A Conserved N-capping Motif Contributes Significantly to the Stabilization and Dynamics of the C-terminal Region of Class Alpha Glutathione S-Transferases

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Helix 9, the major structural element in the C-terminal region of class Alpha glutathione transferases, forms part of the active site of these enzymes where its dynamic properties modulate both catalytic and ligandin functions. A conserved aspartic acid N-capping motif for helix 9 was identified by sequence alignments of the C-terminal regions of class Alpha glutathione S-transferases (GSTs) and an analysis by the helix-coil algorithm AGADIR. The contribution of the N-capping motif to the stability and dynamics of the region was investigated by replacing the N-cap residue Asp-209 with a glycine in human glutathione S-transferase A1-1 (hGST A1-1) and in a peptide corresponding to its C-terminal region. Far-UV circular dichroism and AGADIR analyses indicate that, in the absence of tertiary interactions, the wild-type peptide displays a low intrinsic tendency to form a helix and that this tendency is reduced significantly by the Asp-to-Gly mutation. Disruption of the N-capping motif of helix 9 in hGST A1-1 alters the conformational dynamics of the C-terminal region and, consequently, the features of the H-site to which hydrophobic substrates (e.g., 1-chloro-2,4-dinitrobenzene (CDNB)) and nonsubstrates (e.g., 8-anilino-1-naphthalene sulfonate (ANS)) bind. Isothermal calorimetric and fluorescence data for complex formation between ANS and protein suggest that the D209G-induced perturbation in the C-terminal region prevents normal ligand-induced localization of the region at the active site, resulting in a less hydrophobic and more solvent-exposed H-site. Therefore, the catalytic efficiency of the enzyme with CDNB is diminished due to a lowered affinity for the electrophilic substrate and a lower stabilization of the transition state.

As a consequence of their multifunctional capabilities, glutathione S-transferases (GSTs) contribute toward diverse cellular processes ranging from detoxification reactions to the control of gene expression. These proteins, ubiquitous in aerobes, form a superfamily of species-independent classes that, except for the Kappa GST (2), share a common fold. Typically, the canonical GSTs are soluble, dimeric proteins with each subunit possessing a thioredoxin-like domain 1 fused to an all-a-helical domain 2 (3). The Kappa GST, however, is more closely related to the protein disulfide bond isomerase, dsbA, in that domain 2 is inserted in domain 1 (2). Nevertheless, the active site of GSTs consists of two adjacent regions, a G-site on the thioredoxin-like domain that supports GSH as the thiol substrate or cofactor, and a nonpolar H-site on both domains that contributes to the binding of hydrophobic substrates. Although the molecular recognition of GSH is conserved among GSTs, these enzymes employ different strategies such as the nature of the residue (Tyr, Ser, or Cys) in contact with the thiol group of GSH and the structural contribution by domain 2 to the active site to realize their diverse functionalities (1, 4).

The class Alpha GSTs possess, like many other GST classes, an active site tyrosine residue for the activation of GSH but, unlike other GST classes, they possess an extended C-terminal region that forms an integral part of the active site (5–7). The major structural element in the C-terminal region is the amphipathic helix 9 that, although not directly involved in the chemical mechanism of catalysis, is an important determinant of substrate selectivity (8–11), the binding of substrates (12), rate-determining steps (13), desolvation of the active site (14), and the $pK_a$ of the catalytic tyrosine residue (12, 15). In addition to its contributions to catalysis, the C-terminal region is an important determinant of ligandin function, i.e., the binding of nonsubstrate ligands (16–18). Given these contributions of the C-terminal region to the functions of class Alpha GSTs, the conformational dynamics of the region and their impact on enzyme function have received much attention.

