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Kinetic Characterization of the Endogenous Glutathione Transferase Activity of Octopus Lens S-Crystallin[1]

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The kinetic mechanism of the endogenous glutathione transferase (GST) activity of octopus S-crystallin was investigated by steady-state kinetics. Biphasic double-reciprocal plots were obtained for both glutathione and the hydrophobic substrate 1-chloro-2,4-dinitrobenzene (CDNB). Substrate inhibition was observed only for CDNB with K_{si} value of 29.7± 0.01 mM. The catalytic constant for S-crystallin was three orders of magnitude smaller than that for the digestive gland GST of the same species. The initial-velocity studies indicated that the enzyme reaction might conform to a steady-state random Bi-Bi kinetic mechanism, being similar to the reaction of GST from other sources. The pH-rate profiles also suggest that the same chemical mechanism for the nucleophilic aromatic substitution between GSH and CDNB was employed for S-crystallin. The interaction of Tyr[7] with the bound GSH lowered the pK_a value of the sulfhydryl group of GSH to 6.82-6.85, which is 2.32-2.35 pH unit smaller than that found in aqueous solution. This lowering of pK_a value produces the thiolate anion of GSH, a better nucleophile to attack the *ipso* carbon of CDNB, resulting in formation of Meisenheimer complex intermediate. Removing the chloride ion from this intermediate complex produces the conjugate product. Using the method devised by Wang and Srivastava (*Anal. Biochem.* 216, 15-26, 1994), the functional unit of the dimeric S-crystallin was estimated to be a monomer. The possible biological implications of the endogenous detoxification ability of cephalopods S-crystallin are discussed.

Key words: enzyme crystallin, S-crystallin, glutathione transferase, kinetics, cephalopods.

Crystallins are the major soluble proteins in animal lenses, their presence facilitating the transparency of the lens, thus allowing light to reach the retina and form a clear image. There are two major groups of crystallin, both of which are connected with some stress proteins (*1*). The first group is the ubiquitous crystallins, which are further divided into three subgroups: α-crystallin, a small heat-shock protein (molecular chaperone) (*2*); and β- and γ-crystallins, which are related to the S-protein of *Myxococcus xanthus* induced by osmotic shock (*3*). The second group is the taxon-specific crystallins, which are structurally related to some metabolic enzymes. Of all known taxon-specific enzyme/crystallins, over 70% are oxidoreductases (*4, 5*), *e.g.*, ε-crystallin/lactate dehydrogenase in birds and reptiles, η-crystallin/aldehyde dehydrogenase in elephant shrews, λ-crystallin/hydroxyacyl-CoA dehydrogenase in rabbits and hares, ρ-crystallins/NADPH reductase in frogs, Ω-crystallin/aldehyde dehydrogenase in octopi, ζ-crystallin/quinone oxidoreductase in guinea pigs, and π-crystallin/glyceraldehyde 3-phosphate dehydrogenase in diurnal geckos (*6*). The impact of lens metabolism on the animals with an oxidoreductase as lens crystallin was studied by Zigler Jr. and colleagues (*6-9*). The oxidative stresses suppress the proteolytic capability of lenses and reduce the normal protein turnover rate (*10*). Animals with high levels of reduced pyridine nucleotides are less susceptible to photodamage (*8*). Most significant is the recent finding that diurnal but not nocturnal geckos contain the π-crystallin/glyceraldehyde 3-phosphate dehydrogenase (*6*). In this connection, the development of oxidoreductases as lens crystallins may be an evolutionary transition from marine animals to amphibians to terrestrials.

Photo-oxidation stress may not be a serious problem for cephalopods. The major crystallin of the octopus is not an oxidoreductase but has a structure similar to a detoxification enzyme, glutathione transferase (GST; EC 2.5.1.18) (*1, 4, 5*). Whether S-crystallin has a detoxification function *in vivo* other than the structural role is an interesting question. The present study was thus conducted to characterize the endogenous GST activity of octopus S-crystallin. Glutathione transferase catalyzes the conjugation reaction between GSH and various hydrophobic xenobiotics through the formation of a more water-soluble glutathione thioether (*11-14*). The glutathione S-conjugate may then be eliminated from the cell by a phase III detoxification system glutathione S-conjugate export pump (*15*).

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Abbreviations: GST, glutathione transferase; GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene.

