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Variation in Strength of an Unconventional CH...O Hydrogen Bond in an Engineered Protein Cavity

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ARL-TR-1826

October 1998

19981020 031

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Abstract

We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of CH...O interactions between the ligand and protein. Our study shows that the strength of CH...O interactions can be modulated by over 1 kcal/mol by changes in the C-H bond polarity. Consequently, several such interactions may play a significant role in the stability of macromolecular structures.

Acknowledgments

The authors acknowledge C. L. Brooks III, C. D. Stout, M. R. Ghadiri, J. Rebek, Jr., D. A. Case, Y. Cao, S. K. Wilcox, P. Beroza, and B. N. Dominy for their helpful discussions and critical reading of this report. Support for this work was provided by grants GM-41049 (to D. B. Goodin), GM-1744 (to R. A. Musah), and GM-48495 (to D. E. McRee) from the National Institutes of Health (NIH). S. W. Bunte acknowledges financial support from the U.S. Army Research Laboratory (ARL) Director Research Initiative Program. Support was provided by a grant from the Department of Defense (DOD) High-Performance Computing (HPC) Center, ARL, for time on the Silicon Graphics Inc. (SGI) Power Challenge Array (PCA).

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Table of Contents

	<u>Page</u>
Acknowledgments	iii
List of Figures	vii
List of Tables	vii
1. Introduction	1
2. Background	1
3. Conclusion	6
4. References	7
Distribution List	9
Report Documentation Page	11

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List of Figures

<u>Figure</u>		<u>Page</u>
1.	Electron Density for (A) 234TMT and (B) 345TMT Binding to the W191G Cavity	3
2.	Electrostatic Potential Surfaces for (A) 234TMT and (B) 345TMT	4

List of Tables

<u>Table</u>		<u>Page</u>
1.	Thermodynamic Parameters for Trimethylthiazole Binding to the Buried Cavity of W191G	5
2.	Calculated Electrostatic Contribution to the Relative Binding Energy of 234TMT and 345TMT to the W191G Cavity	5

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1. Introduction

While hydrogen bonds of the type $\text{XH}\cdots\text{Y}$ ($\text{X}, \text{Y} = \text{N}$ and/or O) are essentially found in all macromolecular structures, the role of $\text{CH}\cdots\text{O}$ hydrogen bonds in such systems is uncertain. Recent reports [1–5] have suggested that a significant number of the $\text{CH}\cdots\text{O}$ contacts observed in proteins, RNA, and carbohydrates represent cohesive interactions, which may, in sufficient numbers, be significant to structure, stability, and function. The existence of short intermolecular $\text{CH}\cdots\text{O}$ interactions is well-established in many small-molecule crystals [6–9]. In cases where the $\text{C-H}\cdots\text{O}$ angle is approximately linear and the $\text{C}\cdots\text{O}$ bond distance is less than the combined van der Waals radii (3.3 Å), these interactions have been labeled as hydrogen bonding. Theoretical calculations have estimated the $\text{CH}\cdots\text{O}$ bonding interactions to be worth 1–2 kcal/mol [2, 10, 11]. However, many computational approaches utilizing force-field parameterizations do not explicitly consider such interactions as attractive, and $\text{CH}\cdots\text{O}$ hydrogen bonds are not generally considered in the analysis of structures. The strength of such interactions should vary considerably depending on the C-H bond polarity and may thus be different for the $\text{C}_\alpha\text{H}\cdots\text{O}$ interactions observed in some protein β -sheets than those for others involving the more acidic $\text{C}_\epsilon\text{H}$ protons of histidine [1]. While there is a correlation of the $\text{C}\cdots\text{O}$ distance with the C-H bond acidity for a number of organic compounds [6, 12–14], nothing is known experimentally about the strength of these interactions and how this strength varies with the polarity of the proton donor.

