possible use of oral urate oxidase therapy to reduce high concentrations of uric acid in the plasma. Urate oxidase obtained from *C. utilis* orally administered in the pig model found to reduce the uric acid

concentration in blood plasma. This study suggested that the decline in plasma uric acid concentration occurred due to enhanced gut elimination and not kidney excretion of uric acid. Administration of exogenous urate oxidase has been reported to decrease plasma uric acid concentrations, but there is lack of reports showing the impact of oral urate oxidase therapy on plasma uric acid concentrations. The oral administration of urate oxidase has advantages that it is neither immunogenic nor liver or nephrotoxic, as it is not absorbed into circulation. The urate oxidase given orally would enhance intestinal secretion due to the existence of a uric acid gradient between the circulation and gut lumen. Further studies are needed to estimate the kinetics of orally administered urate oxidase [115, 116]. The study conducted by [114] on oral urate oxidase therapy has certain limitations. The porcine model does not fully mimic human physiology as liver urate oxidase is present in pig. Analysis of concentration of uric acid in the portal vein of nephrectomized pigs and the stool should be performed to fully support the hypothesis that uric acid is eliminated via the intestine in pigs with hyperuricemia.

Future prospects

The discovery of urate oxidase effectiveness to mobilize the already existing uric acid in blood serum has set a milestone in the treatment of chronic gout which is refractory to the available drugs. In contrast, the available drugs have tended to prevent only the future production of uric acid in humans. After this discovery, detailed research on the enzyme has been carried out. The recombinant urate oxidase was found to be effective in lowering urate oxidase without the need for dialysis in patients undergoing treatment. But, it has been noticed that the action of enzyme is coupled with some side effects. So, it created the need to discover new sources and techniques to enhance the yield and decrease the side effects of the enzyme. Moreover, urate oxidase has spread its arms in the food industry also, as an ingredient which significantly reduces the uric acid content in the food items. As gout is a much more complex issue, it is need of time that the pharmaceutical industries should dig a lot more to make it a better drug for mankind.

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Conflicts of interests

The authors have declared that no conflict of interest exists.

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