The three-dimensional structures of the alpha-, mu-, pi-, and theta-classes GST have been determined by X-ray crystallographic analyses (*16-19*). More recently, the detailed structure of the cephalopod digestive gland GST was delineated to 2.4 Å resolution and was assigned a new sigma-class (*20*). Although only 30-35% similarity was found between different types of GST, their overall structural topology is similar and they utilize the same catalytic mechanism, as indicated by structural and kinetic analyses (*16-21*).

The kinetic mechanism of GST is quite complicated. Nonhyperbolic kinetics were reported nearly two decades ago (*22-25*). Many hypotheses have been derived to explain this non-Michaelian behavior, including hysteretic enzyme memory (*26*). Recently, Ivanetich *et al.* (*27*) suggested that a simple steady-state random Bi-Bi kinetic mechanism was enough to explain the kinetic data.

In this paper, the steady-state kinetic studies of the endogenous GST activity of octopus S-crystallin is reported. Results indicate that the enzyme activity of S-crystallin may also conform to a steady-state random Bi-Bi kinetic mechanism, like the activity of GST from other sources. Since the octopus S-crystallin possesses definite endogenous GST activity, it might preserve the detoxification function for the octopus lens protection.

MATERIALS AND METHODS

Materials—GSH (Sigma) and CDNB (Serva) were purchased from the designated sources. S-Crystallin from octopus was purified to apparent homogeneity by a similar procedure to that used for squid S-crystallin (*28*).

Enzymatic Activity Assay—GST activity was assayed with a Perkin-Elmer Lambda 3B spectrophotometer at 25°C. A 1-ml reaction mixture contained potassium phosphate buffer (100 mM, pH 6.5), GSH and CDNB (2 mM each), and an appropriate amount of the S-crystallin (16.5 μg/assay). The formation of glutathione conjugate was monitored continuously at 340 nm. One unit of enzyme activity was defined as an initial rate of 1 μmol conjugate formed per minute under the assay conditions using an absorption coefficient of 9.6 $mM^{-1} \cdot cm^{-1}$ for the GSH-CDNB conjugate (*22*).

The hydrophobic substrate CDNB was added as a dimethyl sulfoxide solution. Addition of CDNB solution into the aqueous assay mixture caused cloudiness of the solution. Shaking the solution by hand was necessary to redissolve the CDNB. Longer times (approx. 5 min) were needed for higher CDNB concentrations. In our laboratory, a CDNB concentration of up to 3.5 mM could be achieved in aqueous solution.

Initial-Velocity Studies—The initial-velocity study was performed by varying the concentration of GSH from 0.04 to 1.0 mM and that of CDNB from 0.2 to 1.0 mM. Concentrations of other components were held constant. The nonenzymatic conjugation of GSH with CDNB was corrected in each assay as described previously (*29*). For assay of very low activity, the protein amount was increased and the absorbance change was monitored for longer time, with the recorder set at slow speed in order to get a measurable slope.

pH Studies—We assayed the GST activities at various pH values with two substrate concentrations at 25°C. The experimental conditions were as described for the octopus digestive gland GST (*30*). When the enzyme was assayed at high substrate concentration, the CDNB and GSH concentrations were 2 and 1.5 mM, respectively. The velocities thus obtained were near maximum velocity (V). Enzyme activity was calculated according to the Michaelis-Menten equation (Eq. 1) to give the apparent first-order rate constant (k_{cat}).

$$v = V[S]/(K_m + [S]) \quad (1)$$

where [S] denotes substrate concentration and K_m is the Michaelis constant. k_{cat} was obtained by dividing V by $[E_t]$, the total concentration of S-crystallin.

When the S-crystallin was assayed at low CDNB concentration, the GSH concentration was fixed at 1.5 mM, which was approximately 4.5-fold its K_m, and the CDNB concentration employed was 80 μM, about 0.04 times its K_m. The initial rates were then approximately equal to $V[S]/K_m$. The second-order rate constant ($k_{cat}/K_{m\ CDNB}$) was obtained by dividing v by $[CDNB][E_t]$. Experiments with high CDNB (2 mM) and low GSH (50 μM) concentrations were performed in a similar manner.

pH profiles were fitted to Eq. 2 to estimate various pK_a values.

$$\text{Log } Y = \log\{C/(1 + [H^+]/K_{a1} + K_{a2}/[H^+])\} \quad (2)$$

where Y is the value of the parameter of interest measured at any pH, C is the maximal value of parameter Y, K_{a1}, and K_{a2} represent the dissociation constants for the protein groups. The points drawn in figures are the experimentally determined values. The lines drawn through these points are calculated from the fits to Eq. 2.

