$$^{-}N{=}N^{+}{=}N^{-} \leftrightarrow N{\equiv}N^{+}{-}N^{2-}$$

This charge distribution is probably responsible for the high nucleophilicity of N_3^- toward carbocations, as given, for example, by the N^+ scale,[23] and polarization of N_3^- by a strong electrophile could be responsible for this high reactivity, so that cationic micelles could have the same effect. However the rate effects in deacylation or S_N2 displacement (Table I) suggest that the micelle is stabilizing the transition state for aromatic nucleophilic substitution but not for the other reactions.

In the absence of micelles N_3^- is unusually unreactive in aromatic nucleophilic substitution, based on the N^+ scale,[15] so it seems that unfavorable transition-state interactions disappear in a reaction in a cationic micelle as compared with reaction in water or alcoholic solvent.

Acknowledgment. We thank the National Science Foundation (Chemical Dynamics Program) for support of this work.

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(Glutathiomethyl)glyoxal: Mirror-Image Catalysis by Glyoxalase I

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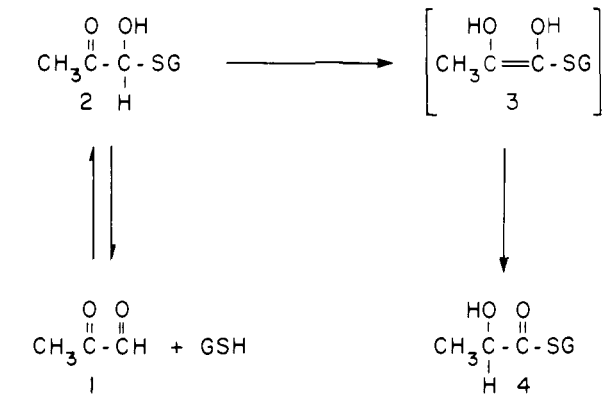
Glyoxalase I [*S*-lactoylglutathione methylglyoxal-lyase (isomerizing) EC 4.4.1.5; GX I] catalyzes the conversion of the thiohemiacetal **2** of α-keto aldehyde **1** and glutathione [*N*-(*N*-L-γ-glutamyl-L-cysteinyl)glycine; GSH] to the thioester **4** of an α-hydroxy acid and GSH (Scheme I).[1] The reaction proceeds via a fast-shielded proton transfer with the intermediacy of enediol **3**,[2] and the resulting acid has been established as the D isomer.[3] Recent ^{1}H NMR studies have suggested that one of the two diastereomeric thiohemiacetals is selectively processed.[4] Two general observations concerning the substrate specificity of the enzyme have been made. First, the specificity for GSH is high; aside from *N*-acyl derivatives of GSH and several related tripeptides, other sulfhydryl-containing compounds are inactive primarily due to poor binding.[5] Second, the specificity for α-keto aldehydes is broad, indicating a high tolerance at that region of the active site.[1,2,3b,6] During our study of β-(alkylthio)-α-keto aldehydes, **6**, we found that one member of this class, (glutathiomethyl)glyoxal (**6a**),[7] exhibits two surprising properties in its reaction with glyoxalase I: *a loss of glutathione specificity for thioester formation and the production of the* L *isomer of the resulting α-hydroxy acid.* We believe that these findings are only

Scheme I



Scheme II

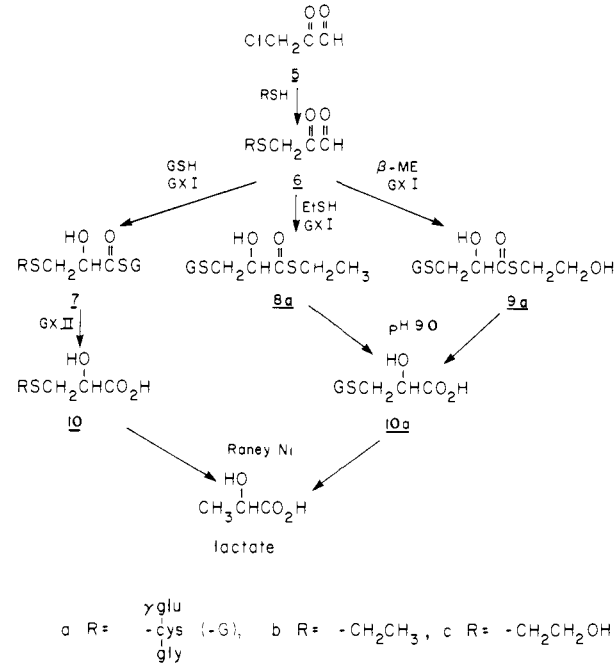


Table I. Relative Velocities of the Reaction of [(Alkylthio)methyl]glyoxals and Thiols with Glyoxalase I and of the Hydrolysis of the Thioesters by Glyoxalase II

