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Kinetic Evaluation of Substrate Specificity in the Glyoxalase-I-Catalyzed Disproportionation of α-Ketoaldehydes†

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ABSTRACT: The dissociation constants, K_{diss}, of the adducts formed in the preenzymic reaction between substrates (α-ketoaldehydes) and coenzyme (glutathione, GSH) in the glyoxalase system have been determined for methylglyoxal, phenylglyoxal, and a series of meta- or para-substituted phenylglyoxals including *p*-CH_3, *p*-OCH_3, *m*-OCH_3, *p*-Br, *p*-Cl, *p*-OH, *p*-NO_2, and *p*-phenyl. For methylglyoxal, $K_{diss} = 3.0 \pm 0.5 \times 10^{-3}$ M in the pH range 5–9; for phenylglyoxal, $K_{diss} = 0.60 \pm 0.05 \times 10^{-3}$ M, increasing somewhat at the higher end of this pH range. At pH 7, all the substituted phenylglyoxals show K_{diss} *ca.* $1–3 \times 10^{-3}$ M. The lack of substituent effects on K_{diss} is reflected also in the similar rates of adduct formation, followed at pH 3. The consistent values observed for K_{diss} when the initial GSH and α-ketoaldehyde concentrations are varied suggest only 1:1 adducts are formed. The data support the idea that the adducts are hemimercaptals. Glyoxalase-I shows very broad specificity in the disproportionation of these adducts into the corresponding GSH thiol esters of the α-hydroxycarboxylic acids. In every case the hemimercaptal is the glyoxalase-I substrate; and all V_{max} values are within a factor of 4 of V_{max} for the methylglyoxal–GSH adduct, again showing complete insensitivity to ring substituents. However, the K_M values for the substituted phenylglyoxals–GSH adducts (2×10^{-4} to 2×10^{-5} M) are smaller than K_M for the methylglyoxal–GSH adduct (3×10^{-4} M). The glyoxalase-I reaction is inhibited competitively by free GSH, $K_i = 5 \times 10^{-3}$ M. Although the glyoxalase-I reaction is known to involve intramolecular hydride transfer, the marked insensitivity of the reaction to variations in the α-ketoaldehydes suggests that hydride transfer may not be the rate-determining step.

The disproportionation of α-ketoaldehydes into the corresponding α-hydroxycarboxylic acids, catalyzed by the glyoxalase system, appears to be a common intracellular reaction (Dakin and Dudley, 1913a,b; Neuberg, 1913; Lohmann, 1932; Knox, 1960) and is of interest for several reasons: (a) the biological importance of the glyoxalase system

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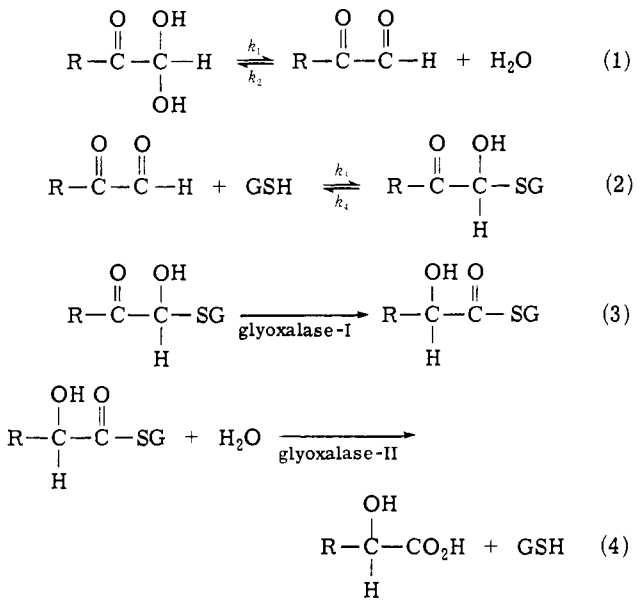
SCHEME I



GSH = γ-L-glutamyl-L-cysteinylglycine

is still unclear, although a number of roles have been suggested such as a role in protection against α-ketoaldehyde toxicity (Salem, 1954), and a role in regulation of cellular growth (Egyud and Szent-Györgyi, 1966a,b; Szent-Györgyi *et al.*, 1967; Szent-Györgyi, 1968); (b) the reaction utilizes glutathione (GSH) as coenzyme, one of many roles for GSH; and (c) the reaction mechanism is unusual in that GSH and α-ketoaldehydes first combine in a nonenzymic reaction to form adducts which are the actual glyoxalase substrates (Cliffe and Waley, 1961; Davis and Williams, 1969). The reactions of the glyoxalase system proposed by Cliffe and Waley (1961) based upon a study using methylglyoxal as substrate are summarized in Scheme I.