Although the C-terminal region in hGST A1-1 is highly dynamic and not observed in the crystal structure of the apo enzyme (5, 7), it is observed in the apo structure of the homologous hGST A4-4 enzyme (8). Rather than being completely disordered, the C-terminal region in apo-hGST A1-1 assumes helix-like conformations close to the surface of the protein (19), where it experiences tertiary contacts that facilitate its folding (16, 20). It is only when the enzyme binds G-site and/or H-site ligands that the C-terminal region becomes fully immobilized on the protein (5–7, 19). However, the structural determinants that maintain the conformational stability of the region in the apo and complex forms of hGST A1-1 (and other class Alpha GSTs) are not clear. Recently, we demonstrated that a bulky,
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hydrophobic residue at position 219 contributes significantly toward the stability of the region in apo and complexed hGST A1-1 (17).

The type of amino acid residue found at the N-terminal end of a helix has a major effect on the stability of the entire helix and is referred to as the N-capping residue (21). All GSTs have a conserved N-capping motif for helix 6 in domain 2 (22) shown to play an important role in the folding and stability of GSTs (23, 24). In this study, we identified a conserved aspartate N-capping motif for helix 9 in all class Alpha GSTs and, given the importance of N-capping motifs in the folding and stability of α-helices (25, 26), investigated its contribution to the dynamics of the C-terminal region of hGST A1-1.

EXPERIMENTAL PROCEDURES

Chemicals—GSH was from ICE Biomedical Inc. (Aurora, OH). TFE (>99% grade), 8-aniline-1-naphthalene sulphonate, ethylenediamine, glutathione sulphonate, p-bromobenzyl GSH, and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich. All other reagents were of analytical grade. Peptides corresponding to the wild-type and D209G forms of the C-terminal region of hGST A1-1 were synthesized by Alpha Diagnostic Inc. (San Antonio, TX), and their molecular masses were determined by analytical reverse phase HPLC. Peptides corresponding to the wild-type and D209G hGST A1-1 (subunit concentration) with 5-μl aliquots of 4.1 mM ANS. Heats of dilution, determined by titrating ANS into buffer alone, were subtracted from the total observed heats, and the corrected data analyzed by nonlinear regression using Origin5 MicroCal Inc. software.

RESULTS AND DISCUSSION

Asp-209, the N-cap Residue of Helix 9—The AGADIR algorithm, based on helix-coil transition theory that explicitly considers specific interactions occurring in helices devoid of tertiary interactions (37), was used to identify N-capping motifs for helices in the class Alpha hGST A1-1. Two such motifs were identified; one for helix 6 and the other for helix 9, both of which are conserved in class Alpha GSTs. The N-capping motif of helix 6 (22) is a class 5 Ser motif (26), which has been shown to play an important role in the folding and stability of GSTs (23, 24). The motif of helix 9 is a class 3 Asp N-capping motif (26) and is conserved in all class Alpha GSTs (Table I). The motif together with its hydrogen-bonding pattern with the main chain of helix 9 is shown in Fig. 1.

Helical Content of C-terminal Region Peptides—Far-UV CD spectra, used to determine the helical content of two peptides corresponding to the sequences of the wild-type and D209G mutant C-terminal regions (WT-pep and DG-pep, respectively) in water, are shown in Fig. 2. The spectra, displaying ellipticity minima at 222 and 201 nm and ellipticity maxima at 218 and 205 nm, are characteristic of a mixture of helical and random coil conformations. The low helical content of WT-pep and DG-pep in water (10 and 4%, respectively) was substantiated by CDNB titrations (26) and is conserved in all class Alpha GSTs (Table I). The motif together with its hydrogen-bonding pattern with the main chain of helix 9 is shown in Fig. 1.