Data Analysis—Fitting of the experimental data to the respective equations was carried out by use of EZ-FIT (*31*), a curve-fitting microcomputer program using the Melder-Mead Simplex and Marquardt nonlinear regression algorithms sequentially. For pH studies, the data were analyzed by use of a generally applicable nonlinear regression program, SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

RESULTS

Effect of Substrate Concentration on the Enzyme Reaction Rate—S-Crystallin isolated from the octopus or squid lens extract was found to have a similar amino acid sequence to glutathione transferase (*32-35*). We found that the octopus S-crystallin possessed endogenous GST activity, albeit of much lower level than the hepatopancreatic GST. Both the S-crystallin and the octopus digestive gland GST were purified to apparent homogeneity (*36*). The apparent specific GST activities of purified S-crystallin and digestive gland GST were found to be 0.096 and 236 μmol/min/mg protein, respectively.

When the enzyme assays were conducted at various concentrations of GSH, the endogenous GST activity of S-crystallin did not follow the Michaelian kinetics, as revealed by the biphasic double-reciprocal plots (Fig. 1). This is common for the GST from various other sources (*26*). Assuming two independent sites for the dimeric S-crystallin, the apparent V (V_1 and V_2) and $K_{m\ GSH}$ (K_1 and K_2) estimated from Fig. 1A according to Eq. 3 were 0.031 ± 0.044 μmol/min/mg, 0.231 ± 0.035 μmol/min/mg, 0.02 ±

0.083 mM, and 0.598±0.268 mM, respectively.

$$v=\frac{V_1[A]}{K_1+[A]}+\frac{V_2[A]}{K_2+[A]} \quad (3)$$

Quite large errors were found for the K_1 and V_1 values. We also fitted the data to a negatively cooperative model for the dimeric protein with an interaction factor of α (Eq. 4), which gave a slightly better fit. V, $K_{m\,GSH}$, and α were evaluated as 0.281±0.032 μmol/min/mg, 0.33±0.047 mM, and 0.032±0.028, respectively. However, with the present data, it is not possible to select one of these two models as the more appropriate.

$$v=\frac{V([A]/K_m+[A]^2/\alpha K_m^2)}{1+2[A]/K_m+[A]^2/\alpha K_m^2} \quad (4)$$

No substrate inhibition was found for glutathione in the concentration range employed. However, substrate inhibition was observed for CDNB at concentrations greater than 3 mM. By fitting the data to Eq. 5, the apparent V, $K_{m\,CDNB}$, and $K_{si\,CDNB}$ were estimated as 0.35 μmol/min/mg, 2.0 mM, and 29.7 mM, respectively.

$$v=\frac{V}{(1+K_m/[A]+[A]/K_{si})} \quad (5)$$

Possible Functional Unit of the Endogenous GST Activity of Octopus S-Crystallin—All types of GST are dimeric proteins with a separate active site in each subunit (*16-20*). Although many scientists suggested that the subunit of GST is enzymatically inactive (*37-39*), using the