To date, very little has been reported on the range of α-ketoaldehydes which are substrates for glyoxalase-I (*S*-lactoylglutathione methylglyoxal-lyase (isomerizing), EC 4.4.1.5), and only methylglyoxal, the α-ketoaldehyde commonly found intracellularly, has been analyzed in detail as a substrate for glyoxalase-I (referred to hereafter simply as glyoxalase). The present study was initiated to answer the following questions. (a) Is the preenzymic reaction between GSH and α-ketoaldehyde consistent with hemimercaptal formation? (b) What is the substrate specificity for glyoxalase in terms of variation of the α-ketoaldehyde? (c) Is the actual glyoxalase substrate the α-ketoaldehyde–GSH adduct, as reported for methylglyoxal? We report here our results from a study of methylglyoxal and a series of substituted phenylglyoxals as glyoxalase substrates (reactions 1–3, Scheme I).

Experimental Section

Materials

GSH (Sigma) was found to be >99% pure by sulfhydryl titration with *N*-ethylmaleimide (Alexander, 1958). Yeast glyoxalase-I (Sigma) was obtained as a 50% glycerol solution. Commercial methylglyoxal, 40% aqueous solution (Aldrich), was purified by distillation (Kermack and Matheson, 1957). Standardization of methylglyoxal solutions was carried out by preparation of the bis-2,4-dinitrophenylhydrazone derivative which in basic solution showed λ_{max} 555 nm, molar extinction coefficient (ϵ) 4.14×10^4 M^{-1} cm^{-1}. Another method involved preparation of the bissemicarbazone derivative which at pH 7 showed λ_{max} 283 nm, ϵ 2.55×10^4 (lit. (Alexander and Boyer, 1971) λ_{max} 286, ϵ 3.20×10^4); the two methods gave good agreement. A series of meta- or para-substituted phenylglyoxals including H, *p*-CH_3, *p*-OCH_3, *p*-Cl, *p*-Br, *p*-phenyl, *m*-OCH_3, and *p*-NO_2 was prepared either by selenium dioxide oxidation of the corresponding acetophenones (Riley and Gray, 1943) or by use of Kornblum's procedure (Kornblum and Frazier, 1966) involving conversion of the substituted phenacyl bromide into the nitrate ester with silver nitrate, followed by treatment with sodium acetate in dimethyl sulfoxide to convert the nitrate ester into the substituted phenylglyoxal. *p*-Hydroxyphenylglyoxal was prepared by oxidation of *p*-benzoylacetophenone, followed by hydrolysis. The products were generally isolated in their hydrated form; structures were confirmed by evaluation of the infrared (ir), ultraviolet (uv), and nuclear magnetic resonance (nmr) spectra as well as elemental analysis of the dioxime derivatives. The uv spectral data of the substituted phenylglyoxal hydrates are given in Table I.

TABLE I: Ultraviolet Data for Substituted Phenylglyoxal Hydrates, Their Adducts with Glutathione, and the Thiol Ester Products of the Glyoxalase Reaction.[a]

Substituent	λ_{max}	ϵ^b	$\lambda_{K_{diss}}$	ϵ_{AD}	ϵ_{xPG}[c]	λ_{iso}	ϵ_{iso}	ϵ_p[d]
H	251	10,300	280	2450	1280	263	6,790	1,100
p-CH_3	263	14,200	320	1250	165	273	10,300	1,010
p-OCH_3	287	15,200	320	4190	1370	296	12,380	970
p-Br	264	14,800	310	1955	280	272	11,500	1,860
p-Cl	260	14,100	320	1350	380	270	10,000	1,020
p-C_6H_5	292	18,000	340	3180	795	303	16,000	2,280
m-OCH_3	255	7,680	310	2060	2550	263	5,230	1,670
p-NO_2	268	13,000	340	1190	575	270	13,000	10,700
p-OH	284	12,600	320	5735	4990	297	9,930	1,140