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region (residues 210–222) of GST A1-1 (6). As expected, replacing the N-cap residue, Asp-209, with a glycine residue had little impact on the global structural features of hGST A1-1 in that its secondary and dimeric structures were unchanged (data not shown). The tertiary environment of the lone tryptophan, Trp-21, is, however, different in the mutant, as indicated by a lower fluorescence intensity, although the maximum emission wavelength was unaltered (data not shown). Trp-21 is located at the domain-domain interface of each subunit where its indole ring is surrounded by helix 1 in domain 1 and helices 6 and 8 in domain 2. Although the Asp-210 N-capping motif of helix 9 is about 18 Å away from Trp-21, it is connected to helix 8 via a 10-amino acid linker. Not only is the fluorescence of Trp-21 sensitive to changes occurring at the domain-domain interface (45), it is also sensitive to changes in the conformational dynamics or deletion of the C-terminal region of GST A1-1 allowing the region to associate with the assembled and folded dimeric protein (20). Aspartate is one of the most stabilizing residues commonly found at the N-cap position in α-helices of proteins, substituting a G1y residue at this position has been found to significantly destabilize proteins (48, 49). Therefore it is expected that the substitution of Asp-209 with a glycine would compromise the stability of helix 9. Tyr-9, the catalytic residue involved in the activation of GSH, displays an unusually low pKₐ of 8.1 (15) and is proposed to control the dynamics of the C-terminal region in GST A1-1, in that the region becomes more dynamic when Tyr-9 becomes ionized (50, 51). This is unlikely a reason for the conformational change in the C-terminal region of D209G GST A1-1 at pH 6.5 because the pKₐ of Try-9 is not perturbed by the mutation (pKₐ is 8.2 for both wild-type and mutant; data not shown).

**GSH Binding and Steady-state Kinetics**—The binding affinity of GSH, determined by tryptophan fluorescence quenching, is unaltered by the D209G mutation, because the Kₛ values for the wild-type and D209G proteins are similar; 0.16 ± 0.01 mM and 0.18 ± 0.01 mM, respectively. This is consistent with the binding of GSH not being significantly influenced by the C-terminal region (12, 16, 17, 52, 53).

Although the C-terminal region does not contribute directly to the chemical mechanism of catalysis, it does form an integral part of the active site, the H-site specifically. Consequently, the catalytic function of GST A1-1 is highly sensitive to perturbations in the conformation and dynamics of the C-terminal region (12, 13, 16, 17, 53). The Kᵣ values of GSH (0.21 mM for wild-type and 0.29 mM for D209G) and CDNB (0.31 mM for D209G) were very similar for the wild-type and mutant. However, an interesting result was obtained with CDNB. The Michaelis constant (Kₘ) for CDNB was 3.3 times lower for wild-type GST A1-1 (0.46 mM) than for the D209G mutant (1.5 mM) which suggests that the C-terminal region may have a role in substrate binding.

**Characteristics of D209G hGST A1-1—Helix 9** (residues 210–220) is the major structural feature in the C-terminal region.
The difference in the free energy change for the formation of the transition states in the mutant and wild-type enzymes ($\Delta \Delta G$), as calculated from Equation 3 (55), is 0.4 kJ mol$^{-1}$ at 20 °C, indicative of a lower stabilization of the transition state on disruption of the conserved N-capping motif in helix 9. The C-terminal region of GST A1-1 plays an important role in guiding the substrates into the transition state (13). The diminished stabilization effect observed for the D209G mutant is, however, not as pronounced as that observed when Met-208, the hydrophobic staple motif at the N terminus of helix 9, is altered (53). It would appear that the reduction in catalytic efficiency is not due to an increase in $K_m^{CDNB}$ but rather a decrease in $k_{cat}^{CDNB}$ (not determined). A decrease in $k_{cat}^{CDNB}$, in turn, would not be due to a decreased rate of product release, because mobility-enhancing perturbations in the C-terminal region generally increase the rate of product release (12, 51, 53). Rather, it is most likely that the affinity of the H-site for CDNB is reduced, which is consistent with the link between productive binding of CDNB and the dynamics of the C-terminal region (9). According to the structure of the complex between hGST A1-1 and S-benzylglutathione (1GUH (6)), the benzene ring of CDNB should be within van der Waals distance of Met-208, Phe-220, and Phe-222 at the H-site. The latter two residues are located in the C-terminal region and changes in their positioning at the H-site would compromise the binding of CDNB (17) and the stabilization of the transition state (13). Both phenyl rings of Phe-220 and Phe-222 contribute to the hydrophobic environment of the H-site (6), whereas the phenyl ring of Phe-222 also reduces the exposure of the site to bulk solvent (17). The calorimetric and fluorescence data presented below suggest that the D209G-induced perturbation in the C-terminal region reduces the hydrophobicity of the active site and increases its accessibility to solvent.

