Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Biological Macromolecules 31 (2003) 155–162
INTERNATIONAL JOURNAL OF Biological Macromolecules STRUCTURE, FUNCTION AND INTERACTIONS
www.elsevier.com/locate/ijbiomac

Salt influence on glutathione—*Schistosoma japonicum* glutathione *S*-transferase binding

Zeyad Yassin, M. José Clemente-Jiménez, Ramiro Téllez-Sanz, Luis García-Fuentes *

Dpto. de Química Física, Bioquímica y Q. Inorgánica, Facultad de Ciencias Experimentales, Universidad de Almería, La Cañada de San Urbano, 04120 Almeria, Spain

Received 14 June 2002; received in revised form 17 September 2002; accepted 1 October 2002

Abstract

There has been some speculation about the salt independence of *Schistosoma japonicum* glutathione *S*-transferase (Sj26GST, EC. 2.5.1.18), but this aspect has not been carefully studied before. To establish the basis for a further development of this dependence, we have performed a methodical study of the influence of some important ions and their concentration on the binding properties of glutathione to Sj26GST by means of isothermal calorimetry and fluorescence quenching. Salts like NaCl, Na_2SO_4 and $MgSO_4$ do not change practically the affinity of the protein for its substrate, whilst $MgCl_2$ has the effect of decreasing the affinity as its concentration rises. However, the enthalpy change is not affected by all the salts studied, and so, the entropy change is the causal factor in dropping the affinity. We also looked at the conformational stability of the protein under different conditions to check the structural changes they provide, and found that the unfolding parameters are practically not affected by the salt concentration. We discuss the results in terms of the chaotropic nature of the ions implied.

Keywords: Glutathione *S*-transferase; *Schistosoma japonicum*; Glutathione; Unfolding; Fluorescence; Binding; Microcalorimetry

1. Introduction

It is known that ionic strength plays an important role in both the binding of ligands to proteins and the stability and function of proteins. Buried salt bridges, [1–3], have been shown to contribute to the binding of ligands to proteins. Studies on solvent-exposed salt bridges have shown similar effects [4]. Electrostatic interactions are known to be important at the subunit interfaces of multimeric proteins, as observed in studies with ionic denaturants such as guanidinium chloride, in comparison to non-ionic denaturants like urea [5]. It has been shown that although the subunit interfaces of proteins are as tightly packed as protein cores, the morphology of interfaces is not always predominantly hydrophobic and that in approximately two-thirds of homodimers of known structure, a mixture of small hydrophobic patches and polar regions occurs [6]. Electrostatic interactions provide the specificity of protein–protein interactions at subunit interfaces, as shown by several site-directed mutagenesis studies on dimeric proteins such as triosephosphate isomerase [7].

The first studies of the effects of ionic strength on proteins were performed by Hofmeister, where he described the effects of different salts on the stability and solubility of proteins [8]. Currently the Hofmeister series is usually given in terms of the ability of the ions to stabilize the structure of proteins.

Glutathione *S* transferases (EC 2.5.1.18) (GSTs) are a supergene family of dimeric multifunctional proteins ($M_r \sim 50\,000$) playing an important role in the biotransformation of xenobiotics, i.e. compounds that are foreign to living cells [9]. GSTs catalyze the addition of reduced glutathione (GSH, γ-Glu-Cys-Gly) to a variety of physiological and xenobiotic electrophiles [10] and are involved in the binding of various compounds for storage and transport [11] purposes. GSTs are classified in several species-independent gene classes, named α, μ, π [12], θ [13] and σ [14,15] on the basis of the N-terminal sequence, the substrate specificity and

* Corresponding author. Tel.: +34-950-015618; fax: +34-950-015008
E-mail address: lgarcia@ual.es (L. García-Fuentes).

0141-8130/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0141-8130(02)00077-6

the immunological properties [16]. Three-dimensional crystal structures have been elucidated for several GST isoenzymes from various classes and reveal a conserved overall folding topology. Each subunit comprises two structurally distinct domains. The smaller N-terminal domain I is the glutathione-binding site (G-site), and the residues involved in this binding belong to this G-site. However, one aspartate (Asp100') must be supplied from the G-site in the adjacent subunit of the dimer [17,18].

The properties and crystal structure of the homodimeric (26 kDa per subunit) glutathione *S*-transferase from *Schistosoma japonicum* (Sj26GST) reveal a number of significant structural and functional differences compared to the other GST classes. The interactions at the subunit interface of Sj26GST are predominantly hydrophobic, similar to those found in the α, μ and π classes GSTs. However, the class σ GST lacks the conserved prominent hydrophobic lock-and-key interaction [19].

Salts have been shown to play an important role in stabilization of proteins via their effect on water structure and thus the hydrophobic effect [20]. Due to the physiological importance of Sj26GST as a detoxification protein, a vaccine and a drug target, and its wide application in protein biotechnology, we have performed the first studies on the dependence of salt type and concentration on the binding of substrate GSH to Sj26GST by fluorescence quenching and isothermal titration microcalorimetry (ITC).

