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The effect of allopurinol on urate oxidase activity

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ALLOPURINOL* [4-hydroxypyrazolyl(3,4-*d*) pyrimidine] is an inhibitor of xanthine oxidase (xanthine: 0_2 oxidoreductase; EC 1.2.3.2), of which it is also a substrate, being oxidised to alloxanthine, also an xanthine oxidase inhibitor. It has been applied in medicine for the relief of hyperuricaemia, and for retardation of metabolic breakdown of 6-mercaptopurine used in treatment of neoplastic diseases[1, 2].

The close structural similarity of AP to other substrates of xanthine oxidase suggests that the effect is one of analogue inhibition. If this is the case, AP could be expected also to inhibit the reaction of oxidation of urate by urate oxidase [urate: 0_2 oxidoreductase; EC 1.7.3.3]. While such an effect would be without significance in the treatment of human subjects, it could affect the validity of determinations of content of purines in blood and urine by the uricase method of Liddle *et al.*[3]

The object of the present research was to examine the effect of AP on urate oxidase activity.

MATERIALS AND METHODS

Enzyme preparations

Three preparations were examined: (1) Extracts of 200 mg of ox-kidney acetone powder in 10 ml of 0·2 M ammonia, (2) solutions in 0·2 M ammonia of the precipitate obtained at 50 per cent saturation of these extracts with ammonium sulphate, (3) extracts of acetone powder in 0·2 M Tris buffer pH 10; these preparations were obtained as described in[4].

Materials

Uric acid was twice recrystallised, by dissolution in dilute NaOH solution, followed by reprecipitation with dilute HCl, and the final product was washed with hot water to remove Cl^-, and then with 97% ethanol, and was then dried at 110°. Allopurinol was donated by the Wellcome Foundations Ltd. (England). Other chemicals were of analytical grade.

Methods

Uric acid was determined by Kalckar's spectrophotometric method, as modified by Praetorius[5], The reaction systems contained: 1 ml of urate solution (containing about 20 μg of uric acid), 1·8–1·9 ml of 0·2 M Tris buffer, pH 9·5, and 0·1–0·2 ml of enzyme solution. The reference cell contained the same components, with water instead of urate solution,

AP was dissolved in water to which a minimum amount of 0·1 M NaOH was added; the substance was not soluble in 0·2 M ammonia. To 1-ml portions of a range of dilutions of 0·1 M AP solution in 0·2 M ammonia we added 1-ml portions of enzyme solution, the mixtures were incubated for 30 min at 37°, cooled to 0°, and assayed for UO activity; the control system, not containing AP was assayed at the beginning and end of the experiment, in order to check that the activity of the enzyme had remained unchanged. Concentrations of AP higher than 40 mM could not be used, because of the excessively high absorbance of AP at 293 mμ, under the given conditions.

It was found, in a separate experiment, that the absorbance at 293 mμ of a 50 mM solution of AP in Tris buffer,pH 9·5, did not change over 2 hr of incubation with UO. It is concluded that AP is not a substrate of UO, and that its presence does not interfere with the spectrophotometric method of assay of UO activity.

RESULTS

The curves of Fig. 1 illustrate the results obtained. Each point of these curves is a mean of the results obtained from 2–4 separate series of experiments.

* Abbreviations used: Allopurinol—AP, urate oxidase—UO.

Curve 1 of Fig. 1, for ammonia extracts, indicates considerable activation of the enzyme, to a maximum of 183 per cent of initial activity in 2·5 mM AP. Activity then fell steeply to the initial value, in 10 mM AP, and then gradually to 40 per cent in 40 Mm AP. Solutions of ammonium sulphate precipitates also showed slight activation in 2·5 mM AP, followed by a steady fall in activity, down to 40 per cent of the initial value, as the AP concentration rose to 40 mM. Tris extracts (curve 3)

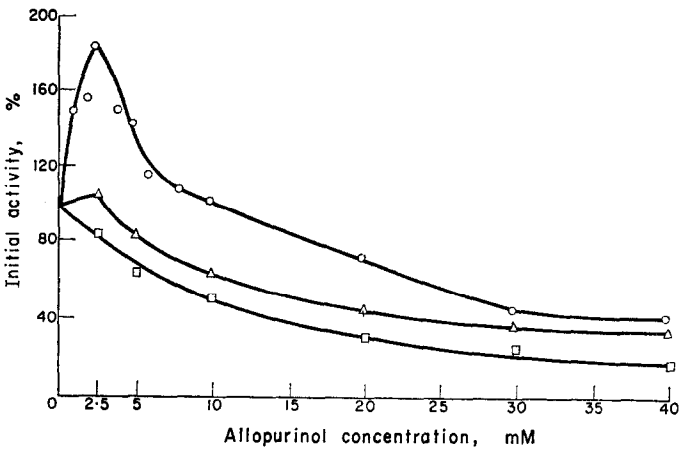

FIG. 1. Effect of allopurinol on the activity of urate oxidase solutions: ○—○ 0·2 M ammonia extracts of ox-kidney acetone powders; △—△ solutions in 0·2 M ammonia of $(NH_4)_2SO_4$ precipitates of these extracts; □—□ 0·2 M Tris extracts. Each point is a mean of 2–4 independent experiments.