**ANS as a Probe of the Active Site**—Although there are no experimental structures available of an ANS-GST complex, molecular docking and ligand-displacement studies indicate the anionic dye to bind the H-site of hGST A1-1. Both Cerius$^2$ (Accelrys Inc.) and LIGP (34) docking software predict ANS to bind the H-site albeit in different binding modes in the absence and presence of GSH, as shown in Fig. 3. Ethacrynic acid, another H-site ligand, has also been shown to bind hGST A1-1 in different modes (5). It should be noted that the docking experiments were done on the assumption that the conformation of the C-terminal region of the protein in the hGST A1-1-ANS complex is the same as that observed in other ligand complexes of the protein. The assumption is supported by the finding that ANS binding, like that for ethacrynic acid, induces the localization of the C-terminal region (see below).

The hydrophobic anilino and naphthyl rings of ANS occupy the nonpolar H-site and are within van der Waals distance of Leu-107, Leu-108, Val-111, Met-208, Leu-213, Ala-216, Phe-222, and Phe-222, whereas the negatively charged sulfonate group is located at the interface between the G-site and H-site and is within van der Waals distance of Tyr-9, Phe-10, Arg-15, and Val-55. These amino acid residues are also found within van der Waals distance of various other ligands bound to the H-site of hGST A1-1 (5–7). The predominantly nonpolar character of the binding site for the aromatic moieties of ANS is consistent with those of other ANS-binding proteins (57–59). Of particular note is that no binding site for ANS could be detected when $\text{GSO}_3^-$ was present at the active site. Although $\text{GSO}_3^-$ binds the G-site in the same way that GSH does, its negatively charged sulfonate group occupies a similar position to that of ANS bound to the H-site. ANS is strongly displaced by ligands that bind the H-site, as shown in Fig. 4, consistent with the H-site being the binding site of ANS in both wild-type and D209G hGST A1-1. The displacement of ANS by $\text{GSO}_3^-$ is explained by their competitive binding due to overlapping bind-
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by BSP, however, is the result of BSP binding to its low affinity site, namely the H-site (69).

Recently, we demonstrated that the energetics of ANS binding can be used to report on changes at the active site of hGST A1-1, including the C-terminal region of the protein, because it forms an integral part of the H-site (17, 70). The calorimetric titration isotherm for the binding of ANS to D209G hGST A1-1, shown in Fig. 5, indicates that complex formation is exothermic.

The binding curve fits well to a model describing one binding site per subunit with a stoichiometry of one ANS molecule per site consistent with that reported for the wild-type protein (18). The affinity of hGST A1-1 for ANS is not significantly affected due to enthalpy-entropy compensation (71), a process characteristic of the weak interactions found between proteins and their ligands (72). This compensatory effect is also observed between wild-type and mutant complex formation. Although their ΔG values are very similar, their ΔHobs and ΔSobs vary greatly. The linear temperature dependence of enthalpy (Fig. 6) indicates that the heat capacity change of D209G-ANS complex formation (ΔCp = 0.3 kJ mol⁻¹ K⁻¹) is not coupled to other structural equilibria with significant enthalpies.

Given the amphipathic nature of ANS, its interaction with proteins cannot be strictly hydrophobic, as often assumed, but will involve van der Waals and electrostatic forces. The importance of van der Waals interactions has been demonstrated for the enthalpically driven formation of the wild-type complex (18), consistent with the putative van der Waals contacts between the aromatic ring systems of ANS and the H-site (Fig. 3), and for the formation of other protein-ANS complexes (57, 73). The role of electrostatic interactions in ANS binding to hGST

![Fig. 3. Molecular docking between ANS and apo-hGST A1-1 (A) and the GSH-hGST A1-1 complex (B). ANS binding sites in hGST A1-1 predicted using the molecular docking program Cerius² is shown in white, and that predicted with LIGIN is shown in black. Hydrogen bonds are depicted as dotted lines. In the absence of GSH (A), the sulfonate group of ANS forms hydrogen bonds with the main chain CO group of Val-55 and the side chain OH group of Tyr-9. In the presence of GSH (B), the sulfonate group of ANS hydrogen bonds with the side-chain NE group of Arg-15 and the carbonyl O of the γ-glutamyl moiety of GSH.](http://www.jbc.org/)