2. Materials, methods and experimental data analysis

2.1. Chemicals

GSH, urea, 1-chloro-2,4-dinitrobenzene (CDNB), *S*-methylglutathione and *S*-butylglutathione were ultrapure grade from Sigma. Dithiothreitol (DTT) was from Pharmacia Biotech and Riedel-Haen, respectively. Tris-(hydroxymethyl)-methyl-amino-propanesulfonic acid (TAPS) buffer was purchased from Sigma. Na_2SO_4, NaCl, $MgCl_2$ and $MgSO_4$ were from Merck. Centriprep 30 concentrators were from Amicon. All other chemicals were of analytical grade of the highest purity available. All solutions were made with distilled and deionized (Milli Q) water. All solutions were degassed and clarified through a 0.45-μm Millipore filter before use.

2.2. Enzyme

Sj26GST was overexpressed in *Escherichia coli* BL21 transformed with the pGEX-2T plasmid (Amersham Pharmacia Biotech) [21] in the presence of 100 μg ml^{-1} of ampicillin, as described elsewhere [22,23]. The protein was purified by glutathione affinity chromatography. In order to use a true Sj26GST in our studies, an additional 9-residue peptide at its C-terminus was removed by adding 19 U mg^{-1} of thrombin protease to the matrix-bound Sj26GST. Then, Sj26GST was eluted with 10 mM of reduced glutathione. The enzyme showed a single-band pattern in polyacrylamide gel electrophoresis in presence of sodium dodecyl sulphate. Purified protein was stored at −80 °C in 20 mM sodium phosphate, pH 7, and containing 2 mM DTT. The protein concentrations were determined spectrophotometrically, using a molar extinction coefficient of 35 050 M^{-1} cm^{-1} [24]. Absorbance measurements were carried out using a Beckman DU-7400 spectrophotometer with cells maintained at 25 °C.

2.3. Enzyme assay

The catalytic activity of Sj26GST was determined spectrophotometrically by monitoring the increase in absorbance at 340 nm. The enzyme assay was composed of 1 ml of 1 M phosphate buffer pH 6.5 containing 1 mM GSH and 100 mM CDNB [25] and corrected for the corresponding nonenzymatic controls. In the unfolding studies with urea, the reactivation of unfolded enzyme was negligible under these conditions.

2.4. Aggregation state

Analytical HPLC was carried out on a Waters apparatus. Effluents were monitored with a UV–Vis detector from Waters, set at 280 nm. The fast protein liquid chromatography column was Superdex 200 HR 10/30 from Pharmacia. The column was calibrated with Blue Dextran (2000 kDa), Albumin (66 kDa), Carbonic Anhydrase (29 kDa), Cytocrome C (12 kDa) and Aprotinin (6.5 kDa), all of them from Sigma. The column was equilibrated in buffer TAPS with and without 300 mM NaCl, at pH 8.5 and 25 °C. Two samples of Sj26GST ($\approx$ 50 μM) were dialyzed in this buffer with or without salt. After, the samples were applied to the column at a volume of 100 μl.

2.5. Fluorescence measurements

Spectrofluorimetric GSH titrations and folding/unfolding studies of *Schistosoma japonicum* GST protein were performed with a Perkin Elmer LS50B spectrofluorometer interfaced to a computer for data collection and analysis.

In the binding studies, the excitation wavelength was 278 nm, and fluorescence was monitored at 339 nm. The temperature of the sample was controlled at 25.0 ± 0.2 °C using a thermostatted cuvette holder and a Frigiterm 6 000 382 Selecta refrigerated circulating water bath. A 2.0 ml solution of protein in a 4.0 ml quartz fluorescence cell was stirred after each addition of

ligand. The fluorescence measurements were corrected for dilution and inner filter effects. The equations and data analysis corresponding to the binding of the substrate to Sj26GST was shown elsewhere [22].

To study the folding/unfolding of Sj26GST under equilibrium conditions, tryptophan and tyrosine fluorescence changes were used as structural and functional probes to monitor changes. The enzyme contains four tryptophan residues per subunit (Trp 7, Trp 40, Trp 200 and Trp 205). The tryptophan emission spectrum for the folded protein (excitation at 295 nm) has a maximal emission at 335 nm, which shifts to 355 nm when the protein unfolds. Refolding of the denatured protein by a 10-fold dilution of the denaturant resulted in both an increase and a decrease of fluorescence at 335 and 355 nm, respectively. No aggregation was detected for the results shown. Urea concentrations were determined from refraction index measurements [26].