[a] Spectra recorded at 25°, phosphate buffer, pH 7.0, μ = 0.2, half of ionic strength from buffer and half from added KCl. [b] ϵ is the molar extinction coefficient (M^{-1} cm^{-1}) at the wavelength for maximum absorption, λ_{max} (nm), for the substituted phenylglyoxal hydrates. [c] ϵ_{AD} and ϵ_{xPG} are the molar extinction coefficients for the GSH adducts and the substituted phenylglyoxals at the wavelengths ($\lambda_{K_{diss}}$) used to determine the dissociation constants. [d] ϵ_{iso} is the molar extinction coefficient at the isosbestic wavelength, λ_{iso}. ϵ_p is the molar extinction coefficient of the thiol ester product of the glyoxalase reaction at the isosbestic wavelength.

Methods

Dissociation Constants. The dissociation constants of the adducts of GSH and α-ketoaldehyde are defined as: K_{diss} = [total α-ketoaldehyde][GSH]/[adduct], where total α-ketoaldehyde at equilibrium is essentially equal to the concentration of the hydrated form, owing to the high degree of hydration of these very reactive aldehydes. The concentrations at equilibrium can be obtained from the absorbance at some wavelength λ if one knows the molar extinction coefficients, ϵ.

For the reaction between methylglyoxal and GSH, K_{diss} was determined at 240 nm, 25°, in the pH range 5–9. For methylglyoxal, ϵ_{MG} 4 M^{-1} cm^{-1} throughout this pH range (lit. (Racker, 1951) ϵ_{MG} 5); for GSH, ϵ_{GSH} 61, 61, 65, 115, 209, 834, 2280 at pH 5.25, 5.74, 6.18, 6.85, 7.47, 8.26, and 8.98, respectively, all at 240 nm. By using very high or very low [GSH]/[methylglyoxal] to push adduct (AD) formation toward completion, ϵ_{AD} was estimated to be 440, pH independent (lit. (Davis and Williams, 1969) ϵ_{AD} 395 and (Cliffe and Waley, 1961) 220). In general, at each pH value the determination of K_{diss} was made at five to ten different concentrations of GSH and methylglyoxal.

For the reaction of GSH with the series of phenylglyoxals, K_{diss} values could be determined at wavelengths where GSH does not absorb. The phenylglyoxal adducts were evaluated over a range of pH, similar to the methylglyoxal study. The dissociation constants for substituted phenylglyoxal adducts were evaluated at pH 7. The λ and ϵ values are summarized in Table I. Absorbance measurements for determinations of K_{diss} were obtained either with a Cary 15 or with a Gilford 222 modified Beckman DU. Both spectrophotometers were temperature controlled with circulating water baths.

Kinetics. Reaction rates for adduct formation were monitored with the spectrophotometers mentioned above. All rate constants were from computer-calculated least-squares lines of first-order plots; correlation coefficients were generally better than 0.999.

The glyoxalase-catalyzed disproportionation of the GSH adduct of methylglyoxal was followed at 240 nm by monitoring the appearance of thiol ester product, ϵ_p 3300 M^{-1} cm^{-1} (lit. (Racker, 1951) ϵ 3370 and (Cliffe and Waley, 1961) 3300). Initial rates were obtained from the initial changes in absorbance as $dP/dt = \Delta A/\Delta t(\epsilon_p - \epsilon_{AD})$. From the initial concentrations and K_{diss} value, the adduct concentrations could be calculated for each initial rate. The data were treated by the Michaelis–Menten (1913) scheme and V_{max} and K_M values obtained from double-reciprocal plots (Lineweaver and Burk, 1934). Methylglyoxal also was used to standardize solutions of glyoxalase. Sufficient concentrations of the GSH adduct of methyglyoxal can be obtained to saturate glyoxalase at concentrations used in the initial rate studies. The V_{max} values from saturation studies agree very well with V_{max} values obtained from the double-reciprocal plots. Commercial yeast glyoxalase (specific activity 200–600 μmoles/min per mg) was generally diluted 250-fold in pH 7.0 phosphate buffer containing 100 μg/ml of bovine serum albumin. In all initial rate studies and in the standardization of the glyoxalase stock solution, 20 μl of enzyme solution/3 ml of reactant solution was used. This amount of enzyme gave convenient initial rates to follow and was in the range where the initial rate is proportional to glyoxalase concentration. At higher enzyme concentrations, the preenzymic reaction to form adduct is rate limiting (Cliffe and Waley, 1961).

