THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 27, Issue of July 2, pp. 19276–19280, 1999
Printed in U.S.A.

Temperature Adaptation of Glutathione *S*-Transferase P1–1

A CASE FOR HOMOTROPIC REGULATION OF SUBSTRATE BINDING*

(Received for publication, January 6, 1999, and in revised form, March 29, 1999)

Anna Maria Caccuri‡, Giovanni Antonini§¶, Paolo Ascenzi¶‖, Maria Nicotra‡, Marzia Nuccetelli‡, Anna Paola Mazzetti‡, Giorgio Federici, Mario Lo Bello‡, and Giorgio Ricci‡ ‡‡**

*From the ‡Department of Biology, University of Rome Tor Vergata, I-00133 Rome, the §Department of Basic and Applied Biology, University of l'Aquila, I-67010 l'Aquila, the ¶Department of Biochemical Sciences A. Rossi Fanelli, University of Rome La Sapienza, I-00185 Rome, the ‖Department of Biology, University of Rome Tre, I-00146 Rome, and the **Ospedale Pediatrico IRCCS Bambin Gesù, I-00165 Rome, Italy*

Human glutathione *S*-transferase P1–1 (GST P1–1) is a homodimeric enzyme expressed in several organs as well as in the upper layers of epidermis, playing a role against carcinogenic and toxic compounds. A sophisticated mechanism of temperature adaptation has been developed by this enzyme. In fact, above 35 °C, glutathione (GSH) binding to GST P1–1 displays positive cooperativity, whereas negative cooperativity occurs below 25 °C. This binding mechanism minimizes changes of GSH affinity for GST P1–1 because of temperature fluctuation. This is a likely advantage for epithelial skin cells, which are naturally exposed to temperature variation and, incidentally, to carcinogenic compounds, always needing efficient detoxifying systems. As a whole, GST P1–1 represents the first enzyme which displays a temperature-dependent homotropic regulation of substrate (*e.g.* GSH) binding.

Although many enzymes are oligomeric proteins, substrate binding to one subunit does not alter generally the catalytic properties of the other one(s). Thus, the enzyme obeys to simple hyperbolic ligand binding behavior and standard Michaelis-Menten kinetics. However, in some oligomeric enzymes, substrate binding to one subunit may modify the catalytic properties of the functionally related one(s). This property, named "homotropic interaction," yields nonhyperbolic ligand binding isotherms and nonstandard Michaelis-Menten kinetics, representing a case of the general phenomenon named "cooperativity" (1, 2).

Some apparently noncooperative oligomeric enzymes display a latent cooperativity. Thus, the dimeric glutathione reductase from *Escherichia coli* shows homotropic catalytic behavior when Gly^{418}, present at the subunit interface, is replaced by Trp (3). Also, tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* displays a cooperative behavior upon mutation of Lys^{233}, involved in the substrate binding, to Ala (4). Furthermore, trimeric aspartate transcarbamoylase from *Bacillus subtilis* and ornithine transcarbamoylase from *Escherichia coli* show a homotropic substrate binding behavior upon mutation of Arg^{99} and Arg^{106}, present at the enzyme active site, to Ala and Gly, respectively (5, 6). Also, the homodimeric human glutathione *S*-transferase P1–1 (GST P1–1),[1] by playing a central role in cellular detoxification processes against toxic and carcinogenic compounds (7, 8), displays latent cooperativity. In fact, the substitution of Gly^{41}, Cys^{47}, or Lys^{54} with Ala, Ser, and Ala, respectively, induces positive cooperativity for substrate binding at 25 °C (9–11)

In this study, we report direct evidence that positive and negative cooperativity occurs for substrate (*e.g.* glutathione; GSH) binding to wild type GST P1–1 above 35 °C and below 25 °C, respectively. This binding mechanism minimizes changes of GSH affinity because of temperature fluctuation. As a whole, GST P1–1 represents the first enzyme displaying a temperature-dependent homotropic regulation of the substrate (*e.g.* GSH) binding.