2. Background

We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of $\text{CH}\cdots\text{O}$ interactions between ligand and protein. The cavity created by the W191G mutation of cytochrome *c* peroxidase (CCP) has been shown to bind a number of cationic heterocyclic compounds [15–17]. Two such compounds, 2,3,4-trimethylthiazole (234TMT) and 3,4,5-trimethylthiazole (345TMT) bind to the

W191G cavity in essentially isosteric conformations (Figure 1).^{*} Crystal structures of the protein soaked in solutions containing these compounds show clear evidence for binding in the omit electron density maps. No significant differences were observed in the structure of the protein between the ligand-free and either of the ligand-bound states. Placement of a ligand model within the omit density implies a close contact between the C5 ring proton of 234TMT and one carboxylate oxygen of Asp-235. An analogous interaction involving the C2 ring proton is observed for 345TMT. Asp-235 is observed to hydrogen-bond to other cavity-binding ligands and helps determine the cation bonding specificity of this cavity [15, 17, 18]. Thus, the absence of standard hydrogen-bond donors in 234TMT and 345TMT evidently results in the substitution of weaker alternative interactions that fulfill a similar role.

The geometries of the interactions implied by the crystal structures are consistent with a CH...O hydrogen bond [8]. The C...O distances between the ligands and the Asp-235 carboxylate oxygen were 2.95 and 2.85 Å for 234TMT and 345TMT, respectively, shorter than the combined van der Waals radii of C and O (3.3 Å). The C-H-O angles estimated from placing the geometrically optimized ligands[†] into the electron density were approximately 150° and 140° for 234TMT and 345TMT, respectively (Figure 2). The deviations of these angles from an ideal linear CH...O hydrogen bond are not outside the range (130–170°) of those observed in organic crystals [8]. Although the two compounds differ structurally only by the interchange of the N3 and C4 atoms in the thiazole ring, this difference serves to increase the acidity of the C2 proton in 345TMT

^{*} Crystal structures of W191G soaked in 50-mM 234TMT or 345TMT were determined as previously described [17]. For each ligand, the orientation was unambiguous due to density observed for the methyl substituents and for the sulfur atom at the higher contour level. The maps were constructed by merging F_{obsd} for the soaked crystal with F_{calcd} from models of the W191G empty cavity. The structures of 234TMT and 345TMT bound to W191G have been submitted to the Brookhaven database (entries 1ac4 and 1ac8, respectively). Diffraction statistics for 234TMT and 345TMT, respectively, included unit cell parameters (103.76, 73.31, 44.64 Å and 106.03, 75.89, 51.13 Å), resolution (2.0 and 2.1 Å), $I/\sigma_{(av)}$ (18.2 and 12.4), $I/\sigma_{(last\ shell)}$ (2.00 and 1.76), number of reflections (18,219 and 19,056), percent completeness (80 and 87%), and R_{sym} (0.048 and 0.062).

[†] Ligand partial charges were calculated as Kollman electrostatic potential (ESP) charges from geometry-optimized structures using density functional theory. The calculations were performed *in vacuo* as previously described [19] using the program Gaussian 94, Becke3LYP functional, and 6-31G* basis set. ESP partial charges were used to generate an ESP grid with the program Delphi (MSI) using a uniform dielectric constant of 1, ionic strength of 0.0, and full Coulombic boundary conditions. This potential grid was mapped onto the solvent-accessible surface of the molecule calculated with a 1.4-Å probe radius as a color spectrum from +135 kT/e (red) to +200 kT/e (blue).

