Partial Characterization and Detergent Solubilization of the Putative Glutathione Chemoreceptor from Hydra

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Received April 17, 1992; Revised Manuscript Received July 23, 1992

ABSTRACT: Feeding behavior in hydra is initiated by the association of glutathione (GSH) with a putative external chemoreceptor. In the present study, the binding of [^{35}S]GSH to hydra membranes has been characterized. Nondisplaceable [^{35}S]GSH binding which compromised previous analyses [Grosvenor, W., Bellis, S., Kass-Simon, G., & Rhoads, D. (1992) *Biochim. Biophys. Acta* (in press)] was eliminated by treating membranes with an inhibitor of GSH metabolism, borate in combination with L-serine. The specific binding which was not inhibited by borate/serine demonstrated many of the characteristics expected of a ligand/receptor interaction. The binding was rapid, reversible, and saturable. A Scatchard analysis of saturation isotherms indicated a dissociation constant (K_D) of 3.4 μM, a value which is in good agreement with concentrations of glutathione which are known to induce feeding behavior. Hydra membranes were detergent-solubilized with 10 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 100 mM KCl, and 10% glycerol. The soluble fraction contained 40% of the original saturable, reversible GSH binding activity. The K_D for GSH binding to the solubilized preparation was estimated as 2.7 μM, a value which is not appreciably different from the K_D for binding to intact membranes. The fidelity of GSH binding in the solubilized preparation suggests that this preparation will be useful in further characterization of the putative glutathione chemoreceptor.

Recent studies have begun to elucidate the molecular mechanisms by which chemoreceptors transduce chemical signals from the environment into a cellular response (Brand et al., 1989). The coelenterate hydra has been a good model for the study of chemoreception because this organism demonstrates a well-defined and quantifiable feeding behavior in response to the water-soluble chemoeffector glutathione (γ-glutamyl-L-cysteinylglycine, GSH). When hydra are exposed to concentrations of glutathione ranging from high nanomolar to low micromolar, tentacle contraction and mouth-opening are observed (Loomis, 1955). These behaviors are inhibited by L-glutamate (Lenhoff & Bovaird, 1961). Characteristics of a putative glutathione chemoreceptor which mediates feeding behavior have been deduced primarily from a quantitative assay of the duration of mouth-opening (Lenhoff, 1961). Only a limited number of studies have employed radioligand binding methodology to attempt to directly probe the ligand/receptor interaction (Koizumi & Kijima, 1980; Venturini, 1987; Bellis et al., 1991; Grosvenor et al., 1992).

In the first of these radioligand binding studies, Koizumi and Kijima (1980) examined the binding of a GSH analogue, *S*-[^{14}C]methylglutathione (SMG), to a nematocyst-rich fraction. It was concluded that SMG binding was not the rapid, reversible type of association which is generally expected of a ligand/receptor interaction. More recently, Grosvenor et al. (1992) identified [^{35}S]GSH binding to a crude membrane preparation. In this study, specific [^{35}S]GSH binding was observed in a concentration range which correlates with concentrations producing behavioral effects; however, only 24% of this binding appeared to be reversible. The remaining 76% of the bound radioligand could not be displaced by adding excess unlabeled GSH after radioligand was allowed to associate with membranes for 15 min. In addition L-glutamate did not compete for any [^{35}S]GSH binding in this study. This is of particular interest because glutathione has been shown to compete for [^{3}H]glutamate binding (Venturini, 1987; Bellis et al., 1991). Clearly, the interaction between GSH and glutamate is more complex than the simple competitive interaction initially envisioned (Lenhoff & Bovaird, 1961).