MATERIALS AND METHODS

Enzyme Preparation—The wild type GST P1–1 (EC 2.5.1.18) was obtained as previously reported (10). The Y49F mutant of GST P1–1 was prepared according to Landt *et al.* (12) in two steps of polymerase chain reaction. In the first step, the 5′ universal primer 5′-TCGAATTCCAGGAAACAGCTATGAC and the 3′ mutagenic primer (Y49F) 5′-AGCTGCCCGAATAGGCAGG were used to generate a double-stranded mutated fragment with p18seq-1 plasmid (10) as a template and the high fidelity polymerase EXPAND (Roche Molecular Biochemicals, Italy). The amplified DNA fragment was purified from agarose gel and used as a primer in the second polymerase chain reaction in combination with the second 3′ universal primer 5′-TCGAATTCGTTTTCCCAGTCACGAC. The amplified DNA was restricted with *Sph*I and subsequently ligated in the expression plasmid pGST-1 to produce the mutant enzyme. Nucleotide sequence analysis was carried out by the chain termination method. The Y49F mutant of GST P1–1 was expressed and purified as previously reported (10).

GSH Association to GST P1–1, Kinetic and Equilibrium Experiments—GST P1–1 steady-state kinetics was carried out at pH 6.5 (0.1 M potassium phosphate buffer) and between 5 and 43 °C, by varying GSH concentration from 10.0 μM to 10.0 mM, in the presence of CDNB as the co-substrate. The CDNB concentration (= 1.0 mM), corresponding approximately to that for the enzyme half-saturation, could not be further increased, accounting for the low co-substrate solubility (13). Kinetic experiments were also carried out at pH 5.0 (0.1 M sodium acetate buffer) and between 5 and 35 °C, by varying GSH concentration from 2.0 μM to 1.0 mM, in the presence of NBD-Cl as the co-substrate. The NBD-Cl concentration (= 0.2 mM) can be considered as saturating for the enzyme (14). The GST P1–1 concentration ranged between 10 nM and 100 nM.

Equilibrium experiments for GSH binding to GST P1–1 were performed at pH 6.5 (0.1 M potassium phosphate buffer), between 5 and 43 °C, by following the quenching of the enzyme intrinsic fluorescence, as a function of GSH concentration (ranging between 10 μM and 10 mM),

* This study is a part of special projects supported by Italian Ministry of University and of Scientific and Technological Research, Italian National Research Council and Italian Ministry of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence should be addressed Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica, I-00133 Rome, Italy. Tel.: +39+06+72594375; Fax: +39+06+2025450; E-mail: riccig@uniroma2.it.

[1] The abbreviations used are: GST, glutathione *S*-transferase; GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

in the absence of any co-substrate (9). The GST P1–1 concentration was 3 μM.

Under all the experimental conditions, no GST P1–1 inactivation was observed within the experimental time. Furthermore, the effect of temperature on the catalytic activity and the spectroscopic properties of GST P1–1 was completely reversible.

Data Analysis—Steady-state kinetics, obtained in the presence of a constant concentration of the co-substrate (*e.g.* CDNB or NBD-Cl) and by varying the GSH concentration, were analyzed in the framework of the minimum overall enzyme reaction scheme (Scheme 1) (15),

$$E + X\text{-}R + GSH \overset{K}{\longleftrightarrow} X\text{-}R\text{-}E\text{-}GSH \xrightarrow{V_{max}} E + GS\text{-}R + HX$$

SCHEME 1

according to the Hill equation (Equation 1):

$$Y = v_i/V_{max} = [S]^n/(K^n + [S]^n) \quad \text{(Eq. 1)}$$

where E is GST P1–1; *GSH* is the substrate; *X-R* is the co-substrate (*e.g.* an electrophilic harmful compound, such as CDNB and NBD-Cl); *X-R-E-GSH* indicates the reaction intermediate(s); *GS-R* and *HX* are the reaction products; K (*e.g.* K_m, the Michaelis constant) is the overall dissociation equilibrium constant, indicating the substrate (*e.g.* GSH) concentration corresponding to the half enzyme saturation (*e.g.* to $V_{max}/2$); V_{max} is the maximum velocity, observed at saturating substrate (*e.g.* GSH) concentrations; v_i is the initial velocity, observed at nonsaturating substrate (*e.g.* GSH) concentrations; [S] is the GSH concentration; and n is the apparent Hill coefficient at the GSH concentration corresponding to the half enzyme saturation (*e.g.* at K). According to Equation 1, values of n may vary from 1, indicating the absence of cooperativity, to a maximum value, corresponding to the number of interacting subunits (= 2 for the homodimeric GST P1–1) and indicating infinite positive cooperativity. Then, n values lower than 1 indicate negative cooperativity.