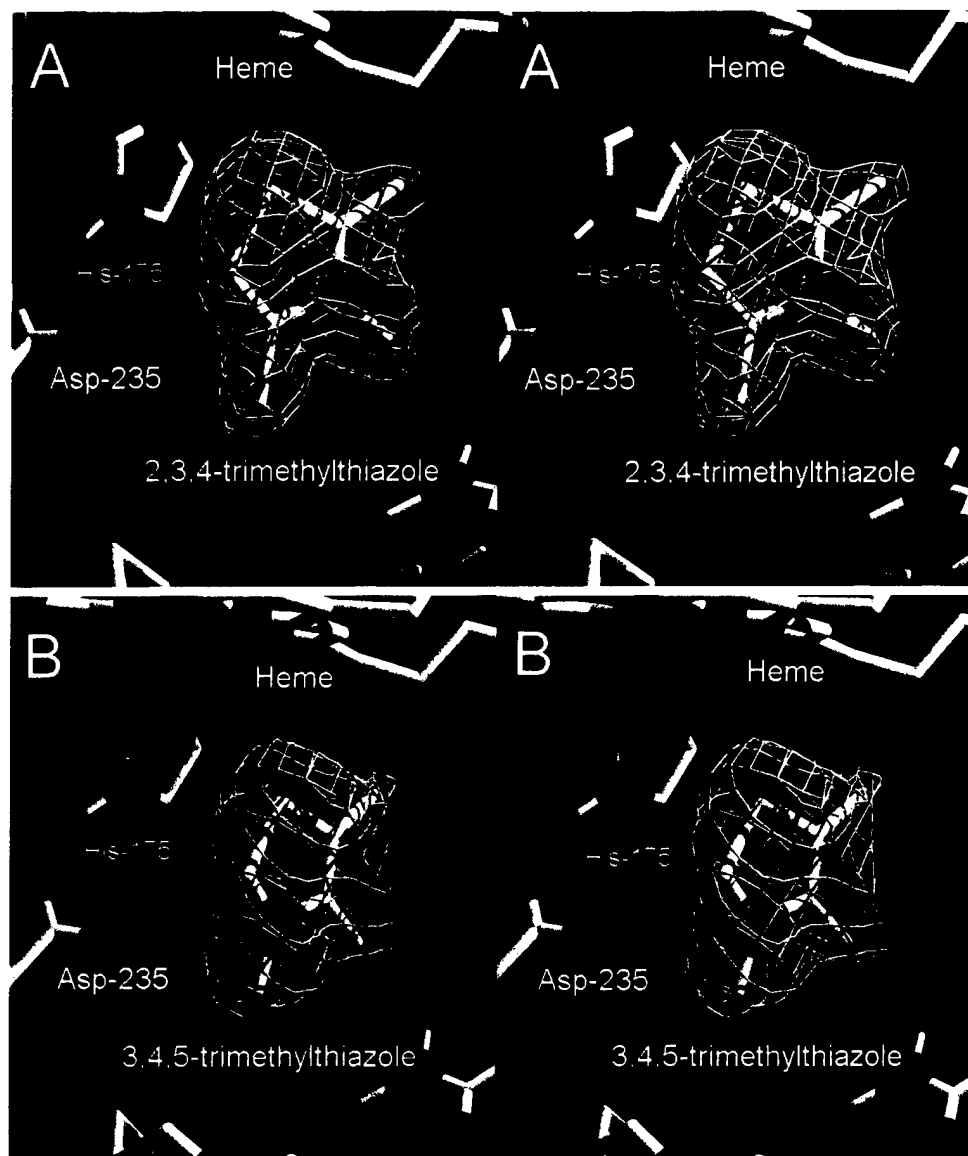


Figure 1. Electron Density for (A) 234TMT and (B) 345TMT Binding to the W191G Cavity. Shown Are Stereoviews of $F_o - F_c$ Fourier Omit Maps Contoured at $+4 \sigma$ (White) and $+9 \sigma$ (Red) Superimposed on a Model of the Ligand That Was Placed Into the Omit Density. (See Footnote [*] on p. 2.)

($pK_a \approx 17-19$) [20, 21] relative to the C5 proton in 234TMT ($pK_a \approx 25-30$). This difference in acidities is manifested in the electrostatic potential calculated for the two compounds and mapped onto the solvent-accessible surface (Figure 2). Since the $CH \cdots O$ hydrogen bond is primarily