Denaturation curves were evaluated according to the linear extrapolation method [27] for dimeric proteins [28] as described elsewhere [26]. The dimeric state must, therefore, be significantly populated in the transition zone. The equilibrium constant, K_U, corresponding to this reaction,

$$A_2 \overset{K_U}{\rightleftharpoons} 2U$$

was calculated at each point in the transition region of the denaturation curve by

$$K_U = \frac{[U]^2}{[A_2]} = 2P_t \frac{f_U^2}{1 - f_U} \quad (1)$$

with P_t being the total protein concentration and f_U the fraction of unfolded protein. If this model provides a reasonable thermodynamic description of the denaturation reaction, then one should calculate the same value for K_U or ΔG_U (calculated as $-RT \ln K_U$) from experiments performed at different protein concentrations. A linear dependence of the Gibbs free energy of unfolding on the denaturant is assumed [29]:

$$\Delta G_U = \Delta G_U^W - m \times [\text{Urea}] \quad (2)$$

where ΔG_U^W represents the difference in Gibbs free energy between the unfolded and folded protein in the absence of denaturant.

The conformational stability parameters ΔG_U^W and m were calculated by iterative fitting of the denaturation curves to the above equations using Scientist (MICROMATH Scientific Software). Values of C_m (the denaturant concentration at which half the population of protein molecules are unfolded) were obtained from the midpoints of the unfolding transitions.

2.6. Isothermal titration microcalorimetry

All calorimetric experiments were conducted on an MCS ITC from Microcal, Inc. (Northampton, MA). A complete description of its predecessor instrument, OMEGA-ITC, experimental strategies, and data analyses are given by Wiseman et al. [30]. The calorimeter was calibrated by known heat pulses as recommended by the manufacturer. During titration, the reference cell was filled with Milli Q water. Prior to the titration experiments, both the enzyme and the ligand were degassed for 10 min under vacuum. The sample cell was filled either with 1.8 ml (effective volume: 1.38 ml) of buffer (for the control experiment) or an appropriately diluted enzyme. During the titration, the reaction mixture was continuously stirred at 400 rpm.

The background titration profiles, under identical experimental conditions, were obtained by injecting GSH or inhibitor into appropriate buffer solutions. The observed heat effects were concentration-independent and were identical to the heat signals detected after complete saturation of the protein. The raw experimental data are presented as the amount of heat evolved per second following each injection of ligand into the enzyme solution (after correction for the ligand heat of dilution) as a function of time. The amount of heat produced per injection was calculated integrating the area under individual peaks using the ORIGIN software provided with the instrument.

A model of two equal and independent sites [22] is able to fit the experimental data, which provides the enthalpy change and the microscopic equilibrium constant. The errors correspond to the standard deviation in the fitting of the curves. After the fitting, the entropy change and the Gibbs energy change are easily calculated from the thermodynamic relations ($\Delta G^0 = \Delta H_{obs} - T\Delta S^0 = -RT \ln K_{obs}$). The standard state is that of 1 mol l^{-1}, and these calculations imply the usual approximation of setting standard enthalpies equal to the observed ones.

Solutions of Sj26GST were prepared by dialysis of the enzyme against several changes of 2 mM DTT and 20 mM TAPS in the presence of a range of X salt concentrations (0–0.5 M) at pH 8.5 and 25 °C, where X is NaCl, $MgCl_2$, Na_2SO_4 and $MgSO_4$. The pH of the buffer solution was adjusted at every experimental temperature.

3. Results and discussion

3.1. Unfolding experiments at different temperatures

Samples of Sj26GST, at a final concentration of 0.1–0.4 μM, were incubated in urea solutions (0–8 M) for a time sufficient to ensure that the denaturation equili-

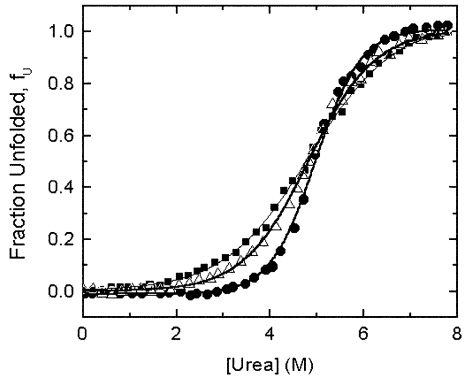

Fig. 1. Examples of urea-unfolding equilibrium profiles for Sj26GST. Fraction unfolded, f_U, is plotted versus urea concentration at 25 °C (●), 35 °C (△), and 45 °C (■) in 20 mM TAPS, 2 mM DTT at pH 8.5. The solid lines represent the best fits of the experimental data to Eq. (1).

brium was established. Profiles of unfolding fraction, f_U, versus urea concentration were obtained at several temperatures within the range of 25–50 °C (Fig. 1) in buffer TAPS 20 mM, pH 8.5. These profiles were analyzed according to the two-state denaturation model shown in Section 2 (as indicated by Kaplan et al. [31]). The fitting curves allowed us to estimate the conformational stability of Sj26GST in the absence of denaturant, ΔG_U^w, and the *m*-value, at different temperatures. It is known that the size of the protein or the amount of its surface area exposed to solvent upon unfolding is the major structural determinant factor for the *m*-value [14,29]. The *m*-values obtained (Table 1) change slightly with increasing temperature, which indicates the area exposed to solvent is practically the same across this temperature range.