Steady-state kinetics were also analyzed in the framework of the minimum two sites Adair model for ligand binding to a homodimeric macromolecule (Scheme 2) (15, 16),

$$E + X\text{-}R + GSH \overset{K_1}{\longleftrightarrow} X\text{-}R\text{-}E\text{-}GSH + GSH \overset{\alpha K_1}{\longleftrightarrow} X\text{-}R\text{-}E\text{-}GSH_2 \xrightarrow{V_{max}} E + GS\text{-}R + H\text{-}X$$

SCHEME 2

according to Equation 2,

$$Y = v_i/V_{max} = ([S]/K_1 + [S]^2/\alpha K_1^2)/(1 + 2[S]/K_1 + [S]^2/\alpha K_1^2) \quad \text{(Eq. 2)}$$

where K_1 is the dissociation equilibrium constant for GSH binding to the substrate-free enzyme, αK_1 (= K_2) represents the dissociation equilibrium constant for GSH binding to the monoligated enzyme, and α is the adimensional interaction parameter coupling the two functionally linked GSH binding clefts (*e.g.* G-sites). According to Equation 2, the value of α may correspond to 1, indicating no functional coupling between identical ligand binding sites (*e.g.* noncooperativity, $K_1 = K_2$). Moreover, values of α may be higher and lower than 1, indicating negative and positive cooperativity, respectively (*e.g.* $K_1 < K_2$ and $K_1 > K_2$, respectively).

Equilibrium fluorescence data for GSH binding to GST P1–1, obtained in the absence of the co-substrate (*e.g.* CDNB and NBD-Cl), were also analyzed in the framework of the minimum overall reaction scheme (Scheme 3) (15),

$$E + GSH \overset{K}{\longleftrightarrow} E\text{-}GSH$$

SCHEME 3

according to the Hill equation (Equation 3),

$$Y = \Delta F_i/\Delta F_{max} = [S]^n/(K^n + [S]^n) \quad \text{(Eq. 3)}$$

where ΔF_i indicates the fluorescence quenching change observed at nonsaturating substrate (*e.g.* GSH) concentrations, and ΔF_{max} is the maximum fluorescence quenching variation detected at saturating substrate (*e.g.* GSH) concentrations.

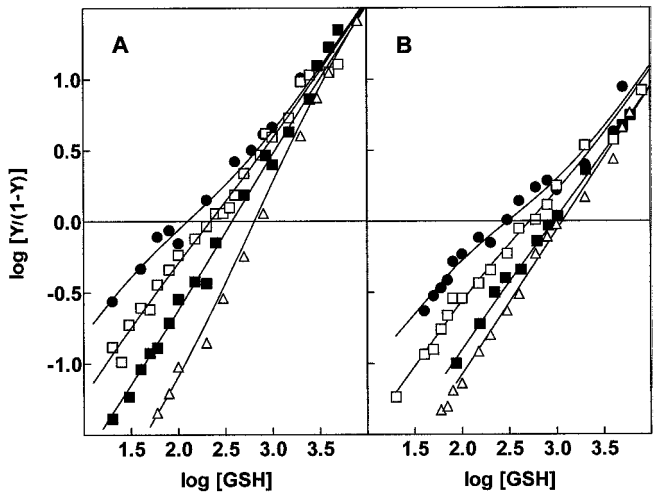

FIG. 1. **GSH association to wild type (*panel A*) and Y49F (*panel B*) GST P1–1, at 5 (●), 25 (□), 35 (■), and 43 °C (△).** The convergence of data on increasing the GSH concentration indicates that the affinity of GSH for the second G-site present in GST P1–1 (*e.g.* αK_1) is unaffected by temperature (*e.g.* $\Delta H_{\alpha K1} \sim 0$ kJ/mol). The *continuous lines* were calculated according to Equation 2 with sets of parameters given in Table II. Data were obtained at fixed CDNB concentration (= 1.0 mM) and pH 6.5. Experiments were performed in triplicate. For further experimental details, see "Materials and Methods."

