Flavin-trapping of the Transient Enediolate Species formed during Glyoxalase I Catalysis

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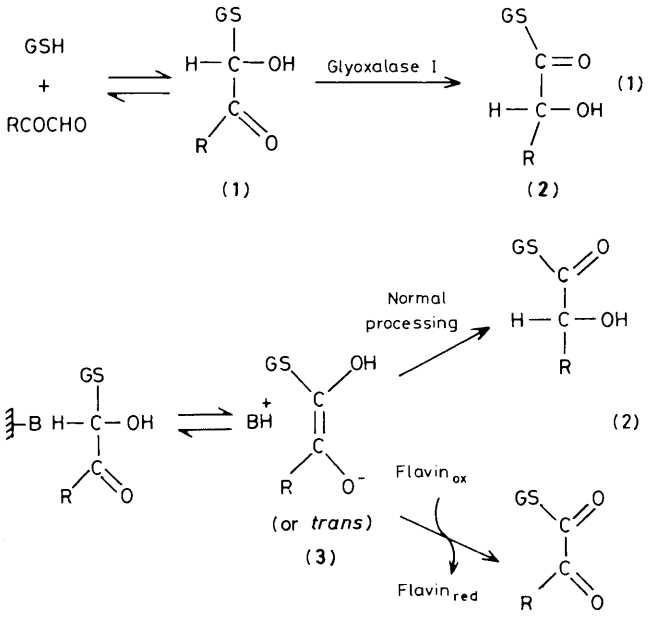
A reactive intermediate, probably an enediolate species, has been oxidatively trapped by reaction with added flavin during catalytic turnover by yeast glyoxalase I of the thiohemiacetal from glutathione and phenylglyoxal under anaerobic conditions.

A number of enzymes have mechanisms which are characterised by the putative intermediacy of enediolate species. One of these is glyoxalase I,[1] which assists in detoxification of cytotoxic α-ketoaldehydes by conversion of the thiohemiacetal,[2] formed nonenzymically between glutathione (GSH) and the α-ketoaldehyde, into the corresponding *S*-D-(hydroxyacyl)glutathione, equation (1). Glyoxalase I (EC 4.4.1.5) is intriguing not only because inhibitors of it may have anticancer activity[3] but also because of its mechanism of action, long cited as a rare example of an enzyme-catalysed hydride transfer but now regarded as more likely to be a proton-transfer,[4] involving an enediolate species. Indeed this high energy enediolate intermediate, which must be close in energy (and hence structure) to the transition-state has been used as a model for design of anticancer agents by transition-state analogy.[5]

The nonenzymic rearrangement of (**1**) to (**2**) has been studied in some detail[6] and shown to occur *via* a carbanionoid enediolate species, the most direct technique[7] involving oxidative trapping of the carbanion (**3**) (enediolate) by means of added flavins [see equation (2)]. We now report on the first use of flavins as oxidative trapping agents for enediolate species formed in enzymic reactions.

Enzymes, proteins, and substrates were obtained from Sigma Chemical Co. (St. Louis, Missouri). Reactions were carried out at 30 °C in 0.02 M phosphate buffer (pH 7.09) in the presence of 5% v/v dimethylsulphoxide (redistilled) and 0.01 wt% of bovine serum albumin. Flavins were from previous studies.[7]

Reactions between flavins and the various components of the glyoxalase I reaction were studied anaerobically under nitrogen in a Thunberg cuvette by following spectrophotometrically the disappearance of flavin.



Velocities were estimated from the initial slopes and extinction coefficients. Glyoxalase I enzymic activity (conversion into the glutathione ester of the thiohemiacetal formed from GSH and phenylglyoxal) was measured in the absence of flavin at 263 nm, the isosbestic point[8] for the phenylglyoxal : GSH hemithiolacetal equilibrium.

When GSH (1.04 mM) and phenylglyoxal (5.00×10^{-4} M) were incubated anaerobically at 30 °C in phosphate buffer (pH 7.09, 0.02 M, 0.01 wt% bovine serum albumin) in the presence of 10-ethylisoalloxazine (9.91×10^{-5} M), flavin was found to be slowly reduced (followed at 431 nm, $\Delta\varepsilon = 8150$ mol^{-1} dm^3 cm^{-1}) with an initial velocity of 0.77×10^{-10} mol dm^{-3} s^{-1}. In the presence of yeast glyoxalase I (9.81×10^{-2} units) the reduction was more rapid, with an initial velocity of 7.92×10^{-10} mol dm^{-3} s^{-1}. In the range of glyoxalase concentrations of 4—20 units per run the velocity of flavin reduction was proportional to the amount of glyoxalase present, after correction for the nonenzymic background. Omission of phenylglyoxal, but not of glyoxalase I, completely abolished flavin reduction. This indicates that the flavin reduction is not caused by the glutathione or the bovine serum albumin in the supporting medium. The time-course for the flavin reduction (10-ethylisoalloxazine) was almost (not strictly) zero-order in the presence of GSH, phenylglyoxal, and glyoxalase I at pH 7.09 (431 nm). When the decrease in absorbance at 263 nm (*i.e.* conversion of normal substrate to normal product) was complete, the flavin reduction also stopped. A number of other flavins were found to be susceptible to reduction by the turnover of the glyoxalase reaction, including FMN, FAD, riboflavin, and 3-methyltetra-*O*-acetylriboflavin.

It is likely that the flavins are reacting with some intermediate formed during the normal catalytic cycle of glyoxalase I. As flavins are very inefficient hydride ion trapping agents,[7,9] ($NaBH_4$, HCHO + HO^-, PhCOCHO + HO^-), the easiest explanation is that in this functioning enzyme system an enediolate species is trapped oxidatively by flavin. This is in close analogy to the reaction previously reported[7] for the nonenzymic model system.

The amount of flavin reduced was typically 1.6% of the total (limiting) phenylglyoxal concentration for 10-ethylisoalloxazine. This indicates that only a small number of enzymic turnovers can be successfully intercepted by this trapping procedure. It has yet to be established whether the enediol trapped is attacked by the flavin whilst still bound to the active-site or whether it is the small (theoretical) amount of free solution enediolate intermediate in equilibrium with this which is intercepted. Whichever the case, these studies provide direct support for an enzyme-generated enediolate species [(**3**), equation (2)] on the glyoxalase I pathway. Oxidative carbanion trapping has been successful in other enzymic systems.[10]

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