To study the influence of salt concentration and type on the unfolding parameters, similar experiments to those mentioned above were performed with two different salts (NaCl and $MgCl_2$) in the range of 10–400 mM. The unfolding curves are similar to those without salt, showing the unfolding is insensitive to the salt type and concentration used. A different behavior was found for class σ GST [32].

Table 1
Unfolding data for *Schistosoma japonicum* glutathione *S*-transferase at pH 8.5 and at different temperatures

T (°C)	*m* (kJ mol^{-1} M^{-1})	ΔG_U^W (kJ mol^{-1})	C_m (M)
25	25.12±0.75	132.67±4.10	5.01
30	21.48±0.37	113.36±1.67	4.73
35	19.56±0.33	105.46±1.67	4.76
40	17.76±0.29	94.26±1.42	4.59
45	17.14±0.21	94.47±1.04	4.73
50	14.00±0.33	88.14±2.01	5.12

The uncertainties are the standard errors in the fit of the curves.

Moreover, the presence of the substrate (glutathione) or *S*-butylglutathione does not alter the unfolding/folding parameters as could be expected due to the increased stabilization of the native state of Sj26GST upon occupation of its active sites by these ligands, a phenomenon observed for other GSTs [33].

3.2. Salt-induced conformational changes at equilibrium

Fig. 2 shows the effect of increasing salt concentration for the salts studied on the intrinsic fluorescence (Fig. 2A) and the enzyme activity (Fig. 2B) of Sj26GST in 20 mM TAPS, pH 8.5 at 25 °C. In Fig. 2A a fluorescence ratio (F_{355}/F_{335}) of ≈0.75 can be observed, which practically does not change with the salt concentration used. Fig. 2B displays the enzyme relative activity loss (as percentages of the total loss) for the four salts analyzed. Stevens et al. [32] claimed that Sj26GST maintained its catalytic activity at up to 2M NaCl, but these authors did not show any data. However, we have found that the enzyme activity decreases when each salt concentration is increased. This relative decrease at a given concentration is in the order $Na_2SO_4 < NaCl < MgSO_4 < MgCl_2$. The analysis of these results as a function of ion class according to Hofmeister's series [8] seems to suggest that the loss of activity is higher as the trend to accumulate molecules of water increases

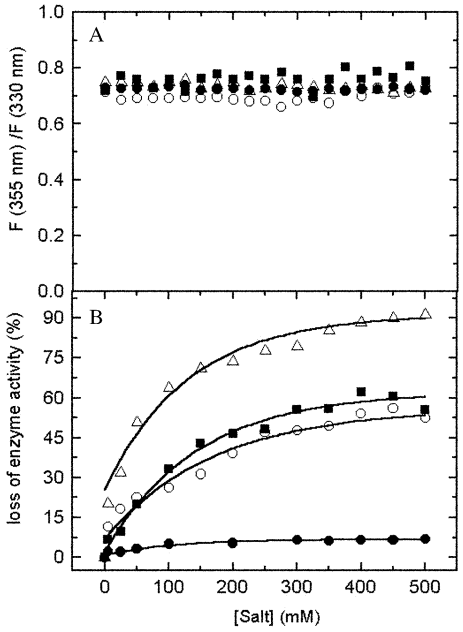

Fig. 2. Influence of salt type and concentration on Sj26GST. The protein concentration was 0.4 μM in 20 mM TAPS, pH 8.5, containing 2 mM DTT at 25.1 °C. Panel A shows the intrinsic fluorescence change with increasing Na_2SO_4 (●), NaCl (○), $MgSO_4$ (■), and $MgCl_2$ (△) concentrations, using an excitation wavelength of 295 nm. Panel B displays the loss of enzyme activity, shown as a percentage. The solid lines correspond to an arbitrary smoothing of the experimental data.

and the ionic radii decrease, i.e. when the ion chaotropic character increases.

3.3. Sj26GST aggregation state

A size-exclusion column of Superdex 200 HR 10/30 at a flow rate of 0.5 ml min^{-1} was used to determine the aggregation state of this enzyme without and at high salt concentrations. The protein was detected by absorbance at 280 nm, and the retention time corresponds to that of the dimer of Sj26GST when you use a column equilibrated under the same salt concentration. Thus, there is no detectable evidence for association–dissociation phenomena caused by the presence of salts.

3.4. Fluorescence binding experiments

The binding of GSH to the Sj26GST dimer was monitored by measuring the intrinsic fluorescence as a function of the substrate concentration at 25 °C and pH 8.5 in 20 mM TAPS and 2 mM DTT in the presence of different salt concentrations. The binding curves under these conditions are non-cooperative, just as we found previously under different conditions [22]. The equilibrium constants obtained are practically independent of salt type and concentration, except for $MgCl_2$, whose equilibrium constant decreases as the $MgCl_2$ concentration increases. The Fig. 3 shows three representative fluorescence titrations of the binding of GSH to Sj26GST protein at 10.2, 200 and 505 mM of $MgCl_2$. When comparing the results for NaCl and $MgCl_2$, the NaCl association constants show values between 2×10^4 and 4×10^4 M^{-1} at all the studied concentrations (0–500 mM), while for $MgCl_2$ the equilibrium constants vary from 11.3×10^4 to 0.6×10^4 M^{-1} in the same salt concentration range. This further confirms our ITC results (below).