substrate	thiol	rel velocity GX I[a]	rel velocity GX II
6a	GSH	83	100
	EtSH	40	0.4
	β-ME	25	0
6b	GSH	100	100
	EtSH	0	
	β-ME	0	
6c	GSH	73	70
	EtSH	0	
	β-ME	0	

[a] Measured spectrophotometrically. Under identical conditions, methylglyoxal gives a relative velocity of 134 (2.5 μmol/min) with GSH and is inactive with EtSH and β-ME.

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(7) The proper (current Chemical Abstracts Index) name for **6a** is *N*-[*N*-L-γ-glutamyl-*S*-(2,3-dioxopropyl)-L-cysteinyl]glycine. We propose the trivial name (glutathiomethyl)glyoxal to reflect the homology with other β-(alkylthio)-α-keto aldehydes.

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Table II. Stereochemical Analysis of Lactate Derived from Raney Nickel Desulfurization of 10

comp	thiol used in GX I reaction	% lactate[a] D	% lactate[a] L
10a	GSH	71	29
	EtSH	19	81
	β-ME	22	78
10b	GSH	75	25
10c	GSH	80	20

[a] The total amounts of D- and L-lactate found were in excellent agreement (±10%) with that predicted by the specific activities of the samples. Controls containing 10 prior to Raney nickel treatment were performed. The percentages are the average of two independent experiments.

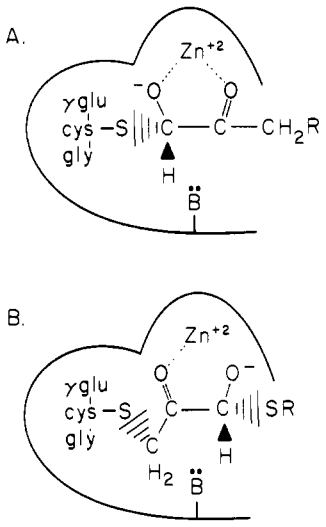
explainable by the binding of **6a** to and its subsequent processing by glyoxalase I in a manner *inverted* with respect to the normal enzymatic reaction.

(Glutathiomethyl)glyoxal (**6a**), [(ethylthio)methyl]glyoxal (**6b**), and [[(β-hydroxyethyl)thio]methyl]glyoxal (**6c**) were prepared under aqueous conditions by the reaction of (chloromethyl)glyoxal (**5**)[8] with GSH, ethanethiol (EtSH), and β-mercaptoethanol (β-ME), respectively (Scheme II).[9] Incubation of the keto aldehydes **6** (2.0 mM) with GSH (4.0 mM) and GX I (3.0 units/mL; yeast, Sigma Grade X) resulted in the rapid formation of thioester products **7** (Scheme II; Table I) as determined by the increase in absorbance at 240 nm.[1,10] The thioesters were immediately hydrolyzed (Table I) by glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase, EC 3.1.2.6; GX II; beef liver, Sigma) and chromatographed on Dowex 1 (formate; 0 → 6 M formic acid) to afford the corresponding β-(alkylthio)-α-hydroxy acids **10** in quantitative yield.[11]

When EtSH or β-ME (4.0 mM) replaced GSH in the GX I reaction, only **6a** was converted to the corresponding thioester **8a** or **9a**, respectively. **6b** and **6c** were neither processed to thioesters (**8b,c** and **9b,c**) nor consumed. The relative velocities of formation of **8a** and **9a** from **6a** compared quite respectably to the reaction of **6a** and GSH by GX I (Table I). **8a** and **9a** were also found to be stable to hydrolysis by GX II, which has been shown to be specific for thioesters of GSH.[12] Hydrolysis of **8a** and **9a** in mild alkaline solution (pH 9) and Dowex 1 chromatography quantitatively afforded **10a**, which was identical by ^{1}H NMR spectroscopy with **10a** obtained from the GX I catalyzed reaction of **6a** and GSH as described above.

