

Inhibition of Partially Purified Xanthine Oxidase Activity from Sera of Patients with Gout

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ABSTRACT

In this research, xanthine oxidase (XO) was partially purified from sera of patients with gout, by dialysis and anion exchange chromatography techniques. One protein peak of XO activity was obtained with specific activity (0.149) unit/mg protein and with purification fold of (496.66) compared to crude enzyme.

Inhibition of partially purified XO was studied by allopurinol, caffeine and allopurinol with caffeine as a prodrug. The lower concentration of each inhibitor which exhibited maximal inhibitory effect was (10^{-7}) mM. The inhibition type of XO by using these inhibitors at these concentrations competitive inhibition.

The results show that K_m value without inhibitors was (40) mM. However, was K_m values were (58.8), (100), and (125) mM with inhibitors respectively, and V_{max} value remained stable (0.135) unit/ml. The inhibition constant K_i values were (0.68), (0.4) and (0.32) mM, respectively.

Accordingly, these results indicate that inhibitory effect of allopurinol with caffeine was better than caffeine, and caffeine was better than allopurinol. We suggest the intake of herbs and plants containing caffeine alone and/or with allopurinol drug to prevent or treat gout.

$$\frac{(496.66)}{(10^{-7})} \cdot \frac{(XO)}{XO} = \frac{(0.149)}{(40)} \cdot \frac{XO}{K_m}$$

$$\frac{(0.32) (0.4) (0.68) K_i}{(125) (100) (58.8) K_m} / \frac{(0.135)}{V_{\max}}$$

INTRODUCTION

Xanthine oxidase (XO) converts hypoxanthine to xanthine, and xanthine to uric acid. In mammals, this enzyme occurs almost exclusively in the liver and small intestine mucosa (Voet and Voet, 1990).

Xanthine oxidase binds to the surface of vascular endothelial cells by sulfated glycosaminoglycans and produces superoxide anion (Adachi et al., 1993). XO plays a role in the vascular dysfunction that occurs in atherosclerosis (White et al., 1996).

Xanthine oxidase plays an important role in the generation of free radicals in diabetes and this enzyme increase in liver (Desco et al., 2002) and it is believed to be a source of reactive oxygen species in the failing heart (Jennifer et al., 2005).

Xanthine oxidase activity was found significantly elevated in chronic obstructive pulmonary diseases (COPD) compared with healthy subjects (Ichinose et al., 2003).

Inhibition of XO with intravenous allopurinol has been demonstrated in animals and human heart failure to improve mechanical efficiency and in some reports contractile function of the myocardium (Cappola et al., 2001; Ukai et al., 2001).

Recent data have suggested that chronic inhibition of XO improves survival and cardiac function in postischemic cardiomyopathy (Engberding et al., 2004; Stull et al., 2004).

The chronic inhibition of XO can alter the progression of heart failure in dilated cardiomyopathy (Jennifer et al., 2005). XO inhibition can inhibit germ cell apoptosis induced by experimental cryptorchidism, and may be infertility associated with heat stress (Kumagia et al., 2006).

The aim of research is partial purification of XO from gout patients serum. Then, study of activity inhibition by drug or existing compounds in many herbs and plants for using as disease treatment or for prevention.

MATERIALS AND METHODS

Collection of blood samples: Venous blood samples were drawn from patients with gout from Ibn-Sina hospital, Mosul city, and by a range (0.5-1) milliliter for one sample.

Blood serum isolation: the serum is isolated by putting tubes in a water bath at (37) °C for (10) min, and centrifuged at 1008xg for (10) min. The supernatant was taken to conserve in freezing (Zhang et al., 1998).

Determination of protein: Biuret method was used to determine total protein concentration (Wootton, 1974).

Xanthine oxidase assay: The rate of formation of uric acid from xanthine is determined by measuring the increased absorbance at 290 nm. A unit is defined as forming one micromole of uric per minute at (25) °C; the molar absorptivity of uric acid is 1.22×10^4 (Westerfield et al., 1959).

Dialysis: six milliliters of serum was dialyzed for about (5) hrs. against (50)mM of phosphate buffer solution of pH=7.5 at (4) °C.

