INHIBITION OF GLYOXALASE I: A POSSIBLE TRANSITION-STATE ANALOGUE INHIBITOR APPROACH TO POTENTIAL ANTINEOPLASTIC AGENTS?

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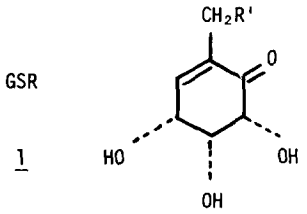
1. Introduction

The glyoxalase system contains two enzymes. Glyoxalase I requires glutathione (GSH) as coenzyme and catalyses the disproportionation of methylglyoxal into the thiol ester of GSH and lactic acid. Glyoxalase II then hydrolyses this ester to free GSH and lactate [1].

There have been suggestions that the ubiquitous [2] glyoxalase enzyme system may aid in cell growth regulation by controlling the level of methylglyoxal [2–4]. Indeed, the proposal [3,5] that selective inhibition of glyoxalase I may provide carcinostatic activity by preventing metabolism of α-ketoaldehydes in tumour cells has considerable experimental support. The carcinostatic activity of α-ketoaldehydes [6] is well-known, but their chemotherapeutic use is obviated by rapid metabolism to α-hydroxyacids (by the glyoxalase system).

Based on the above, approaches to antineoplastic agents have included preparation and screening of substituted-glutathione analogues [5,7,8]. For example, 1, GSR (R = $-(CH_2)_nCO.Ar$, $-CH_2Ar$, $-(CH_2)_n$ H) are powerful competitive inhibitors of glyoxalase I, [3,9,10] and are cytotoxic to L1210 leukaemia and KB cells in tissue culture [5]. In addition, several S- and N- substituted cysteinylglycines have been evaluated as glyoxalase inhibitors [8]. However, rapid metabolism rendered such compounds inactive in vivo [7,8,11] and attempts have been made to find degradation-resistant versions [7,8]. α-Hydroxythiolesters (product-like inhibitors) have no significant antileukaemic activity [12].

Another inhibitor of glyoxalase I, (2), was isolated from *Streptomyces griseosporeus*. This inhibits glyoxalase I if incubated with GSH, presumably by conversion to 3. Compound 2 showed strong inhibition of growth of HeLa cells and Ehrlich ascites carcinoma, weak inhibition of solid type Ehrlich



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2; R' = $-O_2CC{=}C(H).CH_3$

3; R' = GS-

carcinoma and prolonged the survival period of L1210-inoculated mice [13,14]. Another compound (MS-3), from a mushroom, inhibits glyoxalase I and growth of Yoshida rat sarcoma cells in cell culture [15]. Reductones (e.g., ascorbate) suppress the growth of Sarcoma 180 and are also effective glyoxalase inhibitors, suggested to be because of their structural similarity to methylglyoxal [16].

We report here several new inhibitors of glyoxalase I, designed on transition-state analogy as a new approach to potential antileukaemic agents.

2. Materials and methods

Maltol (3-hydroxy-2-methyl-4H-pyran-4-one), rhodizonic acid and 2,3-dihydroxybenzoic acid were from Aldrich. Squaric acid, dihydroxyfumaric acid,

glucono-δ-lactone, D-isoascorbic acid, glutathione, methylglyoxal and glyoxalase I (grade IV, yeast) were from Sigma. Enzyme assays were performed (25°C, 0.05 M phosphate buffer (pH 6.6)) using a thermostatted Pye-Unicam SP8-100 spectrophotometer. Solutions were prepared, on the day of the assays, using distilled, deionized water. Stock inhibitor solutions were prepared in the above buffer and adjusted, when necessary, to pH 6.6. Methylglyoxal, GSH and buffer (and inhibitor) were added to a cuvette and allowed to stand for 5 min in the thermostatted compartment of the spectrophotometer to allow complete hemimercaptal equilibration. Sufficient glyoxalase I solution, in the presence of 0.1% bovine serum albumin (Sigma) as a stabilising agent [20], was added to give an easily measured initial rate (followed at 240 nm). Hemimercaptal substrate concentrations were calculated from the concentrations of GSH and methylglyoxal added, using a value of 3.1×10^{-3} M for the dissociation constant [9] of the hemimercaptal at pH 6.6. Data were analysed using an Exidy Sorcerer Minicomputer (Z80 chip); we are grateful to Mr John Torkington for programming assistance.

3. Results

Figure 1 and table 1 show that maltol is an effective inhibitor of glyoxalase I at pH 6.6. From an Eadie plot of V_o versus V_o/[hemimercaptal], the app. K_m for the hemimercaptal substrate is 3.0×10^{-4} M ($\pm 1.6 \times 10^{-4}$ M) assuming both enantiomers are bound productively. This value agrees well with published values [8,17]. Lineweaver-Burk ($1/V_o$ versus 1/[hemimercaptal]), analysis at various inhibitor concentrations indicated non-competitive inhibition (using the terminology of Cleland [18]); however, the replot of the intercepts against inhibitor concentration may be curved (see fig.1e). The Dixon plot, of $1/V_o$ versus [maltol] (in fig.1) yields 3.94×10^{-4} M for K_i and, again, the pattern is that of non-competitive inhibition. This value of K_i was calculated as the average value of the intercepts on the [maltol] axis, values of which lay between 3.81 and 4.00×10^{-4} M (4 determinations).

Other inhibitors are collected in table 1; dissection of the inhibition kinetics has not yet been carried out for them, but it is apparent that some of these simple compounds are rather effective inhibitors.