The stereochemistry of the α-carbon of the hydroxy acids **10** was determined by desulfurization with Raney nickel,[13] isolation of the resulting lactic acid, and measurement of the relative amounts of D and L isomers by production of NADH with D-lactate dehydrogenase (EC 1.1.1.28) and L-lactate dehydrogenase (EC 1.1.1.27).[14] The results (Table II) show that **10** prepared from the glutathione-dependent GX I reaction afforded mostly D-lactate, as might be expected. However, **10a** derived from the GX I catalyzed reaction of **6a** with EtSH or β-ME, yielding the abnormal thioesters **8a** or **9a**, afforded predominantly L-lactate.[15]

Scheme III



These observations constitute an unprecedented case of inverse substrate processing. Our proposal is outlined in Scheme III. In the normal enzymatic reaction (A), the glutathione moiety of the thiohemiacetal serves as the major substrate binding component and also renders the C-1 proton susceptible to abstraction by the active-site base. A reasonable model then would have the thiohemiacetal of the *S* configuration binding to the enzyme and proton transfer occurring suprafacially to the *si* face of C-2 to yield the D-hydroxy acid. For **6a**, however, binding to the enzyme via the glutathione moiety presents the substrate to the active site rotated 180° (B). Activation of the C-1 proton must still be effected by the addition of a thiol (RSH).[16] Since this thiol now occupies the highly tolerant portion of the active site, the loss of specificity for glutathione is apparent.[17] Finally, the mechanistic constraints of the system necessitate that the proton transfer proceed in a reversed direction and from the *R* isomer of the thiohemiacetal to the *re* face of C-2, generating L-hydroxy acid, thus completing the "looking-glass" effect.[18]

(8) Chari, R. V. J.; Kozarich, J. W., in press.

(9) In a typical reaction (25 mL), **5** (2 mM) and the sulfhydryl compound (2 mM) were maintained at 25 °C in 0.1 M KPO_4 buffer (pH 6.8). The extent of reaction was monitored by an increase in absorbance at 306 nm for the GSH and ethanethiol reactions (ϵ_{306} ~3200 M^{-1} cm^{-1}). The keto aldehyde derived from β-mercaptoethanol absorbed much less strongly at 306 nm presumably due to internal hemiketal formation via addition of the β-hydroxyl group to the keto carbonyl. Reactions were usually complete in ~2.5 h.

(10) Thioester formation could also be determined by the concomitant decrease in absorbance at 306 nm in the case of **6a** and **6b**. The amounts of thioesters formed were quantitative with the assumption ϵ_{240} ~3370 M^{-1} cm^{-1}.[1]

(11) Yields were determined by preparing **6** from [1-[^{14}C]chloromethyl]-glyoxal (**5**; 20 μCi/mmol) and using these labeled compounds to locate chromatographic peaks and to quantitate recoveries. **10a**: ^{1}H NMR (D_2O) δ 4.35 (m, 1 H), 3.83 (dd, 1 H, *J* = 3.9, 6.8 Hz, $CH_2CH(OH)CO_2^-$), 3.40 (m, 3 H), 2.76 (dd, 1 H, *J* = 4.9, 14.1 Hz, SC*H*HCH(NH-γ-glu)(CO-gly)), 2.58 (m, 3 H), 2.16 (m, 2 H), 1.8 (m, 2 H). **10b**: ^{1}H NMR (D_2O) δ 4.05 (dd, 1 H, *J* = 4.1, 6.6 Hz), 2.65 (dd, 1 H, *J* = 4.1, 14.0 Hz), 2.51 (dd, 1 H, *J* = 6.9, 14.1 Hz), 2.25 (q, 2 H, *J* = 7.3 Hz), 0.85 (t, 3 H, *J* = 7.3 Hz). **10c**: ^{1}H NMR (D_2O) δ 3.79 (dd, 1 H, *J* = 3.9, 6.8 Hz), 3.36 (t, 2 H, *J* = 6.2 Hz), 2.60 (dd, 1 H, *J* = 3.9, 13.8 Hz), 2.44 (dd, 1 H, *J* = 6.8, 13.8 Hz), 2.38 (t, 2 H, *J* = 6.2 Hz).