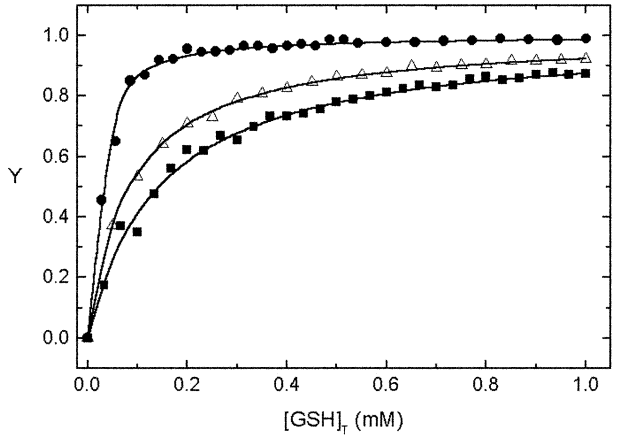

Fig. 3. Representative fluorescence titrations of the binding of GSH to Sj26GST protein in 20 mM TAPS (pH 8.5), 2 mM DTT and 10.2 (●), 200 (△) and 505 mM (■) of $MgCl_2$ at 25 °C. The saturation fraction, Y, is displayed vs the total concentration of GSH. The protein concentration was 0.5 μM. The continuous line is the theoretical curve generated using a non-cooperative model with two sites.

3.5. Isothermal calorimetry experiments

ITC has been used to investigate the thermodynamic influence of electrostatic interactions on the binding of substrate GSH to the enzyme. We have performed a series of calorimetric titrations of Sj26GST binding to its substrate over a range of NaCl, Na_2SO_4, $MgSO_4$ and $MgCl_2$ concentrations from 5 to 500 mM in TAPS buffer at 25 °C and pH 8.5. Fig. 4 shows the titration of 44.22 μM Sj26GST with 25 aliquots (5 μl each) of GSH (stock concentration of 7.48 mM) in 20 mM TAPS, 0.2 M Na_2SO_4 and 2 mM DTT at pH 8.5 and 25.2 °C. The top panel in Fig. 4 shows the raw calorimetric data, denoting the amount of heat produced (negative exothermic peaks) following each injection of substrate into the cell containing the protein solution. The area under each peak represents the amount of heat evolved per injection. Note that, as the titration progresses, the area under the peaks progressively becomes smaller due to an increased occupancy of the enzyme by glutathione. The bottom panel of Fig. 4 shows the plot of the amount of heat generated per mole of ligand injected as a function of the molar ratio of reduced GSH to enzyme. The smooth solid line represents the best fit of the

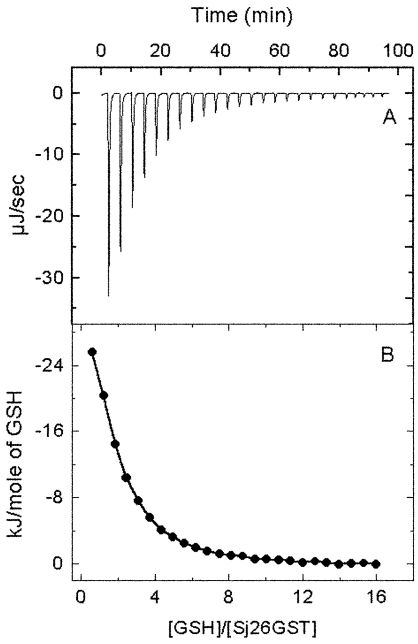

Fig. 4. Titration calorimetry of 44.22 μM Sj26GST by 7.48 mM GSH in 20 mM TAPS (pH 8.5), 0.2 M Na_2SO_4, containing 2 mM DTT at 25.2 °C. Panel A shows the raw data for the titration of 1.8 ml of enzyme with twenty-five 5 μl injections of GSH. The area under each peak was integrated and plotted in panel B against the molar ratio of total GSH to total Sj26GST inside the cell. The solid smooth line represents the best fit of the experimental data to a model of two equal and non-interacting sites.

experimental data to a model of two equal and independent sites, with values for the microscopic association constant (K_{obs}) and the standard enthalpy change (ΔH_{obs}) of 3.73×10^4 M^{-1} and -33.82 ± 0.71 kJ mol^{-1}, respectively. The equilibrium constant obtained is one order of magnitude higher than those obtained at pH 6.5 without salt [22], and is also in good agreement with the equilibrium constant measured by fluorescence titrations. Globally, the observed binding constants (K_{obs}) are practically insensitive to salt concentration for NaCl, Na_2SO_4 and $MgSO_4$. However, K_{obs} depends upon $MgCl_2$ concentration. Fig. 5 shows a titration of 40.51 μM Sj26GST with aliquots (5 μl each) of GSH (stock concentration of 12.55 mM) in 20 mM TAPS, 0.4 M $MgCl_2$ and 2 mM DTT at pH 8.5 and 24.9 °C. By comparing this titration with those in Fig. 4, it can be observed that in this case the saturation is reached at a higher ligand/protein ratio, which indicates a lower affinity constant.