It has been proposed that reversible [^{35}S]GSH binding identified in a crude membrane fraction represents GSH association with the putative chemoreceptor (Grosvenor et al., 1992). Lenhoff (1961) demonstrated that removal of hydra from GSH-containing medium resulted in termination of the feeding response within 1 min, a finding which supports the view that feeding behavior is induced by a rapidly reversible type of GSH association with the chemoreceptor. The dissociation constant (K_D) for reversible [^{35}S]GSH binding to hydra membranes could not be evaluated previously because the presence of apparent nondisplaceable binding in the same preparation compromised Scatchard analysis of saturation isotherms (Grosvenor et al., 1992). Therefore, only limited inferences regarding the correspondence between reversible binding and feeding behavior could be made. The present study addresses this problem.

Among the processes which might generate radioligand binding which cannot be displaced by unlabeled GSH, one must consider the possibility of [^{35}S]GSH metabolism and the subsequent binding of radiolabeled metabolites to sites distinct from GSH binding sites. In hydra, glutathione is metabolized by the sequential action of two membrane-bound enzymes, γ-glutamyl transpeptidase and dipeptidase (Tate & Meister, 1976; Danner et al., 1976). The metabolism of glutathione is not believed to play a role in the activation of feeding behavior because treatment of whole hydra with an inhibitor of γ-glutamyl transpeptidase (azaserine) did not attenuate the GSH-induced mouth-opening response (Heagy

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0006-2960/92/0431-9838$03.00/0

et al., 1982). Furthermore, the GSH binding site of the transpeptidase appears to have a different specificity than that inferred from behavioral studies of the putative chemoreceptor (Danner et al., 1976, 1978).

In the present study, we have determined that nondisplaceable radioligand binding is eliminated by treating hydra membranes with a known inhibitor of γ-glutamyl transpeptidase, borate in combination with L-serine (Revel & Ball, 1959; Tate & Meister, 1978). This treatment has been utilized to perform a kinetic characterization of the reversible [^{35}S]-GSH binding which remains after enzymatic activity has been blocked.

EXPERIMENTAL PROCEDURES

Isolation of Membranes. Specimens of *Hydra vulgaris* were cultured in BVC solution (Loomis & Lenhoff, 1956) at 18 °C and fed with *Artemia nauplii*. The animals belong to a single, asexually reproducing clone (Kass-Simon & Potter, 1971). A crude membrane fraction was prepared as described previously (Bellis et al., 1991; Grosvenor et al., 1992). A total of 1000–2000 hydra were starved for 48 h and then homogenized in cold 50 mM Tris-HCl (pH 7.4) by six strokes using a glass–Teflon biohomogenizer. The homogenate was centrifuged at 1000*g* for 15 min at 4 °C. The pellet (P1) was resuspended in 50 mM Tris-HCl and then further disrupted on ice with five 20-s pulses from a Fisher biohomogenizer. After centrifugation at 1000*g* for 15 min, the supernatant was combined with the previous supernatant (S1) and centrifuged at 30000*g* for 15 min, yielding a crude membrane fraction (P2). The membrane fraction was washed by resuspension into 50 mM Tris-HCl (pH 7.4), followed by centrifugation at 30000*g*.

Detergent Solubilization of Membrane Proteins. The crude membrane fraction was incubated in 50 mM Tris-HCl (pH 7.4) containing one of the following detergents: (1) 10% octyl glucoside; (2) 10 mM CHAPS; or (3) 10 mM CHAPS plus 10% glycerol and 100 mM KCl. The incubation proceeded for 60 min on ice with intermittent vortexing. Solubilized membrane proteins were separated from insoluble material by centrifugation at 100000*g* for 60 min. The concentration of membrane proteins during the solubilization procedure varied from 2 to 7 mg/mL.

Treatment of the Membrane Fraction with Inhibitors of γ-Glutamyl Transpeptidase. Three treatments were used to inhibit γ-glutamyl transpeptidase activity: (1) 5 mM sodium borate plus 5 mM L-serine (Revel & Ball, 1959; Tate & Meister, 1978); (2) 1.0 mM [*S*-(*R**,*R**)]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125) (Allen et al., 1980; Reed et al., 1980; Griffith & Meister, 1980; Gardell & Tate, 1980; Griffith & Tate, 1980); and (3) 1.0 mM phenylmethanesulfonyl fluoride (PMSF) plus 200 mM maleate (Inoue et al., 1978; Elce, 1980). Membranes were incubated with inhibitors for 45 min on ice prior to binding assays. Transpeptidase inhibitors were also included in binding assays.