Fractionation on DEAE-Cellulose column: the dialyzed enzyme solution was applied on DEAE-Cellulose anion exchanger column (2.5×40)cm, which has been equilibrated with gradient phosphate buffer (50-250)mM of pH=7.5. fractions of (6)ml volume were collected. Flow rate was approximately 72ml/hr.

Inhibition of XO: Xanthine oxidase activity was inhibited by addition of 0.2 ml of inhibitor to 0.1ml of enzyme and incubated for 10 min at (25) °C.

RESULTS AND DISCUSSION

Table (1) shows purification steps of XO. The specific activity of crude enzyme was (0.0003) unit/mg protein, after dialysis became (0.0005) unit/mg protein. Figure (1) explains the elution profile of purified XO by ion exchange chromatography. We obtained a one peak at elution volume (54-132) ml with a specific activity (0.149) unit/mg protein.

Table 1 : purification XO steps from serum patients with gout.

Purification steps	Volume (ml)	Protein concentration (mg/ml)	Total protein (mg)	Activity (U/ml)	Total activity U*	Specific activity (U/mg--)	Yield %	Purification fold
Crude	6	75.454	452.724	0.0245	0.147	0.0003	100	1
Dialysis	5.5	72.727	399.99	0.04	0.22	0.0005	149.65	1.66
Ionexchang DEAE-Cellulose peak I	80	7.575	37.875	0.071	5.68	0.149	3863.94	496.66

*U: A unit is defined as the formation of one micromole of uric acid per minute at (25) °C

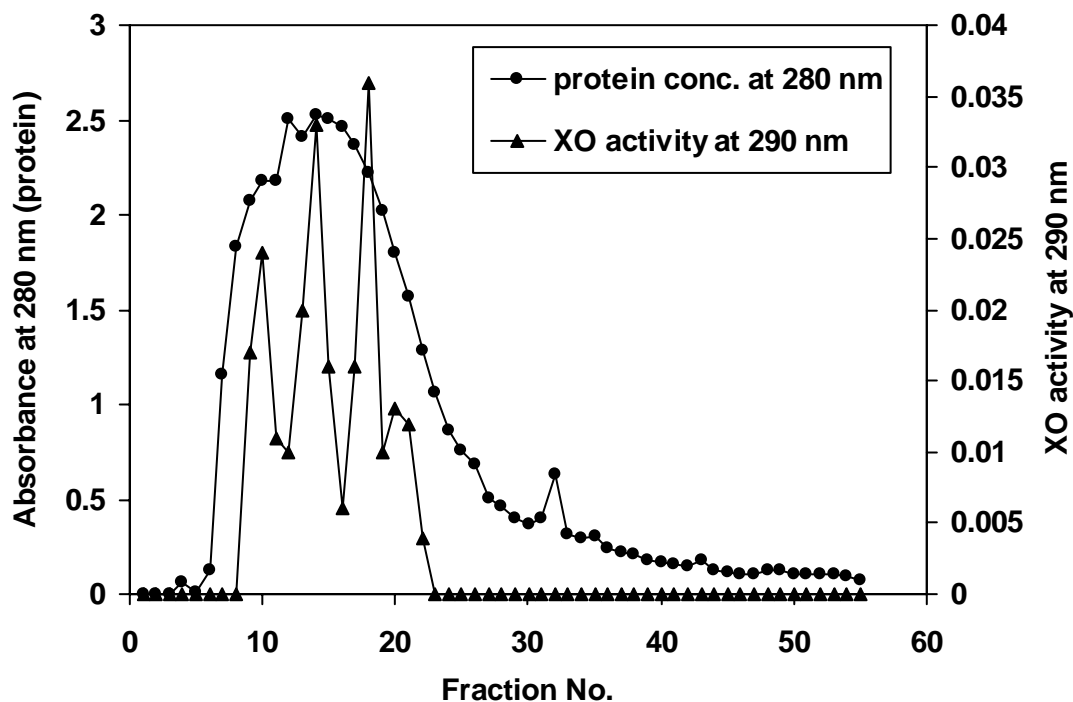


Figure 1 : Purification of XO by DEAE-cellulose chromatography.