![Fig. 4. Displacement of ANS bound to D209G (open triangles) and wild-type (closed circles) hGST A1-1 by various active site ligands. Samples, consisting of 100 μM ANS and 1 μM protein, were titrated with active site ligands and the fluorescence of bound ANS followed at 475 nm (excitation at 390 nm).](http://www.jbc.org/)
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A1-1, however, was previously not clear. Considering the close proximity (within 4 Å) of the negatively charged sulfonate group in ANS to the positively charged guanidinium group of Arg-15 at the interface between the H-site and G-site (see Fig. 3), electrostatic interactions between these moieties could very well be important contributors to complex formation (74). This would certainly apply to addressing the energetically unfavorable desolvation of the highly solvated, negatively charged sulfonate group of free ANS that becomes buried in a predominantly nonpolar environment. Electrostatic interactions between the sulfonate group of ANS and Arg-15 would, at least in part, compensate for this unfavorable energy of desolvation by their large exothermic contributions to enthalpy (74). Furthermore, compared with that of bulk solvent, the lower dielectric environment of the ANS binding site, a consequence of the nonpolar site and anilinonaphthalene moiety of ANS, would enhance the strength of these electrostatic interactions. Conversely, the increased solvent exposure of ANS bound to the D209G mutant (see below) would attenuate the exothermic contribution to enthalpy from the ANS-Arg-15 electrostatic effect resulting in a less favorable ΔH_{abs}. Arg-15 is conserved at the active site of all class Alpha GSTs, providing electrostatic stabilization of the thiolate anion of glutathione (15). The presence of electrostatic interactions between the sulfonate group of ANS and the guanidinium group of an arginine residue is also demonstrated in the structures of other protein:ANS complexes (57–59, 75), supporting the important role that the ion pair plays in determining binding affinity. Because most native proteins do not bind ANS, or at least appear not to because the many ANS molecules bound to surface cationic residues of proteins by electrostatic forces only do not necessarily fluoresce and, thus, go undetected by fluorescence techniques (74), binding specificity appears to be determined largely by the anilinonaphthalene moiety of ANS. Each of the ANS-binding proteins mentioned here possesses a specific nonpolar site for ANS, and binding of the anion occurs with a stoichiometry of one molecule per monomer of protein.

The positive TΔS of the D209G mutant reflects a net favorable entropy change of solvation in that restrained water molecules solvating, at least, the nonpolar surface of the aromatic ring systems and negatively charged sulfonate group of free ANS are released to bulk solvent upon complex formation (76). The TΔS term will, however, be reduced by a smaller, unfavorable change in conformational entropy due to a loss of degrees of freedom at the interface between ANS and protein (18). Free ANS is relatively constrained, because only two angles, one a dihedral angle and the other a torsion angle, define its conformation (57). The positive enthalpy associated with desolvation events will attenuate the favorable enthalpy of binding. Only at temperatures below 17 °C does entropy contribute favorably toward ANS binding to wild-type hGST A1-1 (18), implying that at higher temperatures, desolvation of the interacting surfaces is inadequate to compensate for losses in conformational entropy. These losses will include those associated with ANS and the protein groups involved in binding, including the C-terminal region. Although the binding of another H-site ligand, ethacrynic acid, induces localization of the C-terminal region of the protein (5), the situation with respect to the binding of ANS is not clear. Nevertheless, given that the putative interactions that ANS undertakes with the region (Fig. 3) are similar to those involved in binding ethacrynic acid (5), it is feasible that complex formation with ANS also induces the localization of the C-terminal region in the wild-type protein, thus contributing unfavorably to entropy. Furthermore, localization of the C-terminal region would contribute favorably to enthalpy due to the additional interactions at the interfaces between the region, protein, and ANS. On the other hand, the positive entropy of ANS-D209G complex formation suggests that ANS binding to the mutant does not induce localization of the region resulting in a less favorable enthalpy. Further, ANS bound to the mutant is also more exposed to solvent compared with the wild-type complex (see below). Complex formation between ANS and two other ANS-binding proteins, ALBP and IFABP, display TΔS values of 1.62 kJ·mol⁻¹ at 25 °C (57) and −7.5 kJ·mol⁻¹ at 22 °C (73), respectively. The favorable entropy of the ANS-ALBP complex is likely due to the release of ordered water molecules from the ANS binding site, because binding does not induce major changes in the conformation of the protein. On the other hand, the unfavorable entropy of the ANS-IFABP appears to result from the ordering of ANS (73) and a water molecule at the binding cavity (77). The waters displaced by ANS at this site are short-lived waters that are not highly ordered. Ligand binding also does not significantly affect the structure of IFABP (78).