Assay for Binding to the Crude Membrane Fraction. [^{35}S]-Glutathione binding to hydra membranes was assayed as described previously (Bellis et al., 1991; Grosvenor et al., 1992) in 0.5 mL of 50 mM Tris-HCl (pH 7.4) containing 100 nM radioligand. The amount of protein added to each incubation was 100–200 μg. With the exception of time course experiments, membranes were incubated with radioligand for 15 min. A 15-min incubation with radioligand was shown to be sufficient time to establish equilibrium binding conditions (see Figure 1). Following incubation at 25 °C, the samples were rapidly filtered under vacuum through MSI cellulose filters (0.45 μm). Filters were washed once with 4 mL of cold 50 mM Tris-HCl buffer, and radioactivity was measured by liquid scintillation spectrometry. Specific binding was operationally defined as the binding obtained in the presence of 100 nM [^{35}S]GSH (total binding) minus the binding obtained in the presence of 100 nM radioligand plus an excess, 100 μM, of unlabeled GSH (nonspecific binding). Nonspecific binding accounting for 10–20% of the total binding when assayed at 15 min. For time course experiments (Figure 1), parallel incubations representing total binding and nonspecific binding were performed for each time point. Nonspecific binding was subtracted from total binding to determine the amount of specific binding at each time point. To identify the amount of reversible binding in a given preparation, membranes were incubated with radioligand for 15 min at 25 °C. An excess of unlabeled GSH (100 μM) was then added, and the incubation was allowed to proceed for an additional 10 min (preliminary data suggested that displacement was maximal 10 min after the addition of unlabeled ligand). Protein was quantified by the method of Lowry et al. (1951) using bovine serum albumin as the standard. A statistical analysis of data from saturation experiments was performed by the computer software program EBDA/LIGAND as modified by McPherson (Munson & Rodbard, 1980).

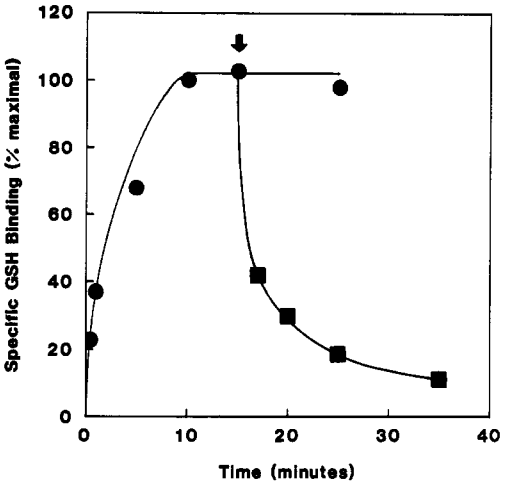

FIGURE 1: Time course of specific [^{35}S]glutathione binding to borate/serine-treated membranes. Parallel incubations representing total binding and nonspecific binding (see Experimental Procedures) were performed for each time point. Nonspecific binding was subtracted from total binding to obtain the values shown in the graph (specific GSH binding). The association reaction (●) was measured by incubating membranes for varying times with 100 nM [^{35}S]-glutathione. Dissociation (■) was assayed at several time points following the addition of 100 μM unlabeled GSH (arrow) to membranes incubated with radioligand for 15 min. Data presented are averages of 2 experiments performed in duplicate using 1000–2000 hydra each. Individual values varied by no more than 20% of mean values.