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(13) In a typical reaction, **10** (5 μmol, ~20000 cpm) and Raney nickel (50 mg dry weight) in H_2O (0.6 mL) was heated to 80–85 °C for 16 h. The suspension was filtered, and the residue was washed with H_2O. The combined filtrate was evaporated to dryness under vacuum and redissolved in H_2O (0.5 mL). Recovery of product (based on cpm) ranged from 50% to 60%.

(14) (a) Gawehn, K.; Bergmeyer, H. V. In "Methods of Enzymatic Analysis", 2nd English ed.; Academic Press: New York, 1968; Vol. 3, p 1492. (b) Gutmann, I.; Wahlefeld, A. W. *Ibid.*; p 1464.

(15) Several explanations exist for the lack of complete stereochemical purity of the lactates. Spontaneous epimerization of thioesters of glutathione (Patterson, M. A. K.; Szajewski, R. P.; Whitesides, G. M. *J. Org. Chem.* **1981**, *46*, 4682), the alkaline conditions of hydrolysis of **8a** and **9a**, and Raney nickel desulfurization are possibilities. While D-lactate does not epimerize upon Raney nickel treatment, this is not strictly analogous to **10**. Nevertheless, the predominance of one isomer is quite clear.

(16) Abstraction of the C-1 proton could have proceeded via the hydrated form of **6a** to afford **10a** directly. This does not occur, suggesting that hydration does not sufficiently activate this proton.

(17) The requirement for glutathione in the reaction of **6b** and **6c** is also obvious. That the reaction of **6a** and GSH affords mostly the D-hydroxy acid suggests that in this case normal binding is favored. The scheme is not intended to imply the existence of an active-site cleft but merely reflects the relative binding specificities for portions of the substrate.

(18) We have recently observed that **6a** deuterated specifically at the aldehydic position yields the L-hydroxy acid **10a** with ~40% retention of ^{2}H at C-2. This excludes the possibility of a two-base mechanism. While Scheme III is based on a *cis*-enediol, an equally plausible mechanism may be drawn for a *trans*-enediol. In this case, the stereochemistry of the thiohemiacetal that binds would be reversed but the overall "mirror-image" effect would be retained. Apparently, the additional methylene group between the glutathione and the dicarbonyl group does not present a serious problem to the relative geometries of the substrate, Zn^{2+}, and active-site base. This has been substantiated by CPK model analysis.

Acknowledgment. We gratefully acknowledge the National Institutes of Health (GM 29204 and GM 26985) for support of this research and Professor John Gerlt for a helpful discussion. R.V.J.C. is a Celanese Fellow.

(19) **Note Added in Proof**: Recent NMR studies indicate that the substrate oxygens probably lie in the second coordination sphere of the metal (Rosevear, P. R.; Sellin, S.; Mannervik, B.; Mildvan, A. S. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 1152).

Minimal Bromate Oscillator: Bromate-Bromide-Catalyst[1]

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While the first homogeneous oscillating chemical reaction discovered, the Bray reaction,[3] is an iodate-containing system, bromate oscillators, especially the prototype Belousov–Zhabotinsky reaction,[4] have until recently dominated both the experimental study and mechanistic understanding of chemical oscillation. Noyes[5] has distinguished five classes of "bromate-driven oscillators", four of which consist of bromate, bromide, a redox couple, and an organic reducing substrate, and a fifth "uncatalyzed" class in which the organic substrate performs some of the functions of the metal as well. The Field–Körös–Noyes[6] and derived mechanisms[7] give predictions in excellent qualitative agreement with experimental results on these systems, though the details of some reactions involving the organic substrate remain a mystery.[8]

Essentially quantitative agreement between theory and experiment has been obtained for the conditions under which bistability is found in a stirred tank reactor (CSTR) when only bromate, bromide, and cerium are present.[9] These calculations also predict the existence of a very narrow region of small-amplitude oscillations in this system.[10] The first experimental search for these oscillations was, however, unsuccessful.[11]

We report here the discovery, within an extraordinarily narrow range of conditions, of oscillations in a CSTR containing bromate, bromide, and cerous or manganous ions. This finding is of major importance not only because it lends further support to the mechanism but also because, as we discuss below and elsewhere,[12] it greatly enlarges the scope of bromate oscillation.

Experiments were performed in two thermally regulated CSTR's of volumes 21 and 28.7 cm^3. The reactors, which have been described elsewhere,[13] were modified to eliminate any air space over the reaction mixture. The potentials of a platinum redox electrode and a bromide selective electrode against a mercurous sulfate reference electrode as well as the optical density at various wavelengths were continuously recorded.