The initial rates for disproportionation of the GSH adducts of substituted phenylglyoxals were followed at the apparent isosbestic points of the substituted phenylglyoxal hydrates and their GSH adducts. Representative spectra for phenylglyoxal are shown in Figures 1 and 2 which summarize the general approach used in studying all of the substituted phenylglyoxals. In all cases, the thiol ester products have significantly lower molar extinction coefficients at the isosbestic points (λ_{iso}) than do the reactants. The reaction scheme for kinetic analysis of the substituted phenylglyoxals is shown in Scheme II.

Initial rates, $dP/dt = \Delta A_{\lambda iso}/\Delta t(\epsilon_{iso} - \epsilon_p)$, where $\Delta A_{\lambda iso}$ is the initial change in absorbance at the isosbestic point in time Δt, and $\epsilon_{iso} - \epsilon_p$ is the difference in molar extinction coefficients of reactant (S) and thiol ester product (P). Owing to the rapidity of the preenzymic reactions (1 and 2 of Scheme I), use of any wavelength other than λ_{iso} makes it difficult to convert initial changes in absorbance into initial rates unless one has knowledge of the rate constants $k_1 - k_4$ of Scheme I. The λ_{iso} and ϵ values are given in Table I.

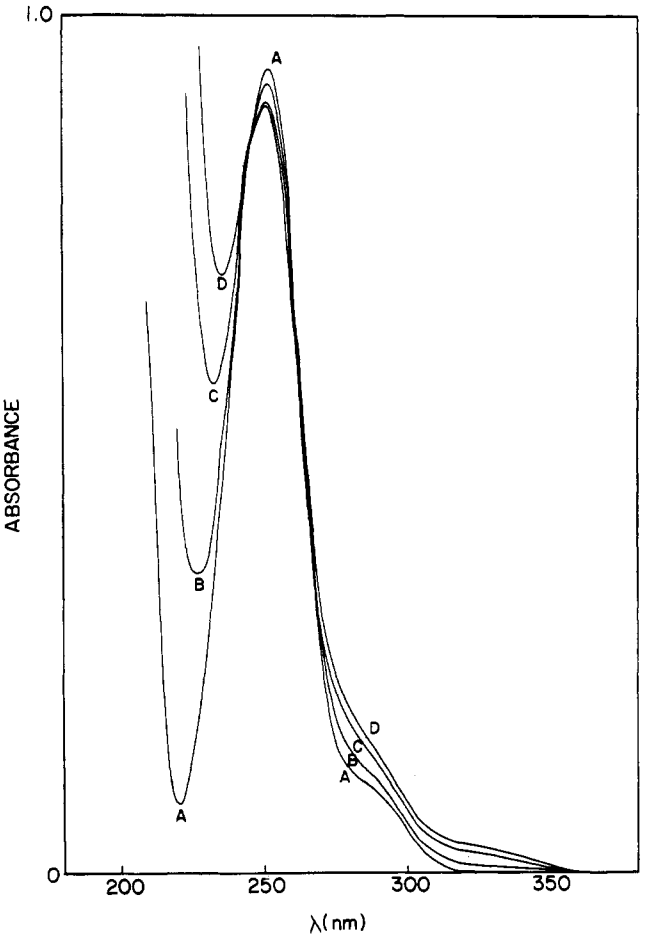
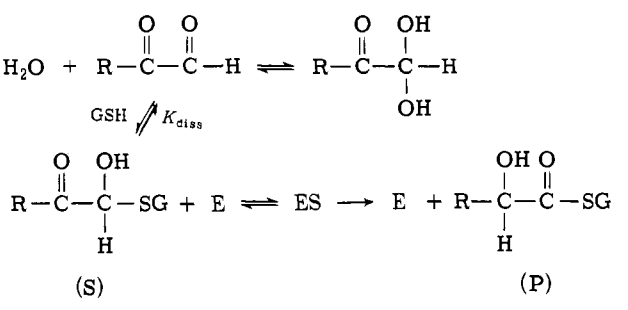