Assay for Binding to Solubilized Proteins. The crude membrane fraction was solubilized as described above in 10 mM CHAPS/10% glycerol/100 mM KCl. After centrifugation at 100000*g*, the supernatant was collected and diluted into 50 mM Tris-HCl (pH 7.4) for the binding assay. The final concentration of detergent in the binding assay was one-tenth the concentration of detergent used for solubilization. Pelleted material was resuspended into solubilization buffer diluted with 50 mM Tris-HCl such that the concentration of detergent in the binding assay was also one-tenth the concentration of the solubilization buffer. The procedure employed to assay radioligand binding to solubilized proteins

Table I: Effects of γ-Glutamyl Transpeptidase Inhibitors on Specific Radioligand Binding

transpeptidase inhibitor	inhibition of radioligand binding (%)[a]
5 mM sodium borate plus 5 mM L-serine	73 ± 5
1.0 mM AT-125	61 ± 8
1.0 mM PMSF plus 200 mM maleate	60 ± 2

[a] Results are expressed as percent inhibition of the specific binding observed in the absence of transpeptidase inhibitors. Values represent the means and standard errors from three experiments performed in duplicate.

Table II: Effects of Transpeptidase Inhibitors on Displaceable and Nondisplaceable Binding[a]

treatment	amount of displaceable binding (pmol/mg)	amount of nondisplaceable binding (pmol/mg)
no treatment (n = 6)	2.4 ± 0.5	6.3 ± 1.5
5 mM sodium borate + 5 mM L-serine (n = 3)	1.6 ± 0.2	0.2 ± 0.1[b]
1.0 mM AT-125 (n = 3)	1.7 ± 0.5	1.6 ± 0.6[b]

[a] After a 15-min incubation with 100 nM [^{35}S]GSH, unlabeled GSH (100 μM) was added to the incubations, and the incubations were allowed to proceed an additional 10 min. The amount of displaceable binding was computed by subtracting the amount of binding remaining after the addition of unlabeled ligand from the amount of specific binding present prior to the addition of unlabeled ligand. [b] These values differed significantly ($p < 0.05$) from no treatment values for nondisplaceable binding as determined by a Student's t test.

was identical to the procedure described above for the crude membrane fraction with the exception of the type of filters utilized to terminate the incubations. Detergent-solubilized proteins were collected on Whatman GF/B filters which were presoaked for 2–10 h with 0.33% poly(ethylenimine) (Bruns et al., 1983).

Materials. [^{35}S]Glutathione was obtained from New England Nuclear (Boston, MA; 30–100 Ci/mmol). 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was purchased from Calbiochem (San Diego, CA). All other reagents, including AT-125 (listed as "acivicin" in vendor catalogue), were obtained from Sigma (St. Louis, MO). MSI cellulose filters (0.45 μm) and Whatman GF/B filters were purchased from Fisher Scientific (Pittsburgh, PA).

RESULTS

The effects of inhibitors of γ-glutamyl transpeptidase on [^{35}S]glutathione binding are shown in Table I. All three treatments greatly reduced the specific radioligand binding. Maximal inhibition of binding, 73%, was observed in membranes treated with a combination of 5 mM sodium borate and 5 mM L-serine. Treatment with 1.0 mM AT-125 resulted in 61% inhibition of binding, and treatment with 1.0 mM PMSF plus 200 mM maleate inhibited 60% of the binding.

The data shown in Table II indicate that treatment of membranes with either borate/serine or AT-125 caused a marked reduction in the amount of nondisplaceable radioligand binding. In the absence of transpeptidase inhibitors, 6.3 pmol/mg of radioligand could not be displaced by adding excess unlabeled GSH 15 min after incubation with radioligand alone. In contrast, only 0.2 pmol/mg of bound radioligand could not be displaced in membranes treated with borate/serine. This corresponds to a 97% reduction in the amount of nondisplaceable [^{35}S]GSH binding. Because nearly all of the nondisplaceable binding was eliminated by borate/serine treatment without statistically significant diminution in reversible binding, the specific binding not inhibited by borate/serine treatment was subjected to kinetic analysis.