Equilibrium data were also analyzed in the framework of the minimum two sites Adair model for ligand binding to a homodimeric macromolecule (Scheme 4) (15, 16),

$$E + GSH \overset{K_1}{\longleftrightarrow} E\text{-}GSH + GSH \overset{\alpha K_1}{\longleftrightarrow} E\text{-}GSH_2$$

SCHEME 4

according to Equation 4.

$$Y = \Delta F_i/\Delta F_{max} = ([S]/K_1 + [S]^2/\alpha K_1^2)/(1 + 2[S]/K_1 + [S]^2/\alpha K_1^2) \quad \text{(Eq. 4)}$$

In the absence of the co-substrate, Scheme 1 reduces to Scheme 3, and Scheme 2 reduces to Scheme 4.

RESULTS

Effect of Temperature on GSH Association to Wild Type GST P1–1, in the Presence of the Co-substrate—GST P1–1 displays standard Michaelis-Menten kinetics, between 25 and 35 °C, in the presence of the co-substrate CDNB (9–11). Accordingly, values of the Hill coefficient n and of the interaction parameter α for GSH binding to GST P1–1 are close to 1 (see Fig. 1, and Tables I and II). However, positive cooperativity for substrate binding to GST P1–1 is observed above 35 °C. Thus, values of the Hill coefficient n and of the interaction parameter α are 1.4 and 0.14, respectively, at 43 °C (see Fig. 1, and Tables I and II). Conversely, negative cooperativity occurs below 25 °C, values of the apparent Hill coefficient n and of the interaction parameter α for GSH binding to GST P1–1 being 0.63 and 5.3, respectively, at 5 °C (see Fig. 1, and Tables I and II). As a whole, data given in Fig. 1 indicate that, at 43 °C, the affinity of GSH for the substrate-free enzyme is lower than that for substrate association to the GSH-monoligated GST P1–1 (*e.g.* $K_1 > \alpha K_1$), but on the other hand, $K_1 < \alpha K_1$, at 5 °C.

The unusual temperature-dependent GST P1–1 properties were observed also in the presence of the co-substrate NBD-Cl, the Hill coefficient n ranging from 0.64, at 5 °C, to 1.2, at 35 °C. Consistently, the value of the interaction parameter α decreases from 5.5, at 5 °C, to 0.42, at 35 °C (see Tables I and II).

Effect of Temperature on GSH Association to Wild Type GST

TABLE I
Effect of temperature on values of the overall parameters K and n for GSH association to GST P1–1

GST P1–1	Co-substrate	Parameter[a]	5 °C	25 °C	35 °C	43 °C
Wild type	CDNB[b]	K (μM)	1.5×10^2	2.2×10^2	3.9×10^2	7.2×10^2
		n	0.63	0.92	1.1	1.4
Wild type	NBD-Cl[c]	K (μM)	3.5	8.3	2.6×10^1	
		n	0.64	0.98	1.2	
Wild type[d]	—	K (μM)	7.9×10^1	1.3×10^2	2.3×10^2	5.1×10^2
		n	0.65	0.95	1.1	1.3
T49F Mutant	CDNB[b]	K (μM)	3.0×10^2	5.2×10^2	9.2×10^2	1.1×10^3
		n	0.64	0.80	0.92	1.0
C47S Mutant[e]	CDNB[b]	K (μM)		1.3×10^3		
		n		1.4		
C47S Mutant[d,e]	—	K (μM)		7.8×10^2		
		n		1.5		

[a] Experiments have been performed in triplicate. The standard error for values of K and n does not exceed ±8%.
[b] The co-substrate concentration (= 1.0 mM) is not saturating. For further experimental details, see "Materials and Methods."
[c] The co-substrate concentration (= 0.1 mM) is saturating. For further experimental details, see "Materials and Methods."
[d] Data have been obtained in the absence of the co-substrate. For further experimental details, see "Materials and Methods."
[e] Data from Ref. 9.