On the other hand, as the salt concentration of any of these salts is increased the enthalpic contribution remains approximately constant (Table 2), and it is the entropic contribution which follows the pattern given by the binding constants for every salt studied.

This behavior can be explained taking into account the chaotropic or kosmotropic nature of the ions involved. The salts used in this study form the following

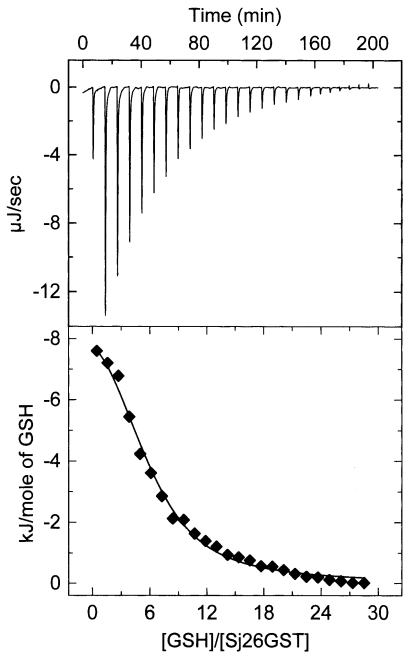

Fig. 5. Titration of Sj26GST by GSH in 20 mM TAPS (pH 8.5), 2 mM DTT and 0.4 M $MgCl_2$ at 24.9 °C. The protein concentration was 40.51 μM, and was titrated with aliquots (5 μl each) of GSH (stock concentration of 12.55 mM). The solid smooth line represents the best fit of the experimental data to a model of two equal and non-interacting sites with values for the microscopic association constant (K_{obs}) and the standard enthalpy change (ΔH_{obs}) of 0.53×10^4 M^{-1}, and -36.57 ± 2.26 kJ mol^{-1}, respectively.

series with respect to kosmotropic nature: anions, sulphate ≫ chloride; cations, sodium ≫ magnesium. From the series, the most inert salt is NaCl, whose ions lack a defined tendency to either one of these characters. This inert nature is in good agreement with our thermodynamic results for NaCl, which are the most even among the salts studied. The opposite role is played by $MgCl_2$. The elevated chaotropic nature of the Mg^{2+} cation and the inert nature of Cl^- favor the solubilization of the hydrophobic spots of the protein and the ligand. In other words, the presence of Mg^{2+} makes water interact more strongly with these hydrophobic zones.

As can be seen in Table 2 and Fig. 6 the affinity of the protein for the substrate decreases with increasing $MgCl_2$ concentration. There are at least two possible reasons for this: (1) the protein denatures partially as a consequence of the high chaotropic nature of Mg^{2+} or (2) the binding is simply hindered because of the presence of the interacting water molecules. We think the first reason does not apply in this particular case, since our denaturing experiments show no significant difference among the results obtained for the various salts we chose. So, the second reason may be the correct one.

Although it is known that the binding of glutathione to Sj26GST is not driven predominantly by hydrophobic forces [22], it is necessary to take them into account when explaining the differences appearing under unlike solution conditions, especially if the latter vary only in free ion types and concentrations.

The hydrophobic interaction between the substrate and the protein is weaker the greater the resistance is. As the Mg^{2+} concentration is raised, more water molecules interact with the hydrophobic spots involved in the substrate-protein binding, and the affinity decreases.

Thermodynamically, this lower affinity is observed only as a more negative entropy change, since the enthalpy change remains practically unaltered under the $MgCl_2$ concentrations studied. This unfavorable entropy change arises from a more ordered water structure relative to the one existing before binding (Fig. 6), as a consequence of the interaction of the water molecules among them after they are released from the hydrophobic patches.

The remaining two salts ($MgSO_4$ and Na_2SO_4) behave similarly to NaCl. In the case of magnesium sulphate, the sulphate anion is a strong kosmotrope and balances the chaotropic magnesium effect, making the system non-dependent upon this salt concentration. Finally, sodium sulphate has also no effect fine-tuning the binding, since none of its ions are chaotropes.