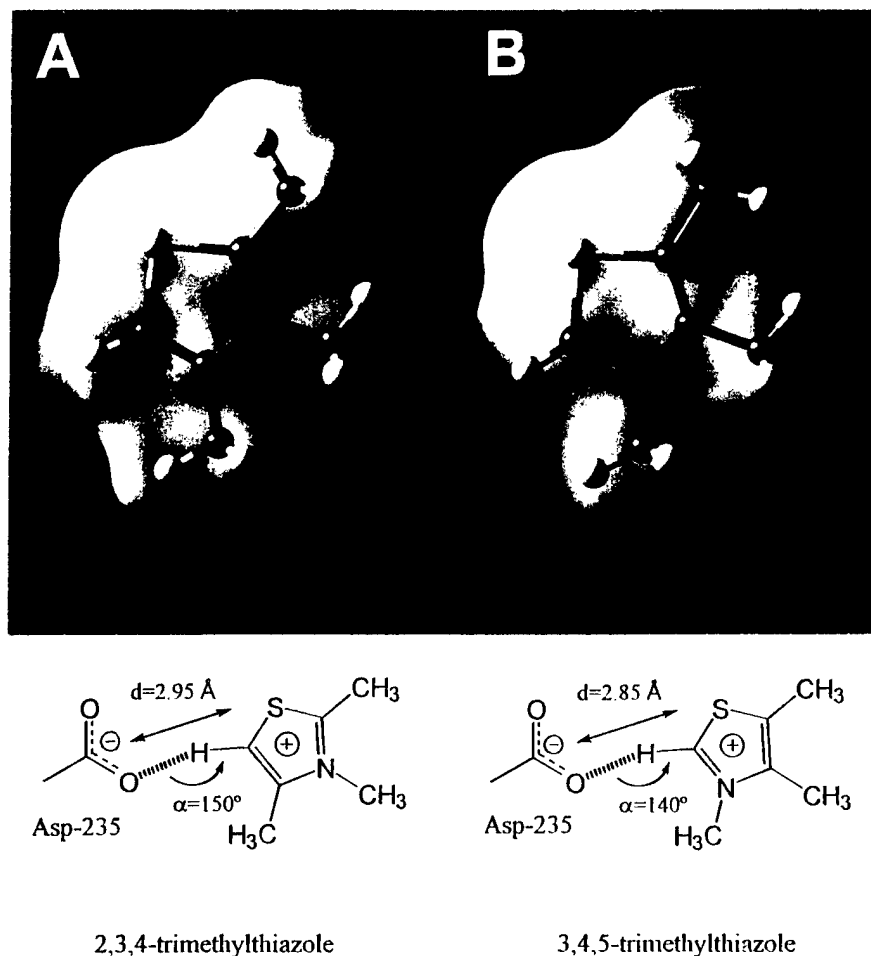


Figure 2. Electrostatic Potential Surfaces for (A) 234TMT and (B) 345TMT. The Color Map Illustrates the Localized Differences Resulting From the Increased Polarity of the C2–H Proton for 345TMT Relative to the C5–H Bond of 234TMT. (See Footnote [†] on p. 2.) At the Bottom Are the Geometries of the Interactions With Asp-235 That Are Inferred From the Placement of the Geometry-Optimized Ligand Into the Omit Electron Density.

electrostatic in nature, the calculated potentials indicate that 345TMT should form a significantly stronger interaction with Asp-235.

Thermodynamic parameters of 234TMT and 345TMT binding to W191G were determined by isothermal titration calorimetry (Table 1). The results show a five-fold decrease in K_d for 345TMT relative to 234TMT, corresponding to 1.2 kcal/mol of additional binding energy for 345TMT. Due

Table 1. Thermodynamic Parameters for Trimethylthiazole Binding to the Buried Cavity of W191G*

	K_d (mM)	ΔH (kcal mol ⁻¹)	ΔS (cal mol ⁻¹ K ⁻¹)	ΔG (kcal mol ⁻¹)
234TMT	1.50 (0.16)	-14 (0.6)	-33 (2)	-3.9 (0.1)
345TMT	0.20 (0.03)	-15 (0.7)	-34 (3)	-5.1 (0.2)

to the absence of structural changes in the protein, the similar contacts made with the protein, and the fact that both compounds desolvate the cavity to the same degree, the observed difference in the free energy of binding for the two compounds should result from differences in the electrostatic interactions between ligand and protein and from differences in their desolvation energies. However, estimates of ligand desolvation energies were very similar (Table 2), indicating that the observed difference in binding free energy for the two compounds can be attributed to protein-ligand interactions.

Table 2. Calculated Electrostatic Contribution to the Relative Binding Energy of 234TMT and 345TMT to the W191G Cavity*

	$-\Delta V_{Q_H}$	$-\Delta V_{Q_\alpha}$	$-\Delta V_{Q_L}$	$-\Delta V_B$	$-\Delta V_{tot}$	$-\Delta G_{solv(water)}$
234TMT	-56.88	3.82	-6.44	-7.26	-66.76	-26.112
345TMT	-57.83	2.98	-5.26	-7.13	-67.24	-26.096
$\Delta\Delta G_{345TMT-234TMT}$	-0.95	-0.84	1.18	0.13	-0.48	-0.016

NOTE: Values are given in kcal/mol.