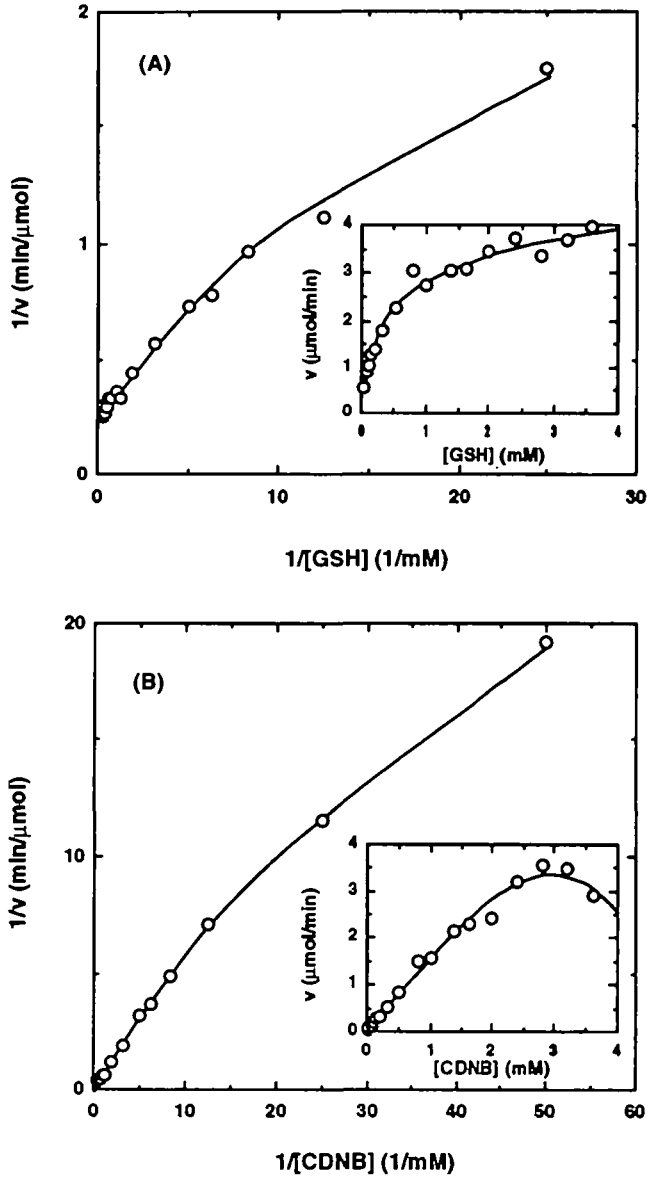


Fig. 1. **Effect of substrate concentration on the endogenous GST activity of octopus S-crystallin.** Double-reciprocal plots of the effect of [GSH] (A) or [CDNB] (B) on enzymatic reaction rate. The effect of [GSH] or [CDNB] on the enzyme reaction rate is shown in the inset by fitting the data to Eqs. 4 and 5, respectively, in (A) and (B). Fitting of the data in Fig. 1A to Eq. 3 gave a similar curve to that shown in the inset but resulted in larger error than did fitting to Eq. 4. The amount of S-crystallin used in each assay was 16.5 μg.

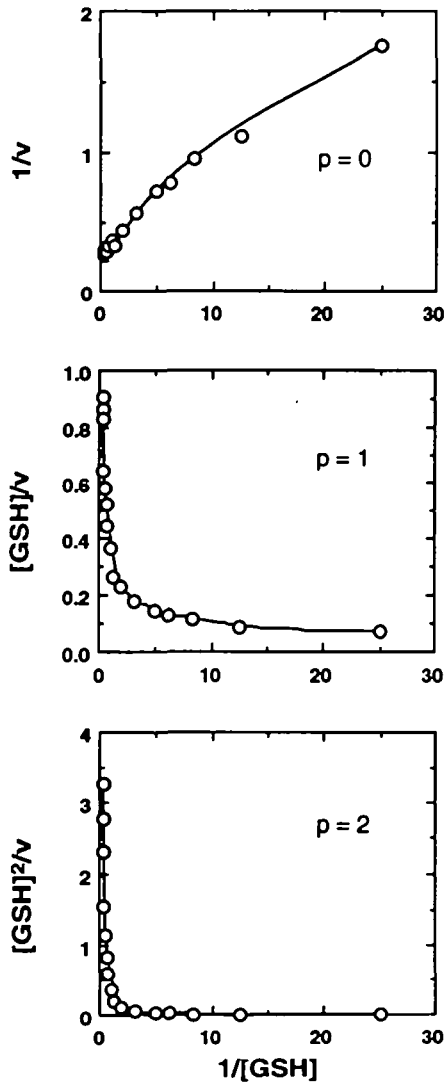


Fig. 2. **Graphical analysis for determining the minimum functional unit of the dimeric S-crystallin.** The minimum subunit number of the functional unit of S-crystallin was determined by plotting $[GSH]^p/v$ *versus* $1/[GSH]$ at various p values. The plot at $p=1$ gives a horizontal asymptote, which suggests that the functional unit of S-crystallin is the monomer.

technique of radiation inactivation, Boyer and Kempner (*40*) have demonstrated that the functional unit of GST-mu is a monomer. The graphical method of Wang and Srivastava (*41*) was used to test the minimum functional unit of the endogenous GST activity of S-crystallin.