Inhibition of XO activity:

Xanthine oxidase activity was inhibited by allopurinol, caffeine and allopurinol with caffeine as a prodrug, with concentration (10^{-1} - 10^{-7}) mM. The lower concentration of all inhibitors that exhibited maximal inhibitory effect was (10^{-7}) mM.

Allopurinol an inhibitor of XO widely used in clinical practice. We suggest that inhibition of XO by allopurinol decreases uric acid and oxidative stress (which induced free radical by enzymatic mechanism of XO), fractions may be effective in preventing oxidation of glutathione and lipoperoxidation which increasing a complications of gout.

The oxidative stress plays a role in the development of complications diabetes (Baynes, 1991).

Treatment with allopurinol decreases oxidative stress in type 1 diabetes patients, glutathione oxidation and the increase in lipoperoxidation are prevented (Desco et al., 2002).

Allopurinol and its metabolite oxypurinol showed considerable promise in the treatment of some disease for examples both experimental animals and in small-scale human clinical trials (Pacher et al., 2006).

Allopurinol could limit infarct size in a reperfusion preparation of myocardial infraction (Reimer and Jennings, 1985). Allopurinol administration of COPD subjects significantly decreases XO activity (Ichinose et al., 2003).

The inhibition of XO activity by caffeine is due to its structure similarity to that of the substrate, xanthine.

Caffeine is the most frequently used psycho-stimulant drug. It exerts its effect by the blockade of adenosine in the brain (Burbiel et al., 2005) as well as that caffeine clinically used for the treatment of peripheral vascular diseases.

Lineweaver-Burk plots (Figures 2,3,4) show a competitive inhibition of XO by using allopurinol, caffeine and allopurinol with caffeine. K_m value without any inhibitor was (40) mM, however K'_m values were (58.8), (100) and (125) mM with above inhibitors, respectively.

V_{max} value is invariable (0.135) unit/ml. the inhibition constant (K_i) values were (0.68), (0.4) and (0.32) mM, respectively.

Accordingly, we found that allopurinol with caffeine had a better inhibition effect than caffeine and the last was better than allopurinol. We suggest that allopurinol with caffeine as a prodrug XO inhibitor, may have effective role for treatment of gout. This prevents oxidation of xanthine to uric acid and H_2O_2 (reactive oxygen species) (Yakugaku, 2005) suggested that a continuous intake of propolis which contain caffeine acid phenetyl ester may be effective for the prevention and treatment of gout and hyperuricemia.

There are many herbs and plants for examples containing caffeine such as coffee that have XO inhibition and thus may be useful in gout treatment.

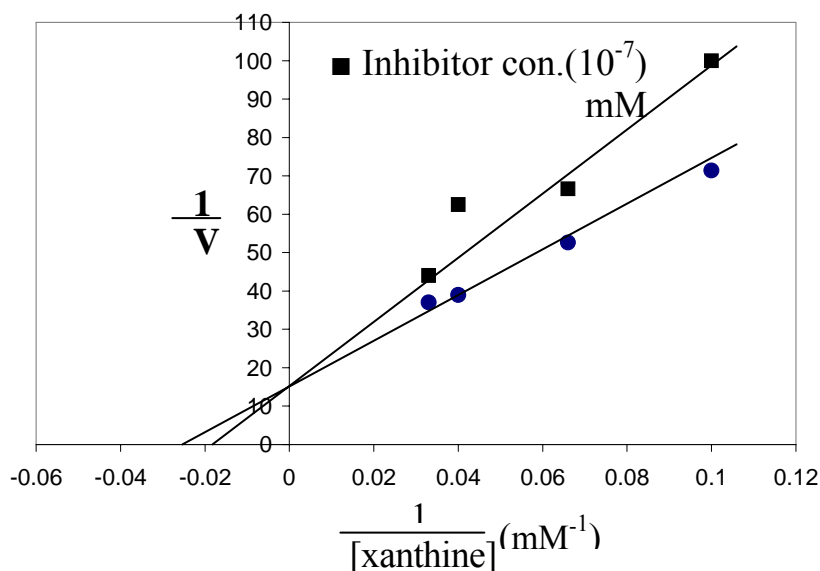


Fig. 2 : Lineweaver-Burk plot shows inhibition type on partially purified XO activity by allopurinol.