The electrostatic contribution to the ligand-protein interaction was estimated for the two ligands bound to the protein cavity. Partial charges were calculated for each ligand (Figure 2) to account for

* Dissociation constants and enthalpies were measured at 25° C in 100 mM Bis-Tris propane pH 4.5 by isothermal titration calorimetry (Microcal MC2 ITC calorimeter). W191G (0.2–0.4 mM) was titrated with 5- μ L injections of ligand (2–8 mM) equilibrated in the same buffer. Error estimates for K_d and ΔH are given as the standard deviation of multiple determinations, and those for ΔS and ΔG were obtained by propagation.

the different C–H bond polarities, and the protein dipoles/Langevin dipoles (PDL) method [19, 22]* was used to account for effects of microscopic atomic polarizabilities of atoms surrounding the ligands. The more favorable electrostatic interaction of 345TMT with the protein relative to that of 234TMT (Table 2) arises from both dipole (ΔV_{Qp}) and induced dipole (ΔV_{Qa}) terms. These interactions are partially canceled by the solvent screening effects represented in the Langevin grid (ΔV_{QL}) and Born (ΔV_B) terms. The net electrostatic contribution to the binding free energy (ΔV_{tot}) is approximately 0.5 kcal/mol of additional stabilization of 345TMT relative to 234TMT. This value is in good agreement with recent *ab initio* quantum-mechanical analysis of CH...O bond energies [2]. Thus, electrostatics alone form a significant contribution to the observed difference in the binding free energy of 1.2 kcal/mol.

3. Conclusion

In conclusion, the observation of untethered ligand binding to this engineered cavity shows that the CH...O interaction is made by choice and is thus a stabilizing interaction. While it is weak, summation of several such interactions may play a significant role in the stability of macromolecular structures. The strength of this interaction can be modulated by over 1 kcal/mol by changes in the C–H bond polarity. Thus, selective inclusion of polar CH...O interactions in refinement algorithms for nuclear magnetic resonance (NMR) and crystal structures may be justified.

* Electrostatic calculations were performed using the PDL method with the program POLARIS [22]. ΔV_{tot} is the sum of four terms, ΔV_{Qp} , ΔV_{Qa} , ΔV_{QL} , and ΔV_B . ΔV_{Qp} is the classical electrostatic interaction of the ligand charges with all of the charges on all protein atoms. ΔV_{Qa} is the interaction energy from the dipole moments induced by the electric field as a result of atomic polarizabilities. ΔV_{QL} is the energy of interaction of the ligand with the field defined by the solvent dipoles. ΔV_B is the Born energy of interaction with bulk solvent beyond the radius used to define the Langevin dipole grid. Values of ΔV have the sign of electrostatic potential, and, thus, a more positive value for ΔV corresponds to a more negative ΔG . POLARIS calculations were performed with Asp-235 and Arg-48 charged, and all other amino acid residues neutral. An estimate of the electrostatic interaction of the ligand charges with the solvent reaction field ($\Delta G_{solv(water)}$) for the two compounds was obtained with a continuum dielectric model (Delphi, MSI) using $\epsilon_{water} = 80$ and $\epsilon_{internal} = 2$, with a grid spacing of 0.25 Å.

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1998	3. REPORT TYPE AND DATES COVERED Final, Jan 96 - Jan 97	
4. TITLE AND SUBTITLE Variation in Strength of an Unconventional CH...O Hydrogen Bond in an Engineered Protein Cavity			5. FUNDING NUMBERS 1L161102AH43	
6. AUTHOR(S) Rabi A. Musah,* Gerard M. Jensen,* Robin J. Rosenfeld,* Duncan E. McRee,* David B. Goodin,* and Steven W. Bunte				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Army Research Laboratory ATTN: AMSRL-WM-BD Aberdeen Proving Ground, MD 21005-5066			8. PERFORMING ORGANIZATION REPORT NUMBER ARL-TR-1826	
9. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES *Department of Molecular Biology, MB8, The Scripps Reseach Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of CH...O interactions between the ligand and protein. Our study shows that the strength of CH...O interactions can be modulated by over 1 kcal/mol by changes in the C-H band polarity. Consequently, several such interactions may play a significant role in the stability of macromolecular structures.				
14. SUBJECT TERMS hydrogen bond, ab initio, calculations, electrostatic modeling			15. NUMBER OF PAGES 15	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

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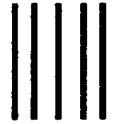
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