This method is based on the theory that the initial rate of an enzyme-catalyzed reaction as a function of ligand concentration ([L]) can be given by the ratio of two polynomials in [L] (Eq. 6).

$$v=\frac{B_k[L]^k+B_{k+1}[L]^{k+1}+\ldots+B_{n-1}[L]^{n-1}+B_n[L]^n}{A_0+A_1[L]+A_2[L]^2+\ldots+A_{n-1}[L]^{n-1}+A_n[L]^n} \quad (6)$$

where k represents the minimal number of ligands bound to an active enzyme molecule. In an oligomeric enzyme, k would be a measure of the minimal number of subunits required for catalysis. The coefficients A_i and B_i consist of combinations of rate constants and reactant concentrations other than [L]. Rearrangement of Eq. 6 gives Eq. 7.

$$1/(v\cdot x^p)=\frac{A_0x^n+A_1x^{n+1}+\ldots+A_{n-1}x+A_n}{B_kx^{n-k+p}+\ldots+B_{n-1}x^{p+1}+B_nx^p} \quad (7)$$

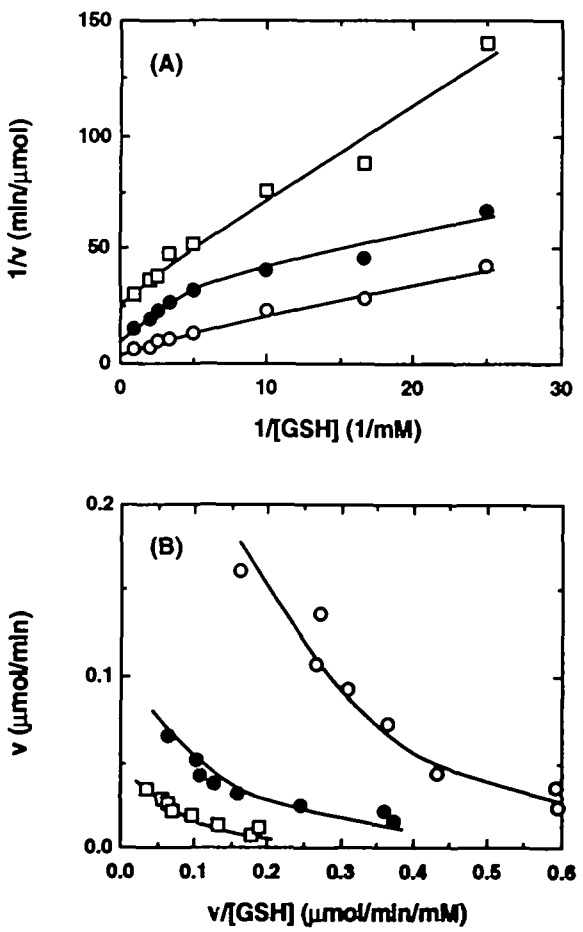


Fig. 3. **Initial-velocity pattern for the endogenous GST activity of octopus S-crystallin.** (A) Double reciprocal plots. The varied-concentration substrate was GSH and the fixed-concentration substrate was CDNB. The concentration of CDNB was fixed at 0.2 (□), 0.4 (●), and 1.0 (○) mM, (B) Eadee-Hofstee plots. The amount of S-crystallin used in each assay was 16.5 μg.

where $x=1/[L]$ when L is a substrate. Series plots of $[L]^p/v$ *versus* $1/[L]$ at $p=0$, 1, 2, *etc.* were constructed. By comparing the preceding and succeeding plots for different values of p, the magnitude of p that gives a horizontal asymptote is precisely assessed. Figure 2 shows such a plot for S-crystallin at $p=0$, 1, and 2. A horizontal asymptote is obtained at $p=1$ and a zero asymptote is obtained when $p=2$. Since Fig. 2, A and B, has entirely different shapes, it is quite clear that $p=1$ is the correct answer. This result suggests that the subunit of S-crystallin is the minimum functional number of the dimeric protein and the subunit is enzymatically active. The same result was obtained for CDNB (data not shown).

Initial-Velocity Studies—The initial-velocity pattern of the S-crystallin-catalyzed conjugation between GSH and CDNB at low concentrations of both substrates is shown in Fig. 3. When GSH was plotted as the varied-concentration