TABLE II
Effect of temperature on values of intrinsic parameters K_1, αK_1 (= K_2) and α for GSH association to GST P1–1

GST P1–1	Co-substrate	Parameter[a]	5 °C	25 °C	35 °C	43 °C
Wild type	CDNB[b]	K_1 (μM)	6.4×10^1	1.8×10^2	4.6×10^2	1.9×10^3
		$\alpha K_1 = K_2$ (μM)	3.4×10^2	2.6×10^2	3.4×10^2	2.7×10^2
		α	5.3	1.4	0.74	0.14
Wild type	NBD-Cl[c]	K_1 (μM)	1.5	8.0	4.0×10^1	
		$\alpha K_1 = K_2$ (μM)	8.2	8.7	1.7×10^1	
		α	5.5	1.1	0.42	
Wild type[d]	—	K_1 (μM)	3.5×10^1	1.0×10^2	2.9×10^2	1.3×10^3
		$\alpha K_1 = K_2$ (μM)	1.8×10^2	1.8×10^2	1.9×10^2	2.0×10^2
		α	5.1	1.8	0.66	0.15
T49F mutant	CDNB[b]	K_1 (μM)	1.1×10^2	3.1×10^2	7.7×10^2	1.2×10^3
		$\alpha K_1 = K_2$ (μM)	8.4×10^2	8.9×10^2	1.1×10^3	1.1×10^3
		α	7.6	2.9	1.4	0.92
C47S mutant[e]	CDNB[b]	K_1 (μM)		4.1×10^3		
		$\alpha K_1 = K_2$ (μM)		2.9×10^2		
		α		0.07		
C47S mutant[d,e]	—	K_1 (μM)		2.4×10^3		
		$\alpha K_1 = K_2$ (μM)		2.7×10^2		
		α		0.11		

[a] Experiments have been performed in triplicate. The standard error for values of K_1, αK_1 (=K_2) and α does not exceed ±8%.
[b] The co-substrate concentration (= 1.0 mM) is not saturating. For further experimental details, see "Materials and Methods."
[c] The co-substrate concentration (= 0.1 mM) is saturating. For further experimental details, see "Materials and Methods."
[d] Data have been obtained in the absence of the co-substrate. For further experimental details, see "Materials and Methods."
[e] Data, from Ref. 9, have been analyzed according to Eqs. 2 and 4.

P1–1, in the Absence of the Co-substrate—GSH binding to GST P1–1, as observed by following the quenching of the enzyme intrinsic fluorescence, displays temperature-dependent cooperativity also in the absence of CDNB and NBD-Cl (see Tables I and II). In fact, the value of the Hill coefficient n increases from 0.65, at 5 °C, to 1.3, at 43 °C. In agreement, the value of the interaction parameter α decreases from 5.1, at 5 °C, to 0.15, at 43 °C. On the other hand, no significant cooperativity for substrate binding to GST P1–1 was detected at 25 and 35 °C.

Equilibrium data, obtained in the absence of any co-substrate, overlap steady-state results (see Tables I and II), indicating that a true homotropic behavior occurs for GSH binding to GST P1–1. In fact, the reliability of kinetic determinations to account for cooperativity is limited. In this respect, a noncooperative homodimeric enzyme, following the steady-state random mechanism, may yield apparent cooperativity, simulating intersubunit communications (15).

As shown in Fig. 1 and in Tables I and II, the affinity of GSH for the substrate-free GST P1–1, expressed by K_1, is temperature-dependent with a positive apparent enthalpy value (*e.g.* $\Delta H_{K1} \sim +50$ kJ/mol). However, as a consequence of the temperature-dependent cooperative GSH binding to GST P1–1, the affinity of the substrate for the monoligated-enzyme (*e.g.* $\alpha K_1 = K_2$) is almost temperature-independent (see Fig. 1, and Tables I and II), the apparent $\Delta H_{\alpha K1}$ (= ΔH_{K2}) approaching zero. As expected, the value of the interaction parameter α decreases from 5.3 at 5 °C, to 0.15 at 43 °C (see Table II), the value of ΔH_α being about −50 kJ/mol.

Effect of the Y49F Mutation on GSH Association to the GST P1–1 in the Presence of the Co-substrate—Tyr^{49} appears to be a crucial residue for the intersubunit communication in GST P1–1, contacting helices 4 and 5 of the opposite chain (17, 18) (see Fig. 2). Upon mutation of Tyr^{49} to Phe, negative cooperativity for GSH binding to the Y49F mutant of GST P1–1 occurs below 43 °C, in the presence of CDNB, as shown for the wild type enzyme below 25 °C (see Fig. 1, and Tables I and II). In fact, values of the Hill coefficient n decrease from 1.0, at 43 °C, to 0.64, at 5 °C (see Table I). In parallel, values of the interaction parameter α increase from 0.92, at 43 °C, to 7.6, at 5 °C (see Table II). As a whole, the affinity of GSH for the substrate-free Y49F enzyme is higher than that for the GSH-monoligated species below 43 °C (*e.g.* $K_1 < \alpha K_1$ (see Table II).