FIGURE 1: Spectrum of a 10^{-4} M solution of phenylglyoxal (curve A), pH 7, 25°, and spectra of equilibrium mixtures of 10^{-4} M phenylglyoxal and added glutathione, 2.3×10^{-4} M (B), 6.9×10^{-4} M (C), and 1.2×10^{-3} M (D). K_{diss} was determined from the changes in absorbance at 280 nm. The glyoxalase-I-catalyzed disproportionation of phenylglyoxal was monitored at the apparent isosbestic point, 263 nm. This general procedure was used for all of the substituted phenylglyoxals.

Kinetics of Product Hydrolysis. The thiol ester products were hydrolyzed at pH 10.5 using a Radiometer SBR2c Tit-

SCHEME II



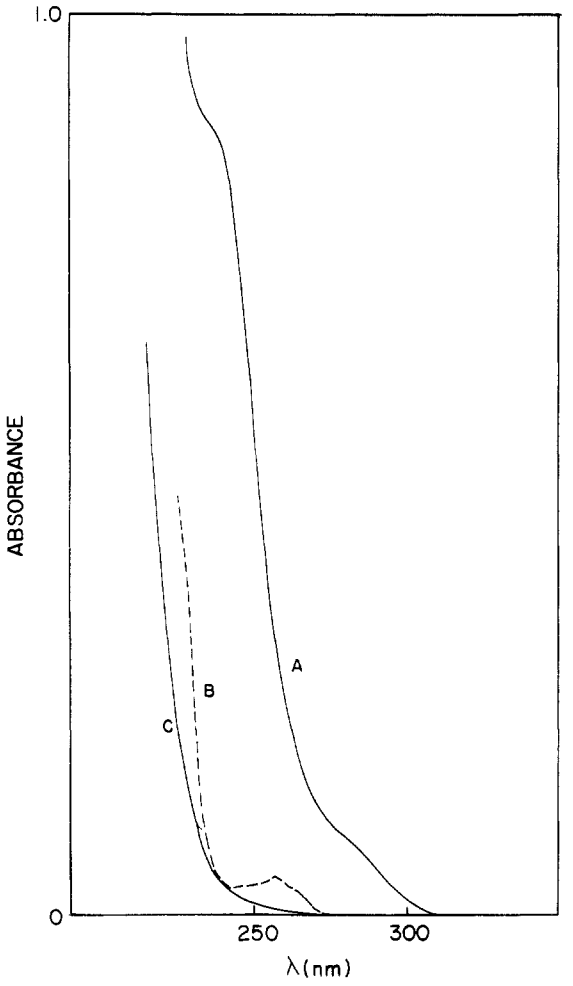


FIGURE 2: Spectrum of the thiol ester product of the glyoxalase-I-catalyzed disproportionation of the GSH adduct of phenylglyoxal (curve A); concentration of thiol ester 10^{-4} M. Spectrum B is for a 2×10^{-4} M solution of mandelic acid. Spectrum C is for a 2.3×10^{-4} M solution of glutathione. All spectra at 25°, pH 7.

rigraph pH-Stat. The esters were prepared by running the enzyme reaction in neutral, unbuffered water.

Results

The dissociation constants for the GSH adducts of methylglyoxal and phenylglyoxal were determined in the pH range 5–9 for various initial concentrations of GSH and α-ketoaldehyde (Table II). For the methylglyoxal adduct, K_{diss} = $3.0 \pm 0.5 \times 10^{-3}$ M throughout the pH range studied. The adduct of phenylglyoxal shows $K_{diss} = 0.60 \pm 0.05 \times 10^{-3}$ M from pH 5 to 7.5 and somewhat larger values as the pH increases. In both cases, the reasonably consistent values obtained at a given pH when the initial concentrations of both GSH and α-ketoaldehyde are varied indicate that only 1:1 adducts are formed.