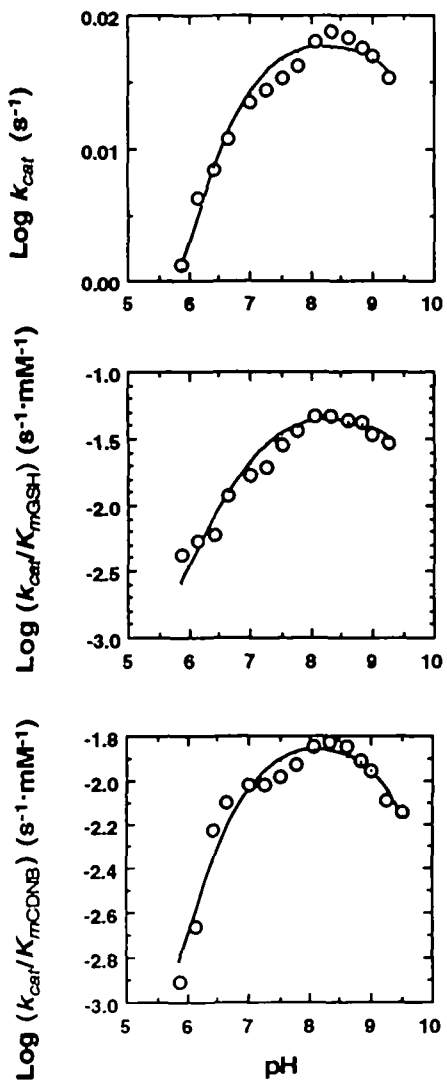


Fig. 4. **Effect of pH on the endogenous GST activity of octopus S-crystallin.** The effects of pH on k_{cat} (A), $k_{cat}/K_{m\,GSH}$ (B), and $k_{cat}/K_{m\,CDNB}$ (C) were determined at various pH values. The symbols represent the experimental data and the solid lines represent the computer fits using Eq. 2. The amount of S-crystallin used in each assay was 23.2 μg.

substrate with different concentrations of CDNB as the fixed-concentration substrate in a double-reciprocal plot, a series of biphasic intersecting patterns was obtained (Fig. 3).

As the initial-velocity experimental data do not give a parallel pattern, this rules out a ping-pong kinetic mechanism (*42*). The nonlinear plot is also not compatible with an ordered or rapid equilibrium random Bi-Bi sequential mechanism. Therefore, the possibility left for GST activity of S-crystallin is a steady-state random Bi-Bi sequential mechanism. A *steady-state random Bi-Bi mechanism* predicts a nonlinear double reciprocal plot for the initial-velocity experiment (*42-44*). Indeed, departure from linearity was detected by the double reciprocal and Eadie-Hofstee plots (Figs. 1 and 3).

pH Studies—The octopus S-crystallin is stable under the assay conditions at pH 4.90-9.27 for at least 10 min. Detailed pH effect on the enzyme activity was studied in this pH range (Fig. 4). Some caution is required in interpreting the results of the pH studies. The pH-rate profile cannot be used directly to determine pK_as for the enzyme when different steps in the overall process depend on different levels of protonation. Furthermore, in our studies, the low and high substrate concentrations used were limited by the low enzymatic activity and the solubility problems, respectively. The assignment of pK_a values thus must remain tentative. With these caveats in mind, we now examine our results.

Careful examination of the data shown in Fig. 4 suggests that there might be two plateaus with three pK_a values involved (*44, 45*). However, fitting the data to an equation including three pK_a values (*46*) resulted in large errors and the resulting fitted data did not seem to match with the actual values. The data were then fitted to a model described by Eq. 2, which gave satisfactory results.

The log k_{cat} *versus* pH plot showed two molecular dissociation groups with pK_a values of 6.85 ± 0.07 and 9.67 ± 0.29, respectively, in the GSH·S-crystallin·CDNB ternary complex involved in catalysis (Fig. 4A). The former group has to be deprotonated and the latter group has to be protonated to give the maximum reaction rate. Fitting of the data to Eq. 2 also indicates that the S-crystallin has a k_{cat} value of $0.2 \pm 0.02\ s^{-1}$, which is at least three orders of magnitude smaller than that for the digestive gland GST ($213 \pm 8.1\ s^{-1}$) (*36*). The kinetic parameters are summarized in Table I.

A plot of log $k_{cat}/K_{m\,GSH}$ *versus* pH suggested two molecular pK_as of 7.11 ± 0.11 and 9.56 ± 0.39 in the S-crystallin·CDNB binary complex (Fig. 4B). The former has to be deprotonated and the latter has to be protonated to give the optimum reaction rate. Similarly, two molecular pK_as of 6.82 ± 0.07 and 9.44 ± 0.14 were detected in the S-crystallin·GSH binary complex (Fig. 4C).