Moreover, in order to figure out the role of the substrate's thiol group of sulfhydryl, ITC experiments were carried out at low and high $MgCl_2$ concentration using S-methylglutathione as ligand. In this case, the

Table 2
Thermodynamic data for the interaction of *Schistosoma japonicum* glutathione *S*-transferase with glutathione at pH 8.5 and 25.2 °C for different salts

[Salt] (mM)	[GSH] (mM)	K_{obs} (10^4 M^{-1})	ΔG^0 (kJ mol^{-1})	ΔH_{obs} (kJ mol^{-1})	$T\Delta S^0$ (kJ mol^{-1})
Sodium sulphate					
26.14	7.48	6.91 ± 0.04	−27.50 ± 0.08	−30.30 ± 1.88	−2.76 ± 0.08
150.97	7.48	4.38 ± 0.10	−26.38 ± 0.50	−29.51 ± 1.38	−3.13 ± 0.50
400.92	7.48	3.12 ± 0.07	−25.54 ± 0.26	−30.10 ± 0.92	−4.51 ± 0.29
Sodium chloride					
11.25	7.48	2.54 ± 0.12	−25.04 ± 0.33	−27.88 ± 1.25	−2.84 ± 0.37
101.10	7.48	2.42 ± 0.02	−24.91 ± 0.08	−26.42 ± 0.29	−1.46 ± 0.08
500.82	7.46	2.30 ± 0.05	−28.97 ± 0.12	−28.42 ± 0.46	−3.59 ± 0.12
Magnesium sulphate					
10.97	12.15	3.82 ± 0.11	−26.04 ± 0.16	−24.83 ± 0.50	1.21 ± 0.12
100.94	12.15	6.14 ± 0.10	−27.21 ± 0.46	−33.31 ± 1.08	−5.10 ± 0.42
500.82	15.00	1.34 ± 0.07	−23.45 ± 0.12	−26.92 ± 0.46	−3.47 ± 0.08
Magnesium chloride					
15.85	8.02	6.80 ± 0.05	−27.46 ± 0.07	−38.12 ± 1.34	−10.66 ± 0.29
150.82	7.22	2.22 ± 0.07	−24.70 ± 0.02	−29.97 ± 0.38	−5.27 ± 0.08
500.78	12.55	0.44 ± 0.07	−12.92 ± 0.01	−36.57 ± 2.30	−23.66 ± 0.04

For further clarity, this table shows only the titrations carried out at three salt concentrations: low, medium and high. However, in Fig. 6 all of the experiments at all the salt concentrations are displayed. The errors shown correspond to the standard deviation of the non-linear least squares fit of the data points of the titration curve.

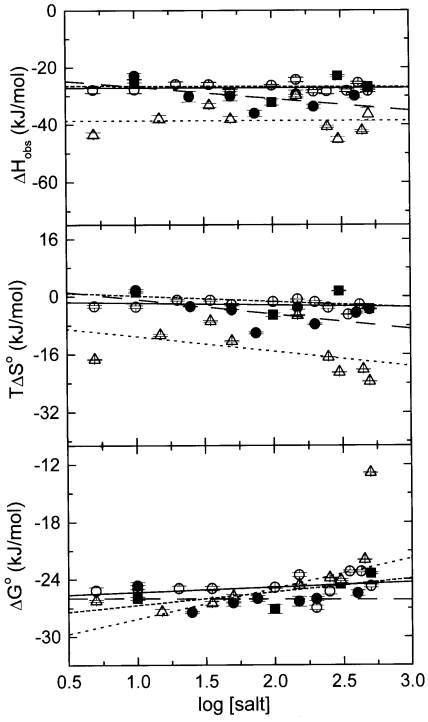

Fig. 6. Dependence of the thermodynamic parameters for the binding of GSH to Sj26GST on Na_2SO_4 (●), NaCl (○), $MgSO_4$ (■) and $MgCl_2$ (△) concentrations. The lines represent the linear regression analysis of the experimental data for the four salts studied: Na_2SO_4 (—·—), NaCl (—), $MgSO_4$ (-··-), and $MgCl_2$ (. . .).

affinity decreases as well ($K_{obs} = (2.00 \pm 0.04) \times 10^3$ and $K_{obs} = (0.10 \pm 0.04) \times 10^3$ at 0.5 and 400 mM, respectively), which indicates the thiol group of sulfhydryl or the enzyme residues interacting with it were not the salt-affected zones for this decreased affinity.

As can be observed in Fig. 6, for the NaCl, Na_2SO_4 and $MgSO_4$ salts, there is no enthalpy-entropy compensation with increasing salt concentration over the range studied, and both the entropy and the enthalpy changes for the binding are practically unaffected under these conditions.

Stevens et al. [32] studied the influence of ionic strength in class σ GST. In this case, the activity and fluorescence of the protein is affected by the NaCl concentration. When comparing the structures of both transferases, there are several likely structural reasons for the insensitivity of Sj26GST to ionic strength, the main origin of which is the hydrophobic (hydrophilic in class σ transferase) nature of the subunit interface. This result shows the low significance of electrostatic interactions in keeping the active-site region active in contrast with class σ transferase. These results are in agreement with our previous studies pointing out that the forces and the contributions responsible for the association between the substrate or several inhibitors and the protein are van der Waals interactions and H-bonds [22,23].