Effect of the C47S Point Mutation on GSH Association to GST P1–1 in the Presence and Absence of the Co-substrate—Point mutation of residues Gly^{41}, Cys^{47}, and Lys^{54}, belonging to helix 2 and forming the G-site (17, 18) (see Fig. 2), induces positive cooperativity for GSH binding to GST P1–1, at 25 °C (9–11). In fact, in the presence and absence of CDNB, the

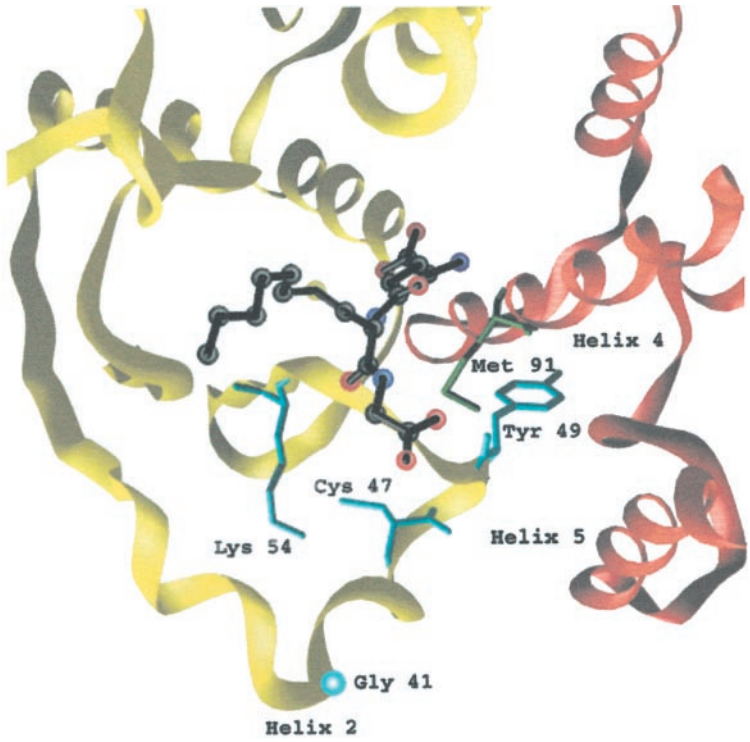

FIG. 2. **Ribbon representation of the GSH binding domain (G-site) of the homodimeric human GST P1–1.** Subunits are represented in *yellow* and *red*. The *S*-hexyl-GSH is shown in *ball* and *sticks*. Residues involved in the cooperative intersubunit communication are shown. The picture was based on the crystal structure of the GST P1–1-*S*-hexyl-GSH complex (17).

replacement of Cys^{47} with Ser triggers homotropic behavior, at 25 °C (*e.g.* $n \sim 1.4$ and $\alpha \sim 0.09$), as observed in the native enzyme at 43 °C (*e.g.* $n \sim 1.3$ and $\alpha \sim 0.14$, at 43 °C) (see Tables I and II). Therefore, the affinity of GSH for the substrate-free C47S enzyme is lower than that for the GSH-monoligated species at 25 °C (*e.g.* $K_1 > \alpha K_1$) (see Table II).

DISCUSSION

Here, reported data indicate that GST P1–1 represents the first enzyme displaying a temperature-dependent homotropic regulation of substrate (*e.g.* GSH) binding to the two G-sites present in the homodimer. In fact, negative cooperativity for GSH binding to GST P1–1 occurs below 25 °C. On the other hand, positive cooperativity is observed above 35 °C (see Fig. 1, and Tables I and II).

Temperature has been previously reported to reverse allosteric phenomena in rat liver fructose-1,6-diphosphatase, in phosphofructokinase from *Bacillus stearothermophilus* and in carbamoylphosphate synthetase from *Escherichia coli*. In fact, when varying the temperature, the AMP, ADP, or IMP action changes from inhibition to activation. Therefore, these enzymes display a temperature-dependent etherotropic regulation for substrate and allosteric ligand binding (19, 20).