The dissociation constants for GSH adducts of the substituted phenylglyoxals, determined at pH 7, are shown in Table II. The data again show that only 1:1 adducts are formed. The effects of substituents on K_{diss} are quite small, suggesting that the rates of adduct formation might also be insensitive to substituents. Cliffe and Waley (1961) reported that the rate of adduct formation between GSH and methylglyoxal depends only upon the methylglyoxal concentration.

TABLE II: Dissociation Constants of the GSH Adducts of Substituted Phenylglyoxals and Methylglyoxal (pH 7.0).[a]

Substituent	K_{diss} (mM)
H	0.60 ± 0.05
p-CH_3	1.1 ± 0.1
p-OCH_3	1.4 ± 0.1
p-Br	1.5 ± 0.2
p-Cl	3.6 ± 0.5
p-C_6H_5	0.89 ± 0.05
m-OCH_3	1.2 ± 0.1
p-NO_2	1.2 ± 0.2
p-OH	0.93 ± 0.05
Methylglyoxal	3.0 ± 0.5

[a] 25°, phosphate buffer, μ = 0.2, half of ionic strength from buffer and half from added KCl.

TABLE III: Apparent First-Order Rate Constants for the Reaction of GSH with α-Ketoaldehydes (pH 3.0).[a]

Substituted Phenylglyoxal[b]	k (10^{-3} sec^{-1})
H	7.22 ± 0.06
p-CH_3	6.93 ± 0.03
p-OCH_3	7.95 ± 0.05
p-Br	8.31 ± 0.03
p-Cl	7.60 ± 0.19
p-C_6H_5	8.04 ± 0.05
m-OCH_3	7.72 ± 0.09
p-NO_2	5.53 ± 0.28
p-OH	6.35 ± 0.11
Methylglyoxal	6.55 ± 0.41

[a] Formate buffer, 25°, μ = 0.2, half of ionic strength from buffer and half from added KCl. [b] [α-ketoaldehyde] = 0.3–0.4 mM; [GSH] = 5 mM.

These authors suggested that dehydration of methylglyoxal (k_1, Scheme I) is rate determining in adduct formation. Thiol additions to reactive aldehydes such as formaldehyde also involve rate-determining dehydration (Lienhard and Jencks, 1966; Kallen and Jencks, 1966). In Table III are shown some apparent first-order rate constants for approach to equilibrium, measured at pH 3, for the reaction of GSH with methylglyoxal and the series of phenylglyoxals. The reaction conditions were similar for each reaction. The effects of substituents on the rates are minor, in agreement with their small effects on K_{diss}. These rate constants are composites of the rate constants for the adduct forming reactions (reactions 1 and 2, Scheme I) and cannot be assigned directly to the dehydration step k_1. However, these apparent rate constants should parallel k_1, suggesting that dehydration proceeds at a similar rate for all of the α-ketoaldehydes studied. A possible explanation for this insensitivity was obtained by analysis of the infrared spectra of the unhydrated α-ketoaldehydes. Solutions of substituted phenylglyoxals in acetonitrile were warmed over molecular sieves to prepare the unhydrated α-ketoaldehydes. Infrared spectra showed that the carbonyl-stretch-

ing frequency for the aldehyde carbonyl was essentially constant at 1727 ± 2 cm^{-1}, totally insensitive to substituent; the ketone carbonyl-stretching frequencies were very sensitive to substituents.[1] If one assumes that the energy of the carbonyl-stretching frequency reflects sensitivity to nucleophilic attack, then the constant value observed in the aldehyde carbonyl frequencies is consistent with the insensitivity to substituents observed in K_{diss} and in the rates of adduct formation, and it is consistent with the conclusion that the 1:1 adducts are hemimercaptals resulting from GSH addition only to the aldehyde carbonyl.