showed a steady fall in activity, to 20 per cent of initial; the curve runs below that for the ammonium sulphate-precipitated enzyme, and parallel with it, from 2·5 to 40 mM AP.

DISCUSSION

The activating effect of AP at concentrations of 0–2·5 mM (curve 1, Fig. 1) might be attributed to neutralisation of some inactivating component of ammonia extracts of acetone powder, which would be supposed to compete with UO for substrate molecules. If it is assumed that this substance possesses purine-binding sites, but is not able to catalyse oxidation of bound substrate the following equilibria could be envisaged:

$$I + U \rightleftharpoons I - U \quad (1)$$

$$I + AP \rightleftharpoons I - AP \quad (2)$$

$$E + U \rightleftharpoons E - U \rightarrow E + \text{products} \quad (3)$$

$$E + AP \rightleftharpoons E - AP \quad (4)$$

where I is inhibitory substance and E is enzyme. If the equilibrium point of reaction (2) is further to the right than is that of reaction (1), the concentration of U available for reaction (3) would rise giving apparent activation of E. Further increase in AP concentration would lead to reaction (4), competing with (3), and so lowering the reaction rate.

The very small activating effect observed for solutions of $(NH_4)_2SO_4$-precipitated enzyme (curve 2) suggests that practically all of component I remained in solution at half-saturation with $(NH_4)_2SO_4$, although a small proportion of it was present in the precipitate.

Finally, Tris extracts (curve 3) behaved as if component I were absent from them; this may be because it is not extracted from the powder, or because the equilibrium point of reaction (1) is dis-

placed far to the left by Tris. This could be expected to occur if some heavy metal, e.g., Cu, were to be an essential component of the binding site of I.

This interpretation of the effects of AP on ox-kidney UO solutions is not purely speculative. Numerous additional items of evidence have been obtained in our Laboratory, pointing to the presence of a factor such as the postulated component I in ammonia extracts; work is still in progress on this subject, and the results will be communicated in a future paper.

An analogous effect was reported by Pontremoli *et al.*[6] for the action of fluorodinitrobenzene on rabbit-liver fructose-1,6-diphosphatase; the reagent activated the enzyme at low concentrations, and progressively inactivated it at higher ones. These authors attributed the activating effect to a conformational change following dinitrophenylation of a particular thiol group, postulated as being in an allosteric site of the enzyme. Subsequent inactivation at higher concentrations of reagent was ascribed to dinitrophenylation of a component of the catalytic site of the enzyme. While this interpretation of the results may have been applicable to Pontremoli's enzyme preparation, which was crystalline, and apparently homogeneous, it seems more likely that our interpretation would apply to our findings, inasmuch as the activating effect of AP was largely abolished by partial purification of UO.

As regards possible interference by AP with the determination of the urate content of blood and urine by the uricase method, as applied by Watts *et al.*[2], this would not seem to be of any significance, even at a daily dosage level of 1 g. If all of this were to be excreted in the urine, its concentration therein would amount to 5 mM (assuming a 1500-ml 24-hr output). Since urine is diluted 10-fold for determination of its urate content the AP concentration would fall to 0·5 mM, and this value would be lowered still further by addition of the other components of the reaction mixture. It is evident from the curves of Fig. 1 that such concentrations of AP could not significantly affect the activity of UO preparations used for urate determinations.

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Chlorpromazine-*N*-oxide formation by subcellular liver fractions

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CHLORPROMAZINE (CP) has been shown to be metabolized by several species. Urine samples of different species[1–4] show the following metabolites of CP: CP sulfoxide, nor$_1$- and nor$_2$-CP sulfoxides, nor$_1$-CP and nor$_2$-CP, 7-hydroxy CP, nor$_1$- and nor$_2$-7-hydroxy CP, glucuronides of 7-hydroxy CP, and CP-*N*-oxide (CPNO). Ziegler and Pettit[5] have shown the formation of *N*-oxide in the oxidative demethylation of *N*,*N*-dimethylaniline with a liver microsomal system. CP-*N*-oxide has also been