The inspection of the three-dimensional model of GST P1–1 (17, 18) (see Fig. 2) fulfills a structural interpretation of the homotropic binding behavior of GSH. Structural perturbation occurring at the G-site by GSH binding would be transmitted to the G-site present in the adjacent subunit *via* helix 4 (residues 90–109), which represents the monomer-monomer interface and contains residues that interact with the active site. A key residue involved in the intersubunit communication may be Tyr^{49}, which contacts, with its aromatic ring, the hydrophobic pocket located between helices 4 and 5 of the adjacent subunit. In particular, in the presence of GSH, the Tyr^{49} residue forms 14 van der Waals contacts with the adjacent monomer. Next, the hydroxyl group of Tyr^{49} is hydrogen bonded with the carbonyl oxygen atom of the Met^{91} residue of the opposite subunit. However, in the absence of the substrate, Tyr^{49} forms only 8 van der Waals contacts and loses some intersubunit interactions. Site-directed mutagenesis experiments of GST P1–1 show that the replacement of Tyr^{49} with Phe induces a perturbation of the homotropic GSH binding behavior (see Fig. 1, and Tables I and II). In fact, the Y49F GST P1–1 mutant exhibits negative cooperativity below 43 °C. Therefore, the mechanism for intersubunit communication still exists in the mutant, but the global effect undergoes a remarkable change. More precisely, it appears that Tyr^{49} is involved in the positive homotropic interaction which is absent in the Y49F mutant at 43 °C. Conversely, G41A, C47S, and K54A point mutations at 25 °C revert negative cooperativity induced by Tyr^{49} to Phe substitution (see Fig. 1, and Tables I and II). The positive cooperativity resulting from Gly^{41}, Cys^{47}, and Lys^{54} point mutations could be explained by the structural perturbation of helix 2 and by the resulting effects being transmitted through residue Tyr^{49} to the opposite subunit (9–11, 17, 18). In this respect, Cys^{47} acts as a hinge which limits the extent of frequency of conformational transitions involving helix 2. In its absence, helix 2 would become more flexible (21). A similar effect may be obtained by a higher temperature-enhancing motion of this flexible enzyme region.

As a whole, GST P1–1 somewhat behaves as a thermodynamic system which obeys the Le Chatelier principle. In fact, whenever a physical or chemical factor forces the G-site to assume a low-affinity conformation for substrate binding, GST P1–1 opposes this perturbation by developing positive cooperativity, thus increasing the GSH affinity for the substrate-monoligated enzyme (*e.g.* $K_1 > \alpha K_1$). This scenario is observed in the wild type GST P1–1 above 35 °C as well as in G41A, C47S, and K54A enzyme mutants at 25 °C (9–11). On the other hand, negative cooperativity occurs when the G-site is forced toward a high-affinity conformation, *e.g.* in the wild type enzyme at below 25 °C and in the Y49F GST P1–1 mutant below 43 °C.

The physiological advantage of the GSH binding homotropic modulation mechanism becomes evident when considering that GST P1–1 is present in the human skin (*e.g.* in the upper layers of epidermis) (22) and may suffer very low and high tempera-

tures. Thus, at the human skin GSH concentration (about 1–3 mM) (23), GST P1–1 displays an almost unchanged affinity for GSH in a wild range of temperatures (*e.g.* between 5 and 43 °C; see Fig. 1). This aspect is particularly relevant considering the increased skin cancerogenesis observed in mice lacking Pi class GST, and indicates the crucial role of these enzyme(s) in cancer prevention (24).

Finally, the maintenance of the enzyme-substrate affinity is a well known phenomenon for evolutionary biochemists and, in organisms living at different temperatures, is pursued by the expression of homologous enzymes able to keep unchanged substrate affinity at the average body temperature of each species (25, 26). In this respect, GST is of particular interest as the conservation of the substrate (*e.g.* GSH) affinity is recovered in the same enzyme by triggering positive and negative cooperativity, representing a case of molecular adaptation and evolution.

Acknowledgments—We thank Prof. M. Brunori, Prof. M. Coletta, and Dr. J. Z. Pedersen for helpful discussions.