Figures 1–3 represent a kinetic characterization of the specific binding present in borate/serine-treated membranes. The time course of specific [^{35}S]GSH binding is shown in Figure 1. Sixty-seven percent of the maximal binding was attained within 5 min. The binding reached equilibrium by 10 min and was stable through 25 min. Up to and including 15 min, essentially all (≥90%) of the specific binding to borate/serine-treated membranes was displaceable. After a 25-min incubation with radioligand, however, a significant amount of nondisplaceable binding was observed (data not shown). Only the amount of displaceable binding at 25 min is shown in Figure 1 to demonstrate that the equilibrium level of displaceable binding was stable from 10 to 25 min. The addition of unlabeled GSH to membranes incubated with radioligand alone for 15 min resulted in a rapid loss of bound radioligand (Figure 1). Greater than 50% displacement was observed at 2 min following the addition of unlabeled ligand.

We selected at 15-min incubation to perform saturation isotherms of GSH binding to borate/serine-treated membranes because at this time point essentially all of the specific binding was reversible and the reversible binding was at equilibrium (Figure 1). In Figure 2A, the specific GSH binding is shown to be saturable, and a Scatchard transformation of saturation isotherms (Figure 2B) yields a linear slope, suggesting the presence of only one class of binding sites. The dissociation constant (K_D) was determined to be 3.4 ± 0.6 μM, and the maximal capacity of binding sites (B_{max}) is 118 ± 22 pmol/mg. A Hill analysis of data from saturation experiments (not shown) estimated a Hill coefficient of 1.0 ± 0.02, a value which indicates that there is no cooperativity between binding sites.

It was previously shown that glutamate does not compete for [^{35}S]glutathione binding, and it was proposed that the inhibition of feeding behavior by glutamate is mediated by something other than a competitive mechanism (Grosvenor et al., 1992). In that study, however, only 24% of the binding was reversible, and it is possible that a selective effect on reversible binding by glutamate may have been masked by the presence of large amounts of nondisplaceable binding. In the present study, we have performed glutamate competition experiments using borate/serine-treated membranes to eliminate nondisplaceable binding. Inclusion of an excess of L-glutamate (100 μM) in a standard binding assay (15-min incubation with 100 nM [^{35}S]GSH) had no significant effect on [^{35}S]GSH binding. In two trials using borate/serine-treated membranes, glutamate inhibited specific [^{35}S]GSH binding by only 6%. Such a minute amount of inhibition is not consistent with the actions of a competitive inhibitor given that the concentration of L-glutamate was (1) 1000× the concentration of [^{35}S]GSH and (2) 10× the reported K_D (10 μM) for glutamate binding to hydra membranes (Bellis et al., 1991).

As a first step toward purification of the putative glutathione chemoreceptor, the crude membrane fraction was detergent-solubilized with both CHAPS and octyl glucoside (Table III). Although CHAPS and octyl glucoside were reasonably effective at solubilizing membrane proteins (51% and 42% protein yield, respectively), only a limited amount of the binding activity was retained (less than 10%), suggesting the possibility of inactivation of GSH binding proteins. The addition of stabilizing agents, 100 mM KCl and 10% glycerol (Hjelmeland, 1990), to the CHAPS incubation enhanced the amount of binding activity without significantly altering the