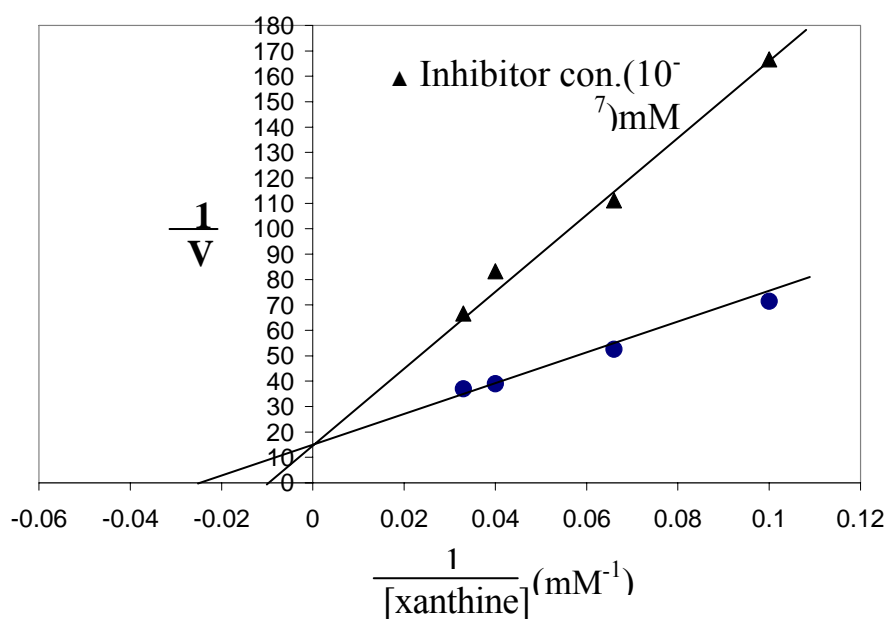


Fig. 3 : Lineweaver-Burk plot shows inhibition type on partially purified XO activity by caffeine.

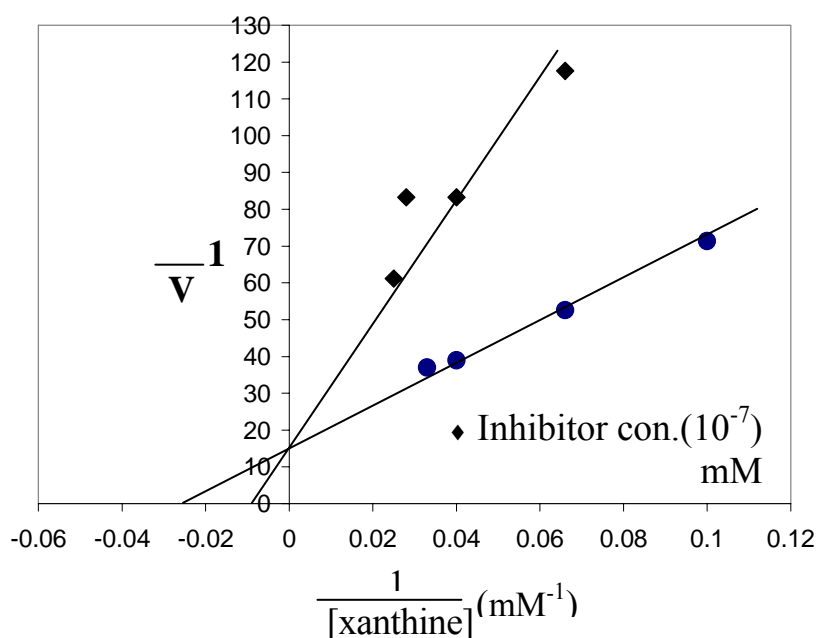


Fig. 4 : Lineweaver-Burk plot shows inhibition type on partially purified XO activity by allopurinol with caffeine.

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