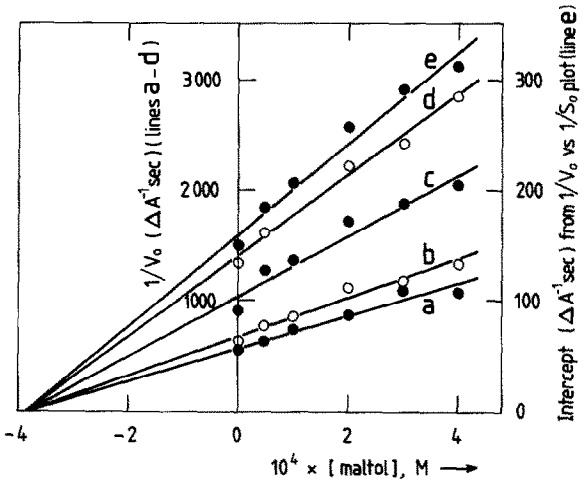

Fig.1. Dixon plot of $1/V_o$ versus [maltol] at various concentrations of hemimercaptal substrate for glyoxalase I at pH 6.6. Initial concentrations of hemimercaptal were (a) 2.51×10^{-4} M; (b) 1.76×10^{-4} M; (c) 1.07×10^{-4} M; (d) 7.76×10^{-5} M. Line (e) represents a replot of the intercepts ($1/V_o$ axis) from a Lineweaver-Burk plot ($1/V_o$ versus $1/S_o$) of the same data. Points are experimental: lines were obtained by linear least squares regression analysis of the data: it is possible that line (e) is actually curvilinear. The value of K_i, obtained from the common intersection on the [maltol] axis is 3.94×10^{-4} M (see text).

4. Discussion

The effective inhibition of yeast glyoxalase I by a molecule as simple as maltol is surprising, especially in view of the rather low K_i ($\sim 4 \times 10^{-4}$ M), comparable with the K_m value. Most other inhibitors with comparable K_i values are derivatives of glutathione or cysteinylglycine. However, a group of reductones has recently been reported as glyoxalase I inhibitors, although detailed inhibition studies or inhibition constants were not published [16]. Particularly effective was ascorbic acid. The structural feature common to the reductones was 4, the enediol (or part of it). The compounds of table 1 were selected on the basis of their resemblance to a putative enediol intermediate.

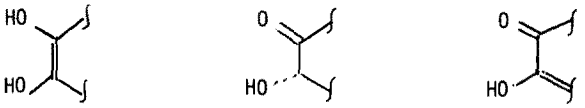

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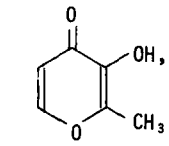
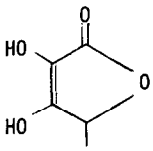
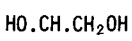
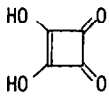
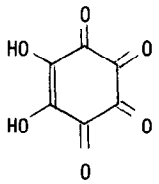
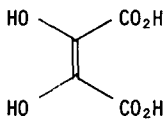
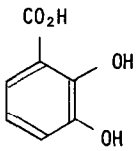
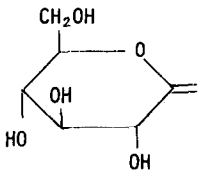
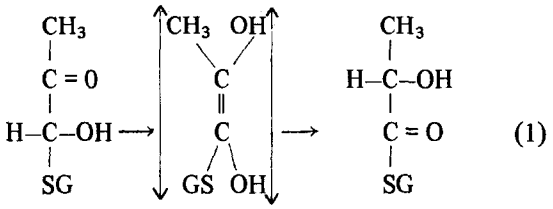
Table 1
Inhibitors of yeast glyoxalase I (pH 6.6, 25°C)

Compound	Conc. (mM)	% inhibition
 OH, maltol	0.40	50
 O, D-isoascorbate 	10.00	100
 squaric acid	0.25	70
 rhodizonic acid	1.00	50.4
 dihydroxy-fumaric acid	10.00	50
 2,3-dihydroxy-benzoic acid	0.583	72
 D-gluc[illegible]-δ-lactone	10.00	9

The concentration of hemimercaptal substrate was 2.51×10^{-4} M

The glyoxalase inhibitor from *Streptomyces griseosporeus*, 2, 2-crotonyloxymethyl-4,5,6-trihydroxycyclohex-2-enone [13,14], contains structural unit 5, very close to 4 but non-coplanar. Maltol contains the analogous element 6, coplanar by virtue of the sp^2 hybridised carbon to which the –OH group is attached; the other compounds in table 1 also contain either an enediol or a *paene*-enediol structure.

Until recently, the mechanism of action of glyoxalase I was believed to be an example of an intramolecular hydride shift [19]. However, the observation (by NMR) of incorporation of solvent protons is consistent with a mechanism involving an enediol [20] form of the substrate, possibly chelated to Mg^{2+} at the active-site (eq. (1)) [21].



(1)

Inhibition by some of the compounds in table 1 (as well as by the reductones and crotonate, 2) could be ascribed to simple similarity of the inhibitor to the substrate (hemimercaptal) structure in the Michaelis complex (i.e., before significant progress along the reaction coordinate has occurred), as suggested for the reductones [16]. However, it is possible that these inhibitors are mimicking to some extent, some of the binding features distinctive of the transition-state (presumably physically and energetically approximate to the enediol intermediate). The concept of 'transition-state analogues' has led to some powerful enzyme inhibitors [22]. The lack of coplanarity of the structural element, 5, found in D-gluconic-δ-lactone may explain its relatively low inhibitory effect. More detailed study is required to distinguish transition-state from ground state (Michaelis-complex) analogy.

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