The heat capacity change of complex formation with the
D209G mutant is positive (0.3 kJ·mol⁻¹·K⁻¹), whereas it is negative (−0.84 kJ·mol⁻¹·K⁻¹) for the wild-type protein (18), indicative of significant differences in the type and amount of solvent-exposed surface areas buried on binding. Because the change in heat capacity originates primarily from the changes in the hydration of nonpolar and polar molecular surfaces areas (79), the positive change observed for the mutant suggests the burial of a smaller nonpolar surface area and the burial of a larger polar surface area on complex formation (80). Because electrostatic interactions generate positive heat capacity changes (76, 81), the interactions between the negatively charged sulfonate group of Arg-15 at the H-site offers an explanation for the positive ΔCₚ values of ANS complex formation with the D209G mutant (this study) and the ΔPhe-222 mutant (18). Electrostatic effects, therefore, play a larger role in ANS binding to the D209G mutant than in binding to the wild-type protein in which a localized C-terminal region is an important contributor to the burial of nonpolar surfaces and the formation of van der Waals contacts. The affinity displayed by ANS toward wild-type and D209G hGST A1-1 is similar. However, the D209G mutation renders the formation of the ANS·hGST A1-1 complex enthalpically less favorable by 29.2 kJ·mol⁻¹ and, due to the compensatory effect, entropically more favorable by 29.8 kJ·mol⁻¹ at 25 °C. This is consistent with fewer van der Waals interactions and higher conformational entropy in the mutant complex as a result of a more dynamic C-terminal region.

Like that for wild-type hGST A1-1, the binding of ANS to I-FABP (73) and A-LABP (57) is also associated with similar, complex as a result of a more dynamic C-terminal region.  

In light of the thermodynamic data and the low helix propensity of the sequence corresponding to the C-terminal region, it is not unreasonable to assume that disruption of the stabilizing N-capping motif of helix 9 increases the mobility of the C-terminal region preventing it to become properly localized on binding of ANS. This is supported by ANS fluorescence data. ANS experiences a blue shift in its maximum emission wavelength from 545 to 474 nm on complex formation, demonstrating that the ANS binding site is nonpolar. However, because water quenches ANS fluorescence (57, 73), a 30% reduction in the quantum yield of ANS bound to the D209G mutant (data not shown) indicates that the dye is more exposed to solvent than when the dye is bound to wild-type hGST A1-1. The quantum yield of ANS is not affected by the conformation of its bound form (57). ANS complexes with the ΔPhe-222 and I219A mutants of hGST A1-1 also displayed lower quantum yields of ANS fluorescence (17, 18). These studies demonstrated the important role of the phenyl side chain of Phe-222 in reducing the solvent accessibility of the active site to solvent. In fact, a crystal structure of the I219A mutant indicates that the side chain of Phe-222 no longer occupies a position over the H-site, thus exposing it to solvent.