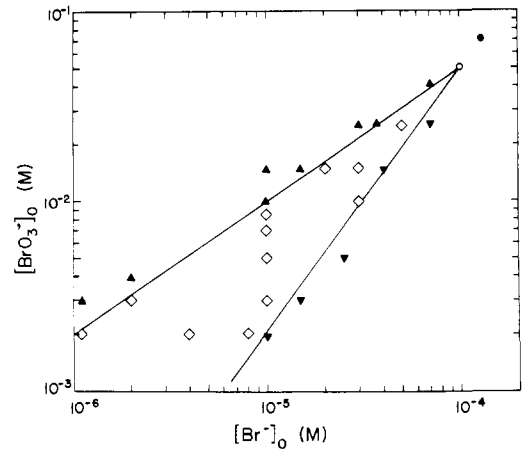

Figure 1. Section of the phase diagram in the $[BrO_3^-]_0$–$[Br^-]_0$ plane with flow rate $k_0 = 0.0128\ s^{-1}$, $[Mn^{2+}]_0 = 1.02 \times 10^{-4}$ M, $[H_2SO_4] = 1.5$ M, $T = 25$ °C: (▲) high potential (low bromide) state; (▼) low potential (high bromide) state; (◇) bistability; (○) oscillation (critical point); (●) critical point (oscillatory composition) when Mn^{2+} is replaced by 1.02×10^{-4} M Ce^{3+} and all other constraints as above.

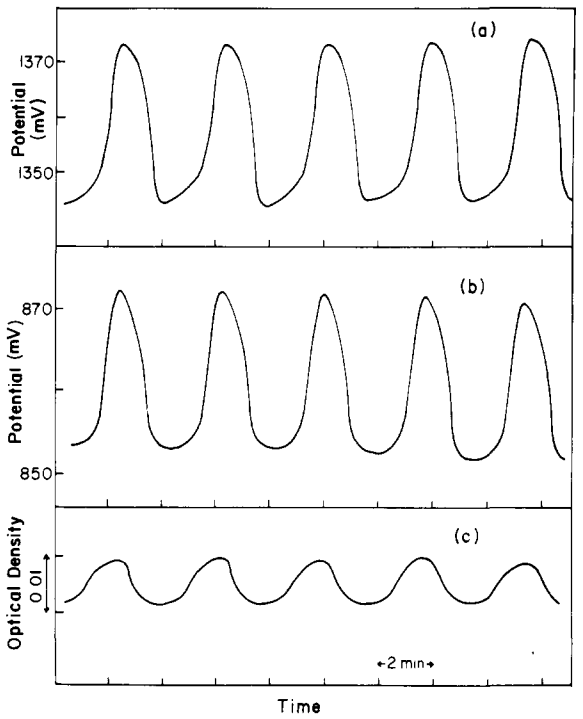

Figure 2. Oscillations at the critical point ○ of the phase diagram in Figure 1 ($[BrO_3^-]_0 = 0.048$ M, $[Br^-]_0 = 9.8 \times 10^{-5}$ M): (a) potential of Pt electrode; (b) potential of bromide selective electrode (uncalibrated, but $[Br^-] < 10^{-7}$ M); (c) absorbance at 350 nm (primarily due to Mn(III) and Br_2, though absorbance oscillations at Br_2 absorption maximum at 390 nm are considerably smaller).

In Figure 1 we show the phase diagram at fixed flow rate, temperature, and input flux of Mn^{2+}, for the bromate–bromide–manganous system in the $[BrO_3^-]_0$–$[Br^-]_0$ plane. A relatively broad region of bistability narrows as the bromate and bromide input concentrations are increased until it disappears at the critical point $[BrO_3^-]_0 = 0.048$ M, $[Br^-]_0 = 9.8 \times 10^{-5}$ M. With manganous replaced by cerous ion, the critical point occurs at $[BrO_3^-]_0 = 0.072$ M, $[Br^-]_0 = 1.22 \times 10^{-4}$ M. In an *extremely narrow*

(1) Part 10 in the series Systematic Design of Chemical Oscillators. Part 9: Grant, J. L.; De Kepper, P.; Epstein, I. R.; Kustin, K.; Orbán, M. *Inorg. Chem.*, in press.

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