CHEMISTRY LETTERS, pp. 1323-1326, 1979.

FACILE OXIDATION OF HEMITHIOL ACETALS BY FLAVIN: EVIDENCE FOR THE ENEDIOL MECHANISM OF GLYOXALASE I

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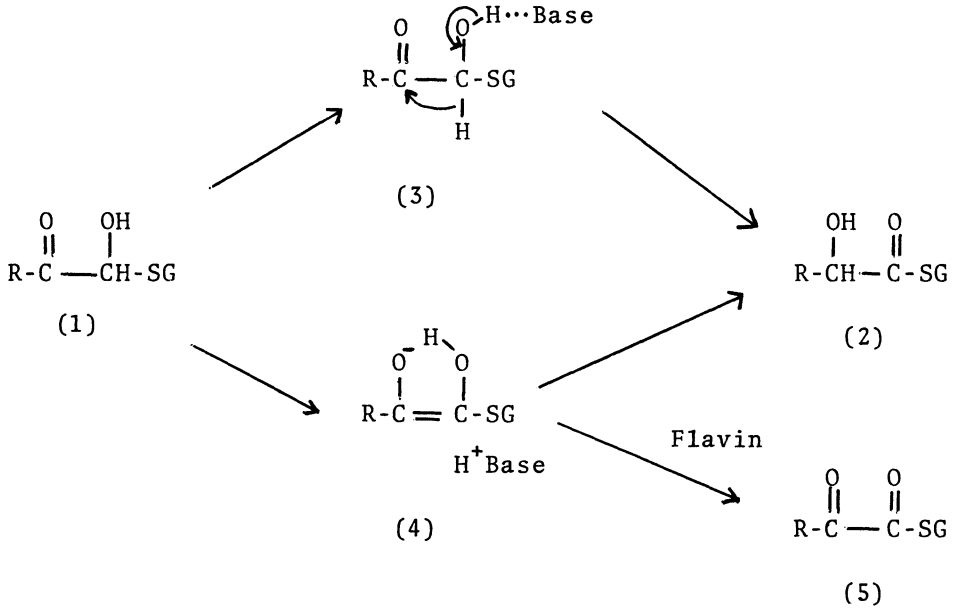
The rearrangement of the hemithiol acetal to the α-hydroxythiol ester is almost completely inhibited on the addition of flavin due to the occurrence of the flavin-trapping of the enediol intermediate. This result suggests that the enzymic catalysis by glyoxalase I occurs via the enediol intermediate.

The glyoxalase enzyme catalyzes the conversion of glyoxals to corresponding α-hydroxy acids with the aid of glutathione (GSH) as cofactor.[1] The most interesting step in the reaction sequence is the rearrangement of the hemithiol acetal (1) to the α-hydroxythiol ester (2) which is catalyzed by glyoxalase I.



Scheme I

The mechanism has been a controversial problem. On the basis of solvent incorporation studies, Franzen[2] and Rose[3] proposed the 1,2-hydride shift mechanism (i.e.,

(3) in Scheme I), like that of the Cannizzaro reaction, whereas Hall et al.[4] contended on the basis of the same solvent incorporation study that the true intermediate is the enediol (4) which is formed by deprotonation of the hemithiol acetal.

We recently found that an enediol intermediate is efficiently trapped by flavin to afford the corresponding oxidation product[5]. Hence, if the enediol mechanism proposed by Hall et al.[4] is correct, it follows that the hemithiol acetal (1) would be converted to the α-ketothiol ester (5) due to the oxidative trapping of the intermediate (4) by flavin. It is expected, therefore, that the flavin-trapping technique would provide the unequivocal conclusion for the long controversy.

We used 3-methyltetra-O-acetylriboflavin (6)[6], and the oxidation of phenylglyoxal (R=C_6H_5-) was carried out anaerobically in an aqueous thiol solution at 30°C for 5 hr in the dark. The solution was then treated with 1 N KOH to complete the hydrolysis of the thioester and analyzed by high-speed liquid chromatography. Typical examples of the product analysis are recorded in Table 1.

Examination of Table 1 reveals that the main product in the absence of (6) is mandelic acid, whereas the formation of mandelic acid is inhibited almost completely on the addition of (6) and benzoylformic acid becomes the main product. The formation of benzoic acid in low yields is probably attributed to the further oxidation of benzoylfomic acid. The result clearly indicates that the intermediate which exists in the course of the rearrangement of (1) to (2) is readily trapped by flavin to afford (5).