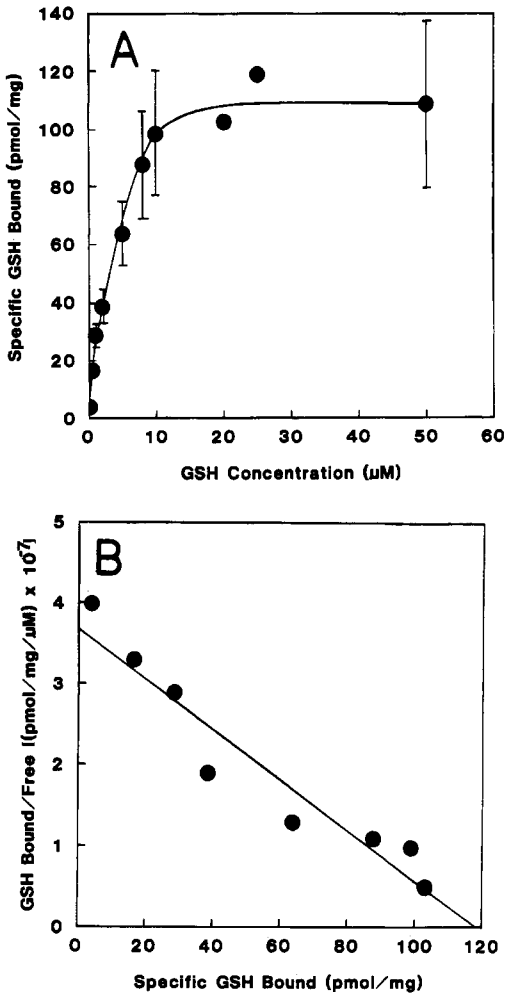

FIGURE 2: (A) Saturation curve of specific [^{35}S]glutathione binding to borate/serine-treated membranes. The crude membrane fraction was incubated for 15 min with 100 nM radioligand and sufficient concentrations of unlabeled GSH to bring the final concentration of GSH to the concentrations shown in the figure. The amount (picomoles per milligram) of GSH bound at each concentration was calculated by adjusting the specific activity of [^{35}S]GSH to account for the addition of unlabeled ligand. Data presented represent the means and standard errors of three experiments performed in duplicate. The values shown for the amount of GSH bound at 20 and 25 μM are from a single determination. (B) Scatchard analysis of saturation isotherms. The data generated from saturation experiments (A) were transformed into a Scatchard plot. The K_D and B_{max}, 3.4 μM and 118 pmol/mg, respectively, were evaluated from three saturation experiments using the computer software program EBDA/LIGAND (Munson & Rodbard, 1980).

Table III: Detergent Solubilization of the Crude Membrane Fraction (P2)

	supernatant		pellet	
treatment	binding act. (% of P2)	protein yield (% of P2)	binding act. (% of P2)	protein yield (% of P2)
10 mM CHAPS (n = 1)	7	51	ND[a]	ND
10% octyl glucoside (n = 1)	5	42	ND	ND
10 mM CHAPS + 100 mM KCl + 10% glycerol (n = 3)	40[b]	42 ± 5	48 ± 5	51 ± 6

[a] Not determined. [b] A 40% yield was observed in all three trials.

protein yield. A consistent 40% yield in binding activity and a concomitant protein yield of 42% were obtained from membranes solubilized in 10 mM CHAPS/10% glycerol/100 mM KCl. Resolubilization of the pelleted material with CHAPS/KCl/glycerol yielded a negligible amount of additional binding activity (3%).