4. Conclusions

The *Schistosoma japonicum* GST is not completely insensitive to the ionic strength, since its binding properties depend upon the chaotropic nature of the ions in solution. Thus, the enzyme activity decreases as the salt concentration increases, being the relative

decrease at a given concentration in the order $Na_2SO_4 < NaCl < MgSO_4 < MgCl_2$, i.e. the decrease is higher as the chaotropic nature increases. The binding constants of GSH to Sj26GST, obtained by fluorometric and calorimetric experiments, are practically insensitive to salt concentration for NaCl, Na_2SO_4 and $MgSO_4$ but not for $MgCl_2$ where the affinity decreases as the salt concentration increases. The binding of substrate to enzyme is favored enthalpically, and this enthalpic contribution is unaffected by salt type and concentration. Therefore, the lower Sj26GST-ligand affinities found when these salts are present are due to changes in the entropy change, as a result of the chaotropic nature of the solution ions. This unfavorable entropy change arises from a more ordered water structure, as a consequence of the interaction of the water molecules among them after they are released from the hydrophobic areas.

Acknowledgements

We would like to thank Dr E. Ortiz-Salmerón for her useful suggestions. This research was supported by Grant SAF2001-2067 from CICYT (co-financed by FEDER), Ministerio de Ciencia y Tecnología, Spain. Z. Yassin was supported by fellowships from the AECI, Spain.

References

[1] O'Brien R, DeDecker B, Fleming KG, Sigler PB, Ladbury JE. J Mol Biol 1998;279:117.
[2] Bolen DW, Flogel M, Biltonen RL. Biochemistry 1971;10:4136.
[3] Eftink MR, Anunsiem AC, Biltonen RL. Biochemistry 1983;19:3884.
[4] Marqusee S, Sauer RT. Protein Sci 1994;3:2217.
[5] Gokhale RS, Ray SS, Balaram H, Balaram P. Biochemistry 1999;38:423.
[6] Larsen TA, Olson AJ, Goodsell DS. Structure 1998;6:421.
[7] Mainfroid V, Terpstra P, Beaurengard M, Frere JM, Mande SC, Hol WGJ. J Mol Biol 1996;257:441.
[8] Hofmeister F. Arch Exp Pathol Pharmakol (Leipzig) 1888;24:247.
[9] Jakoby WB, Habig WH. Enzymatic basis of detoxification. In: Jacoby WB, editor. Glutathione transferases, vol. 2. New York: Academic Press, 1980:63–94.
[10] Armstrong RN. Chem Res Toxicol 1991;4:131.
[11] Takahashi Y, Campbell EA, Hirata Y, Takayama T, Listowsky I. J Biol Chem 1993;268:8893.
[12] Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M. Proc Natl Acad Sci USA 1985;82:7202.
[13] Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B. Biochem J 1991;274:409.
[14] Meyer DJ, Thomas M. Biochem J 1995;311:739.
[15] Ji X, von Rosenvinge EC, Johnson WW, Tomarev SL, Piatigorsky J, Armstrong RN. Biochemistry 1995;34:5317.
[16] Salinas AE, Wong MG. Curr Med Chem 1999;6:279.
[17] McTigue MA, William DR, Tainer JA. J Mol Biol 1995;246:21.
[18] Lim K, Ho JK, Keeling K, Gilliland GL, Ji X, Rüker F. Protein Sci 1994;3:2233.
[19] Stevens JM, Hornby JAT, Armstrong RN, Dirr HW. Biochemistry 1998;37:15534.
[20] Collins KD, Washabaugh MW. Q Rev Biophys 1985;18:323.
[21] Smith DB, Johnson KS. Gene 1988;67:31.
[22] Ortiz-Salmerón E, Yassin Z, Clemente-Jiménez MJ, Las Heras-Vázquez FJ, Rodriguez-Vico F, Barón C. Eur J Biochem 2001;268:4307.
[23] Ortiz-Salmerón E, Yassin Z, Clemente-Jiménez MJ, Las Heras-Vázquez FJ, Rodríguez-Vico F, Barón C. Biochim Biophys Acta 2001;1548:106.
[24] Klotz P, Slaoui-Hasnaoi A, Banéres JL, Duckert JF, Rossi JC. J Med Chem 1998;41:2278.
[25] Mannervik B, Danielson UH. CRC Crit Rev Biochem 1988;23:283.
[26] Pace CN, Shirley BA, Thomson JA. In: Creighton TE, editor. Protein Structure: a practical approach measuring conformational stability of a protein, 2nd ed.. Oxford, UK: IRL press at Oxford University Press, 1989:311.
[27] Pace CN. Meth Enzymol 1986;131:266.
[28] Bowie JU, Sauer RT. Biochemistry 1989;28:7139.
[29] Schellman JA. Biopolymers 1978;17:1305.
[30] Wiseman T, Williston S, Brandts JF, Lin L-N. Anal Biochem 1989;17:131.
[31] Kaplan W, Hüsler P, Klump K, Erhardt J, Sluis-Cremer N, Dirr H. Protein Sci 1997;6:399.
[32] Stevens JM, Armstrong RN, Dirr H. Biochem J 2000;347:193.
[33] Erhardt J, Dirr H. Eur J Biochem 1995;230:614.