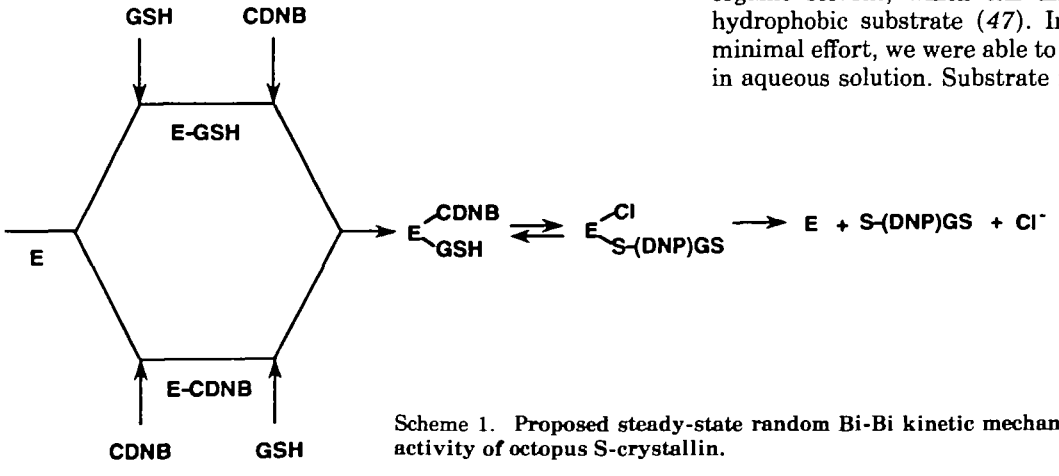
TABLE I. **Kinetic parameters of the endogenous GST activity of octopus S-crystallin.**

Constant	Description[a]	Value[b]
V	Apparent maximum velocity	0.281 ± 0.032 $\mu mol \cdot min^{-1} \cdot mg^{-1}$
K_a	Apparent Michaelis constant for A	0.33 ± 0.047 mM
α	Interaction factor between two subunits	0.032 ± 0.028
K_b	Apparent Michaelis constant for B	2.0 ± 0.1 mM
K_{bi}	Substrate inhibition constant for B	29.7 ± 0.01 mM
$pK_{EAB}1$	Molecular pK_{a1} value in the EAB complex	6.85 ± 0.07
$pK_{EAB}2$	Molecular pK_{a2} value in the EAB complex	9.67 ± 0.29
$pK_{EB}1$	Molecular pK_{a1} value in the EB complex	7.11 ± 0.11
$pK_{EB}2$	Molecular pK_{a2} value in the EB complex	9.56 ± 0.39
$pK_{EA}1$	Molecular pK_{a1} value in the EA complex	6.82 ± 0.07
$pK_{EA}2$	Molecular pK_{a2} value in the EA complex	9.44 ± 0.14.

[a]Abbreviations: A, GSH; B, CDNB; E, S-crystallin. [b]Values shown are averages ± SE.

DISCUSSION

Kinetic and Chemical Mechanisms of the Endogenous GST Activity of Octopus S-Crystallin—We have characterized the kinetic mechanism of the endogenous GST activity of octopus S-crystallin. This was similar to the kinetic mechanism of the digestive gland GST of the same species, although some differences were also observed. Substrate inhibition of S-crystallin was observed for CDNB. GST has a GSH binding site (G-site) and a hydrophobic H-site for CDNB or other xenobiotics (*16-20*). We have unequivocally demonstrated the strong substrate inhibition by CDNB in a reverse micellar system composed of H_2O/detergent/organic solvent, which can dissolve a large amount of hydrophobic substrate (*47*). In the present study, with minimal effort, we were able to use [CDNB] up to 3.5 mM in aqueous solution. Substrate inhibition in aqueous solu-



Scheme 1. **Proposed steady-state random Bi-Bi kinetic mechanism for the endogenous GST activity of octopus S-crystallin.**

tion was also demonstrated. High concentrations of CDNB could have bound to other sites, thus inhibiting the catalytic activity.

The biphasic nature of the $1/v$ *versus* $1/[GSH]$ double-reciprocal plot was more pronounced for the S-crystallin than for the digestive gland GST (*30*), which suggests a steady-state random Bi-Bi kinetic mechanism (*27*). The rapid-equilibrium random Bi-Bi kinetic treatment as with the digestive gland GST would be not valid for S-crystallin (*30*). However, the steady-state analysis for a random Bi-Bi system would yield a complex equation containing $[GSH]^2$ and $[CDNB]^2$, which are difficult to work with (*42*). We have tried to fit the data shown in Fig. 3 to Eq. 8, which contains ten parameters; however, some of the parameters showed high dependency on each other, which means that Eq. 8 may be overparameterized and the model too complicated.

$$v=\frac{V_1[A][B]+V_2[A]^2[B]+V_3[A][B]^2}{K_1+K_2[A]+K_3[B]+[A][B]+K_4[A]^2+K_5[B]^2+K_6[A]^2[B]+K_7[A][B]^2} \quad (8)$$