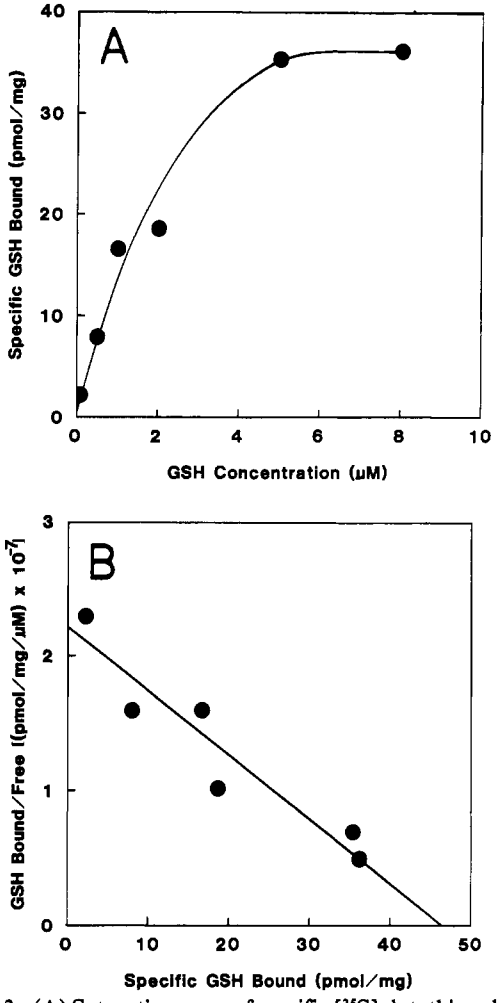

FIGURE 3: (A) Saturation curve of specific [^{35}S]glutathione binding to borate/serine-treated CHAPS-solubilized proteins. Membrane proteins solubilized in 10 mM CHAPS/100 mM KCl/10% glycerol were incubated with 100 nM [^{35}S]GSH and sufficient concentrations of unlabeled GSH to bring the final concentration of GSH to the concentrations shown in the figure. The amount (picomoles per milligram) of GSH bound at each concentration was calculated by adjusting the specific activity of [^{35}S]GSH to account for the addition of unlabeled ligand. Data shown are averages of two experiments performed in duplicate. Individual values varied by no more than 20% of mean values. (B) Scatchard analysis of saturation isotherms. The data generated from saturation experiments (A) were transformed into a Scatchard plot. The K_D and B_{max}, 2.7 μM and 49 pmol/mg, respectively, were evaluated with the computer software program EBDA/LIGAND (Munson & Rodbard, 1980).

Similar to [^{35}S]GSH association with intact membranes, both reversible and nondisplaceable radioligand binding were identified in the solubilized preparation. The treatment of solubilized proteins with borate/serine resulted in a substantial reduction in nondisplaceable binding (data not shown). Using the procedure described for borate/serine treatment of the crude membrane fraction, we treated solubilized proteins with borate/serine to eliminate nondisplaceable binding, and then characterized the reversible binding which remained after borate/serine treatment. The characteristics of reversible binding to solubilized proteins were very similar to characteristics of reversible binding to intact membranes. An initial time course indicated that equilibrium binding was attained within 10 min; therefore, as with intact membranes, a 15-min incubation time was used to perform saturation isotherms. [^{35}S]GSH binding appeared to be saturable (Figure 3A), although only a limited number of data points were tested due

to the difficulty in obtaining large quantities of solubilized membrane proteins. A Scatchard analysis of saturation isotherms (Figure 3B) revealed values of 2.7 μM for the dissociation constant (K_D) and 49 pmol/mg for the maximal capacity of binding sites (B_{max}). The Hill coefficient was computed as 0.95, a value which indicates a lack of cooperativity between binding sites.

DISCUSSION

The correspondence between the behavioral effects of GSH and reversible binding of [^{35}S]GSH to hydra membranes could not previously be assessed because of the presence of substantial nondisplaceable [^{35}S]GSH binding in the same preparation (Grosvenor et al., 1992). In this study, we have hypothesized that apparent nondisplaceable [^{35}S]GSH binding is the result of metabolic activity. In the initial stages of the binding assay, a portion of the added [^{35}S]GSH may associate with the GSH-metabolizing enzyme γ-glutamyl transpeptidase. As the incubation proceeds, radiolabeled metabolites such as [^{35}S]-cysteine may accumulate and bind to a site(s) distinct from GSH binding sites. The binding of radiolabeled metabolites to sites with a different specificity than the GSH binding site would not be expected to be displaced by the addition of excess GSH. In support of this hypothesis, preliminary data suggest that cysteine is a potent inhibitor of radioligand binding. In a single experiment, inclusion of an excess of unlabeled cysteine (20 μM) in a binding assay (20 nM [^{35}S]GSH) resulted in 78% inhibition of the specific radioligand binding. This percentage is comparable to the amount of nondisplaceable binding observed under these conditions. In this same experiment, glutamate and glycine had no effect on [^{35}S]-GSH binding (Bellis, unpublished data). Other processes could account for the observation of apparent nondisplaceable [^{35}S]GSH binding. However, this binding is clearly eliminated by inhibitors of γ-glutamyl transpeptidase, particularly borate/serine (Table II). Therefore, the use of borate/serine to eliminate nondisplaceable binding provides a tool for the selective characterization of the reversible binding.