That the hemimercaptals are the actual glyoxalase substrates, as suggested by Cliffe and Waley (1961) for methylglyoxal, was confirmed by measuring initial rates of disproportionation of the GSH–methylglyoxal adduct under a wide range of different initial concentrations of GSH and methylglyoxal. Glyoxalase solutions generally were standardized under conditions of high initial methylglyoxal/GSH where the amount of free GSH at equilibrium is very small. Under these conditions the glyoxalase reaction proceeds very well. However, the reverse is not true; high initial GSH/methylglyoxal conditions give inconsistent initial rates. The presence of free GSH inhibits the glyoxalase reaction (Kermack and Matheson, 1957). Determination of the enzyme–inhibitor dissociation constant, K_i, and type of inhibition by direct evaluation of double reciprocal plots is difficult because GSH is involved in the preenzymic equilibrium reaction. Therefore, K_i was determined by working under saturation conditions. Various methylglyoxal/GSH ratios were used to form the adduct where in each case the concentration of adduct was sufficient to saturate the enzyme. V_{max} could be measured directly if the concentration of free GSH at equilibrium was low. At that ratio where the observed rate is $V_{max}/2$, $K_i = K_m$[GSH]/[adduct]. This ratio of [GSH]/[adduct] is about 16. For the methylglyoxal adduct, $K_m = 3 \times 10^{-4}$ M, obtained from double-reciprocal plots in runs where at each point the concentration of adduct exceeded the concentration of free GSH. The value of K_i for GSH inhibition is therefore about 5×10^{-3} M, and the inhibition must be competitive since high adduct concentrations overcome the inhibition.

Analysis of the adducts of the substituted phenylglyoxals as substrates demonstrated very broad specificity in the glyoxalase reaction. All of the GSH adducts of the substituted phenylglyoxals were comparable to the GSH adduct of methylglyoxal as substrates. Relative to the V_{max} for methylglyoxal, all α-ketoaldehydes examined fall within a fourfold range, reflecting almost complete insensitivity to substituents. Results are shown in Table IV. The K_M values are smaller than K_M for the methylglyoxal adduct, ranging down to 2×10^{-5} M for *p*-phenylphenylglyoxal. Consequently, many of the substituted phenylglyoxals are better substrates than methylglyoxal if one considers both binding to the enzyme (reflected in K_M) and the catalytic rate constant (reflected in V_{max}). Although many of the substituted phenylglyoxals show limited solubilities, in each case the initial rates could be determined at adduct concentrations which span K_M for that particular substrate.

To test whether the commercial enzyme preparation used in this study contained one or several glyoxalase species, preparations of different specific activity to methylglyoxal were tested with several different α-ketoaldehydes. In no case did the relative specificities for several substrates show any variation from one preparation to another. Furthermore, commercial yeast glyoxalase preparations are available with specific activities within a factor of 2 of the highest activity reported for any yeast glyoxalase preparation (Davis and Williams, 1966). For these reasons, it was concluded that the broad specificity observed in the glyoxalase reaction probably represents the specificity of a single enzyme species.

[1] Details will be reported elsewhere in a paper on some chemical reactions of α-ketoaldehydes.

TABLE IV: Kinetic Parameters K_M and V_{max} for the Glyoxalase-I Disproportionation of GSH Adducts of Methylglyoxal and Substituted Phenylglyoxals.

α-Ketoaldehyde	K_M (M)	V_{max} (rel)[a]
Methylglyoxal	3×10^{-4}	1.00
Substituted phenylglyoxal		
H	2×10^{-4}	0.93
p-CH_3	4×10^{-5}	0.27
p-OCH_3	4×10^{-5}	0.24
p-Br	3×10^{-5}	0.46
p-Cl	2×10^{-5}	0.45
p-C_6H_5	2×10^{-5}	0.30
m-OCH_3	6×10^{-5}	0.88
p-NO_2	9×10^{-5}	0.72
p-OH	7×10^{-5}	0.31

[a] V_{max} values all relative to methylglyoxal; all data at pH 7.0, 25°, phosphate buffer, $\mu = 0.2$, half of ionic strength from buffer and half from added KCl.

In addition to the spectral changes which occur during the disproportionation reactions, and the observed broad tailing absorption around 240 nm (Figure 2) characteristic of thiol esters (Racker, 1951), products were checked by hydrolysis of the thiol esters at pH 10.5. The rates were quite similar as expected for these compounds because the variations in the side groups are quite far removed from the ester linkages.