Table 1. Product analysis for the flavin-trapping of the hemithiol acetal of phenylglyoxal[a]

Thiol		[(6)]	% Yield[b]		
	mM	mM	$ArCH(OH)CO_2H$	$ArCOCO_2H$	$ArCO_2H$
$HO(CH_2)_2SH$	130	0	61	3.3	0
$HO(CH_2)_2SH$	130	13	0	81	5.6
$Me_2N(CH_2)_2SH$	130	13	0	55	0.3
Glutathione	65	13	0	13	3.0
C_6H_5SH	130	13	0	6.1	1.7

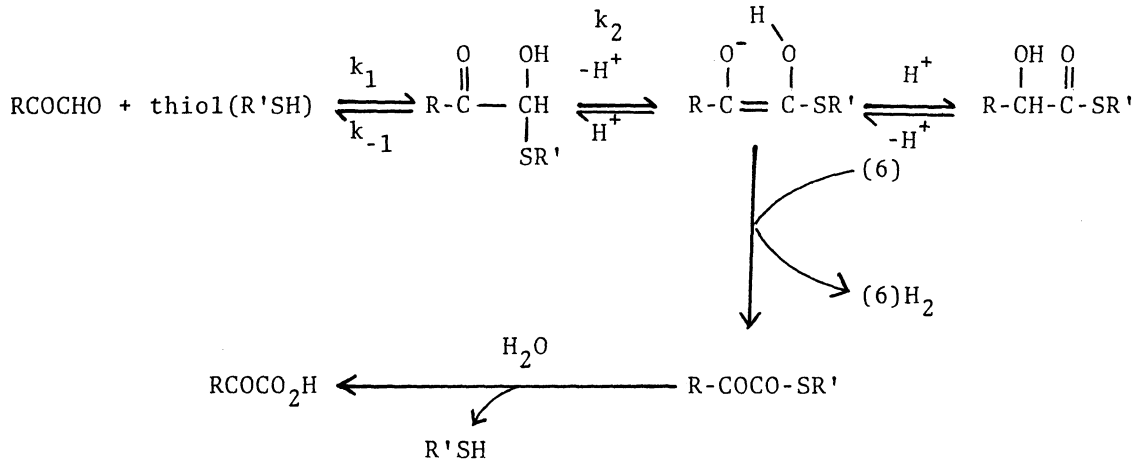
a) [Phenylglyoxal] = 1.3×10^{-2} M, pH 9.35 with H_3BO_3 (0.1 M) and KOH (0.05 M). b) Ar = C_6H_5-.

Kinetic measurements were performed at 30°C and pH 9.5 under anaerobic conditions. The relatively low pH was chosen to suppress the Cannizzaro reaction of glyoxals. The reaction was monitored by the disappearance of (6) at 448 nm. It is well-known that (6) was slowly reduced by thiols. Under the present reaction conditions, however, the rate constants for the reduction by thiol were smaller by more than two orders of magnitude than those for the reduction by hemithiol acetals, so that the reduction by thiols was kinetically neglected. It was found that the oxidation of glyoxals (p-chlorophenylglyoxal, phenylglyoxal, and methylglyoxal) is zero-order in (6) up to 80% reaction and first-order in glyoxals and thiols (N,N-dimethylaminoethanethiol, 2-mercaptoethanol, and glutathione). The apparent second-order rate constants (k_2' (M^{-1} s^{-1}) = v_{obs} / $[RCOCHO][thiol]_{total}$) for the oxidation of p-chlorophenylglyoxal were, for example, 0.640 for N,N-dimethylaminoethanethiol, 0.0114 for 2-mercaptoethanol, 0.00031 for glutathione, and ~0 (the rate not detected) for thiophenol. No catalytic efficiency of thiophenol is well compatible with the fact that in the thiophenol catalyzed system benzoylformic acid was afforded only in 6.1% yield, and this trend is accounted for by the low nucleophilicity of thiophenol. Also suggested from the kinetic measurements is the low catalytic efficiency of glutathione. The spectral examination indicated that in the glutathione catalyzed system a new absorption band appears at around 280 nm which is characteristic of an imine. Thus, the suppression of the glutathione activity is ascribable to the formation of the nonproductive imine between the terminal amino group and the substrate.

These observations can be elucidated by the reaction sequence in the Scheme II. The zero-order disappearance of (6) is rationalized in terms of rate-limiting deprotonation (k_2) of the hemithiol acetal followed by rapid oxidation of the enediol intermediate by (6). The rate equation for the Scheme II is expressed by Eq. 1,

$$v_{obs} = k_2K[RCOCHO][thiol]_{total} / (1 + K[thiol]_{total}) \qquad (1)$$

were $K = k_1 / k_{-1}$. Since v_{obs} was first-order in glyoxal and thiol, $K[thiol]_{total} \ll 1$ is assumed under these conditions (that is, $k_2' = k_2K$).



Scheme II

Since (i) flavin does not serve as an efficient acceptor for hydride[7] ($NaBH_4$, $HCHO + OH^-$, and $C_6H_5COCHO + OH^-$), (ii) neither (1) nor (2) cannot be oxidized by flavin in neutral pH solutions, and (iii) the occurrence of the flavin-trapping of (4) is supported by the kinetic and the product analysis, one may conclude that the rearrangement of (1) to (2) (and also glyoxalase I catalysis) proceeds via the enediol intermediate. The high catalytic efficiency observed for N,N-dimethylaminoethanethiol is attributed to the intramolecular general-base catalysis of the dimethylamino group. One should note, however, that the amine base catalyzes the deprotonation of 2-C-H, but not the deprotonation of 2-O-H as proposed by Franzen.[2]

The authors thank Professor T. Kunitake for helpful discussions.

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(Received July 25, 1979)