The time course of GSH binding to membranes treated with borate/serine (Figure 1) was consistent with the time required for initiation of feeding behavior. In behavioral experiments paralleling the binding study, mouth-opening generally occurred within 3–5 min in response to 2 μM glutathione (Bellis, unpublished data). Similar response times were reported by Lenhoff (1961) when behavior was assayed at a concentration of 2 μM or less (higher concentrations elicited faster responses). As shown in Figure 1, a significant fraction of radioligand (100 nM) was bound by 5 min, and binding was at equilibrium by 10 min. The binding was rapidly reversible, as evidenced by the 58% displacement of bound ligand within 2 min. This finding is consistent with the observation that mouth-opening terminates within 1 min after removal from GSH-containing medium (Lenhoff, 1961).

Essentially all of the specific GSH binding to borate/serine-treated membranes is reversible up to, and including, 15 min. Nondisplaceable binding does begin to appear by 25 min, however. It is possible that the appearance of nondisplaceable binding represents the inception of detectable levels of enzyme activity. Borate/serine is a reversible inhibitor of the enzyme (Tate & Meister, 1981); therefore, longer incubation times would be expected to favor enzymatic activity. AT-125, an irreversible inhibitor of the enzyme (Tate & Meister, 1981), was not used to characterize the reversible binding because AT-125 eliminated a smaller percentage of the nondisplaceable binding than borate/serine (Table II).

GSH binding to borate/serine-treated membranes had many of the characteristics expected of ligand association with a membrane-bound receptor. The specific binding was rapid, reversible, and saturable. A Scatchard analysis of saturation isotherms (Figure 2B) revealed a value of 3.4 μM for the dissociation constant. This value is in good agreement with concentrations of GSH which induce feeding behavior. Using an assay of the duration of mouth-opening behavior, Lenhoff estimated that a half-maximal response was elicited by 1 μM glutathione (Lenhoff, 1961). The correspondence between behavioral observations and characteristics of reversible GSH binding supports the hypothesis that the reversible GSH binding observed in our preparation represents GSH association with the putative chemoreceptor.

Treatment of the crude membrane fraction with 10 mM CHAPS/10% glycerol/100 mM KCl resulted in a consistent yield of active, solubilized glutathione binding proteins (Table III). The characteristics of reversible GSH binding to solubilized proteins are very similar to the characteristics of binding to intact membranes. In particular, the K_D for GSH binding to solubilized proteins (2.7 μM) closely matches the K_D for binding to intact membranes (3.4 μM), indicating that GSH binding proteins are not adversely affected by our detergent solubilization procedure.

In summary, the identification and detergent solubilization of GSH binding sites which demonstrate characteristics consistent with the behavioral actions of the putative glutathione chemoreceptor hold promise for further characterization and purification of the glutathione chemoreceptor. Previously, identification of the glutathione chemoreceptor was hindered by the observation of both reversible and a large amount of nondisplaceable binding in the same preparation. The use of γ-glutamyl transpeptidase inhibitors to eliminate nondisplaceable binding has allowed the selective characterization of the reversible GSH binding which has numerous characteristics expected of GSH association with the glutathione chemoreceptor.

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Registry No. GSH, 70-18-8; CHAPS, 75621-03-3; KCl, 7447-40-7; PMSF, 329-98-6; AT-125, 42228-92-2; glutamate, 56-86-0; glycerol, 56-81-5; γ-glutamyl transpeptidase, 9046-27-9; boric acid, 10043-35-3; L-serine, 56-45-1; maleate, 110-16-7.