REFERENCES

1. Monod, J., Wyman, J., and Changeux, J.-P. (1965) *J. Mol. Biol.* **12,** 88–118
2. Koshland, D. E., Neméthy, G., and Filmer, D. (1966) *Biochemistry* **5,** 365–385
3. Scrutton, N. S., Deonarain, M. P., Berry, A., and Perham, R. N. (1992) *Science* **258,** 1140–1143
4. First, E. A., and Fersht, A. R. (1993) *Biochemistry* **32,** 13651–13657
5. Stebbins, J. W., and Kantrowitz, E. R. (1992) *Biochemistry* **31,** 2328–2332
6. Kuo, L. C., Zambidis, I., and Caron, C. (1989) *Science* **245,** 522–524
7. Armstrong, R. N. (1994) *Adv. Enzymol. Relat. Areas Mol. Biol.* **69,** 1–44
8. Hayes, J. D., and Pulford, D. J. (1995) *Crit. Rev. Biochem. Mol. Biol.* **30,** 445–600
9. Ricci, G., Lo Bello, M., Caccuri, A. M., Pastore, A., Nuccetelli, M., Parker, M. W., and Federici, G. (1995) *J. Biol. Chem.* **270,** 1243–1248
10. Lo Bello, M., Battistoni, A., Mazzetti, A. P., Board, P. G., Muramatsu, M., Federici, G., and Ricci, G. (1995) *J. Biol. Chem.* **270,** 1249–1253
11. Lo Bello, M., Nuccetelli, M., Chiessi, E., Lahm, A., Mazzetti, A. P., Battistoni, A., Caccuri, A. M., Oakley, A. J., Parker, M. W., Tramontano, A., Federici, G., and Ricci, G. (1998) *J. Mol. Biol.* **284,** 1717–1725
12. Landt, O., Grunert, H. P., and Hahn, U. (1990) *Gene* (*Amst.*) **96,** 125–128
13. Habig, W. H., and Jakoby, W. B. (1981) *Methods Enzymol.* **77,** 398–405
14. Caccuri, A. M., Ascenzi, P., Antonini, G., Parker, M. W., Oakley, A. J., Chiessi, E., Nuccetelli, M., Battistoni, A., Bellizia, A., and Ricci, G. (1996) *J. Biol. Chem.* **271,** 16193–16198
15. Segel, I. H. (1975) *Enzyme Kinetics*, John Wiley & Sons, Inc., New York
16. Coletta, M., Ascenzi, P., Polizio, F., Smulevich, G., del Gaudio, R., Piscopo, M., and Geraci, G. (1998) *Biochemistry* **37,** 2873–2878
17. Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Lo Bello, M., Federici, G., and Parker, M. W. (1992) *J. Mol. Biol.* **227,** 214–226
18. Oakley, A. J., Lo Bello, M., Ricci, G., Federici, G., and Parker, M. W. (1998) *Biochemistry* **37,** 9912–9917
19. Taketa, K., and Pogell, B. M. (1965) *J. Biol. Chem.* **240,** 651–662
20. Braxton, B. L., Tlapak-Simmons, V. L., and Reinhart, G. D. (1994) *J. Biol. Chem.* **269,** 47–50
21. Ricci, G., Caccuri, A. M., Lo Bello, M., Rosato, N., Mei, G., Nicotra, M., Chiessi, E., Mazzetti, A. P., and Federici, G. (1996) *J. Biol. Chem.* **271,** 16187–16192
22. Shimizu, K., Toriyama, F., and Yoshida, H. (1995) *Virchows Arch.* **425,** 589–592
23. Halprin, K. M., and Ohkawaro, A. (1967) *J. Invest. Derm.* **48,** 149–152
24. Henderson, C. J., Smith, A. G., Ure, J., Brown, K., Bacon, E. J., and Wolf, C. R. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5275–5280
25. Hochachka, P. W., and Somero, G. N. (1984) *Biochemical Adaptation*, Princeton University Press, Princeton, NJ
26. Somero, G. N. (1996) *News Physiol. Sci.* **11,** 72–77

Temperature Adaptation of Glutathione *S*-Transferase P1–1: A CASE FOR HOMOTROPIC REGULATION OF SUBSTRATE BINDING

Anna Maria Caccuri, Giovanni Antonini, Paolo Ascenzi, Maria Nicotra, Marzia Nuccetelli, Anna Paola Mazzetti, Giorgio Federici, Mario Lo Bello and Giorgio Ricci

J. Biol. Chem. 1999, 274:19276-19280.
doi: 10.1074/jbc.274.27.19276