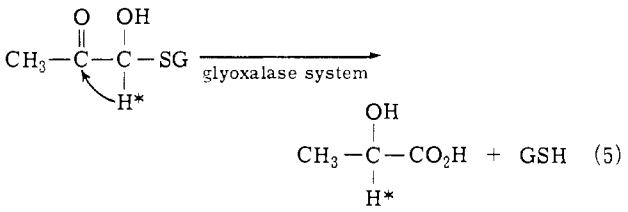
Discussion

The glyoxalase system consists of two enzymes, glyoxalase-I (*S*-lactoylglutathione methylglyoxal-lyase (isomerizing), EC 4.4.1.5) and glyoxalase-II (*S*-2-hydroxyacylglutathione hydrolase, EC 3.1.2.6). The present study of the substrate requirements for glyoxalase-I supports the conclusion of Davis and Williams (1969) that glyoxalase-I is an oxidoreductive isomerase rather than a lyase, owing to the fact that the enzyme catalyzes the disproportionation of the hemimercaptals rather than using both GSH and the α-ketoaldehydes as substrates. The enzyme was named on the basis of the only well-known intracellular α-ketoaldehyde, namely, methylglyoxal. In view of the broad specificity of glyoxalase-I and in view of reports of other intracellular α-ketoaldehydes (Sparkes and Kenny, 1969), which may very well be substrates for the enzyme, the official name for glyoxalase-I may be doubly in error.

Although methylglyoxal, phenylglyoxal, glyoxal, and hydroxypyruvaldehyde have been reported to be substrates for glyoxalase-I (Racker, 1952; Hopkins and Morgan, 1948), only methylglyoxal has been studied in any detail. Early estimates of the extent of adduct formation between GSH and methylglyoxal (Platt and Schroeder, 1934) gave values for K_{diss} *ca.* 10^{-2} M; this procedure involved rapid titration

of the sulfhydryl groups in an equilibrium mixture of GSH, adduct, and methylglyoxal. Kermack and Matheson (1957) determined K_{diss} spectrophotometrically at 240 nm, pH 6.6, and reported a value of 5×10^{-3} M. Cliffe and Waley (1961) used the same procedure and reported a value of 2×10^{-3} M. These authors also estimated K_{diss} from their kinetic data in studies of glyoxalase-I and reported values of $1\text{–}2 \times 10^{-3}$ M, increasing somewhat as the initial concentrations of GSH and methylglyoxal were lowered; they offered no explanation for this observation. These variations in reported values for K_{diss} of the GSH–methylglyoxal adduct raised the possibility that other than 1:1 adducts form, in which case the species distribution at equilibrium would depend both upon the initial GSH/methylglyoxal ratio and upon their actual concentrations. The results of the present study indicate that for all of the α-ketoaldehydes examined, only 1:1 hemimercaptals form to any significant extent.

The very broad substrate specificity shown by glyoxalase-I and the absence of any significant substituent effects in the disproportionation of the substituted phenylglyoxals raise questions about the mechanism of the reaction. It has been well established (Franzen, 1956; Rose, 1957) that the GSH–methylglyoxal adduct is disproportionated without loss of the aldehydic hydrogen (eq 5), presumably by intramolecular



(5)

hydride migration. If intramolecular hydride migration is rate determining and sensitive to the polarity of the α-ketone carbonyl, one might expect to observe some substituent effects. The lack of sensitivity to substituents observed in our study of substituted phenylglyoxals as substrates may mean that hydride migration is not the rate-determining step. However, it is possible the hydride migration is facilitated through polarization of the α-ketone group by the enzyme, in which case the added effects on polarization by the substituents may be small, and hydride migration could still be rate determining.

The smaller K_M values for the substituted phenylglyoxal adducts generally parallel the size and hydrophobic nature of the substituents, K_M being smallest for the *p*-phenyl and *p*-chloro substituents. This observation is in accord with a recent report (Vince and Wadd, 1969) of a nonpolar region at or near the active site of glyoxalase-I, based upon the observed ability of *S*-alkylglutathiones to increasingly inhibit glyoxalase-I as the size of the alkyl chain increased.

The question of the biological role of glyoxalase remains open. The broad specificity of the enzyme for many α-ketoaldehydes is consistent with a detoxification role. However, if regulation of cellular growth involves intracellular α-ketoaldehydes presently unrecognized, these are likely to be substrates for glyoxalase.

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