Since the octopus S-crystallin has very low GST activity, the poor fit to Eq. 8 could be due to the data. Further investigation of the kinetic mechanism of the endogenous GST activity of S-crystallin by collecting more data to give a better fit to Eq. 8 was not successful due to experimental difficulty. The small enzymatic activity of S-crystallin also precluded detailed studies of product-inhibition by the conjugate product or Cl^-. From the series of concave down curves (Fig. 3), we suggest that the endogenous GST activity of octopus S-crystallin qualitatively might conform to a steady-state random sequential kinetic mechanism, although precise quantitative evaluation of individual parameters is not possible (Scheme 1). Alternatively, the results shown in Figs. 1 and 3 could be due to anticooperativity between the two subunits of S-crystallin. Although our data can be fitted to an anticooperative two-site model, this possibility needs further characterization.

The N-terminal amino acid sequence of octopus S-crystallin is 40% identical and 85% similar to that of the octopus digestive gland GST (*36*). The essential Tyr^7 and Arg^{13} are conserved in octopus S-crystallin (*36*). We believe that the endogenous GST activity of octopus S-crystallin catalyzes the conjugation reaction between GSH and CDNB in a similar manner to other GST. According to our previous analysis (*30*) and analogous study on the mammalian GST isoenzymes (*48*), the pK_a values of 6.85 and 6.82 observed in GSH·S-crystallin·CDNB ternary and S-crystallin-GSH binary complexes, respectively, are for the enzyme-bound GSH, which has a pK_a value 2.32-2.35 pH units smaller than that in aqueous solution (9.169± 0.115) (*49*). This lowering of the pK_a value of the bound GSH was presumably due to interaction with Tyr^7, which has a pK_a value of 9.67, 9.56, and 9.44, respectively, in the GSH·S-crystallin·CDNB, S-crystallin·CDNB, and S-crystallin·GSH complexes (Table I). The pK_a value of 7.11± 0.11 observed in Fig. 3B was assumed to be that for the free GSH in the active center of the protein. This value is about 2 pH units smaller than that in aqueous solution. The local environment such as the positive charge of Arg^{13} might have contributed to lowering the pK_a value of the sulfhydryl group of GSH. The ionized thiolate anion of GSH is a better nucleophile to attack the *ipso* carbon of CDNB, forming a negatively charged Meisenheimer complex intermediate. Removing a chloride ion from this intermediate complex produces the conjugate product.

Possible Biological Significance of the Endogenous GST Activity of Cephalopod S-Crystallin—Our results suggest that the subunit of S-crystallin may be enzymatically active (Fig. 2). Our recent experimental results indicated that the octopus digestive gland GST was dissociated into monomers but was enzymatically active in the AOT/isooctane/H_2O reverse micelles (*47*). The catalytic constant of the endogenous GST activity is three orders of magnitude smaller than that for the hepatopancreatic GST of the same species. At this level, the enzyme activity *per se* might no longer have biological significance, and the recruitment of an enzyme to a new structural role might simply be because of its stability (*4, 5, 50*) or its ability to pack well and form a transparent aggregate (*51*). However, considering the total amount of S-crystallin in cephalopods' lens (approx. 90% of total lens protein) (*33*) and the detectable enzymatic activity *in vitro*, the possible biological role of the enzymatic activity of S-crystallin *in vivo* has to be addressed. This enzyme system may play a role in the protection of the eye against endogenous oxidative stress (*12*), which is implicated in some deleterious effects such as cataractogenesis in the lens (*52*).

GST is a detoxification enzyme in cells, involved in the elimination of toxic compounds from the cells. The constraints for a detoxification catalyst are quite different from those functioning in the intermediate metabolism. In contrast to metabolic enzymes, which need high specificity, high reaction rate and fine tuning by metabolites to fulfill the metabolic requirements, the enzymes for detoxification are sluggish, of broad specificity, and essentially unidirectional catalysts capable of reacting with a broad spectrum of xenobiotics that cells may encounter (*11-14*). Lens is a specialized tissue. During their life span, the lens epithelial cells lose their nuclei and other cell organelles. The lens thus lacks the homeostatic controls through hormones or neural responses. In this context, the GST is an ideal protein to be recruited as lens crystallin. Crystallins may function as constitutive stress proteins, exerting a protective function against the endogenous or exogenous oxidative threats to which the lens might be exposed.

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