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REFERENCES

1. Sheehan, D., Meade, G., Foley, V.M., and Dowd, C.A. (2001) Biochem. J. 360, 11–16
2. Ladner, J.E., Parsons, J.F., Rife, C.L., Gilliland, G.L., and Armstrong, R.N. (2004) Biochemistry 43, 352–361
3. Derr, H., Reineimer, P., and Huber, R. (1984) Eur. J. Biochem. 220, 645–681
4. Armstrong, R.N. (1997) Chem. Res. Toxicol. 10, 2–18
5. Cameron, A.D., Sinning, I., L’Hermitte, O., Glin, O., Birk, P.G., Mannervik, B., and Jones, T.A. (1995) Structure 3, 717–727
6. Sinning, I., Klaywget, G.J., Cowan, S.W., Reineimer, P., Dirr, H.W., Huber, R., Gilliland, G.L., Armstrong, R.N., Ji, X., Board, P.G., Olin, B., Mannervik, B., and Jones, T.A. (1993) J. Mol. Biol. 232, 192–212
7. Le, T., I, Stenkamp, R.E., Ibarra, C., Atkins, W.M., and Adman, E.T. (2002) Proteins 48, 616–627
8. Bruns, C.M., Hubatsch, I., Ridderset, M., Mannervik, B., and Tainer, J.A. (1999) J. Mol. Biol. 289, 427–439
9. Allender, C.S., McDonagh, P.D., Liu, L.Y., Wolf, C.R., and Roberts, G.C. (1999) Biochem. J. 343, 525–531
10. Nilsson, L.O., Gustafsson, A., and Mannervik, B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9408–9412
11. Pal, A., Gu, Y., Pan, S.S., Ji, X., and Singh, S.V. (2001) Biochemistry 40, 7047–7053
12. Gustafsson, A., Ekhadi, M., Jemth, P., and Mannervik, B. (1999) Biochemistry 38, 16268–16275
13. Nilsson, L.O., Edelal, M., Pettersson, P.L., and Mannervik, B. (2002) Biochem. Biophys. Acta 1597, 157–163
14. Nieslanik, B.S., Ibarra, C., and Atkins, W.M. (2001) Biochemistry 40, 3536–3543
15. Bjornestedt, R., Stenberg, G., Widersten, M., Board, P.G., Sinning, I., Jones, T.A., and Mannervik, B. (1995) J. Mol. Biol. 247, 765–773
16. Derr, H.W., and Wallace, L.A. (1999) Biochemistry 38, 15631–15640
17. Mosebi, S., Sayed, Y., Burke, J., and Derr, H.W. (2003) Biochemistry 42, 15326–15332
18. Sayed, Y., Hornby, J.A., Lopez, M., and Derr, H.W. (2002) Biochem. J. 363, 341–346
19. Zhan, Y., and Rule, G.S. (2004) Biochemistry 43, 7242–7254
20. Wallace, L.A., and Derr, H.W. (1999) Biochemistry 38, 16688–16694
21. Serrano, L., and Fersht, A.R. (1989) Nature 340, 296–299
22. Aceto, A., Dragan, B., Melino, S., Allocati, N., Masulli, M., Di Ilio, C., and Aceto, A. (2001) J. Biol. Chem. 276, 32177–32183
23. Dragan, B., Stenberg, G., Melino, S., Petruzzelli, R., Mannervik, B., and Aceto, A. (1997) J. Mol. Biol. 265, 239–234
24. Cocco, R., Stenberg, G., Dragan, B., Rossi, P.D., Paludi, D., Mannervik, B., and Aceto, A. (1997) J. Mol. Biol. 265, 239–234
25. Schultz, J. M., and Baldwin, R. L. (1992) Annu. Rev. Biochem. 61, 95–118
26. Wan, W., and Milner-White, E. J. (1999) J. Mol. Biol. 290, 1633–1649
27. Chakrabarty, A., Kortemeyer, T., Padmanabhan, S., and Baldwin, R.L. (1993) Biochemistry 32, 5550–5565
28. Brandts, J. F., and Kaplan, L. J. (1973) Biochemistry 12, 2011–2024
29. Stenberg, G., Bjornestedt, R., and Mannervik, B. (1992) Protein Expr. Purif. 3, 80–84
30. Roh, C. A., Chakrabarty, A., and Baldwin, R. L. (1996) Protein Sci. 5, 2625–2637
31. Sluijs-Cramer, N., Naidoo, N. N., Kaplan, W. H., Manoharan, T. H., Fahd, W. E.,…
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