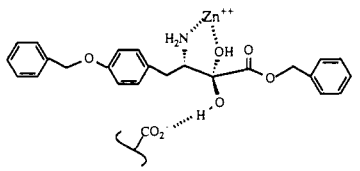
Scheme III. Proposed Inhibitor-Enzyme Complex



matory agents. LTA_4 and its analogs (e.g., LTA_3 and LTA_5)[6] are irreversible inhibitors, and some inhibitors of other Zn^{2+}-containing amino peptidases and angiotensin-converting enzymes are also reversible inhibitors of LTA_4 hydrolase,[7] supporting the proposed mechanism.

Our previous studies on the inhibition of this enzyme with more than 10 peptide-based, synthetic transition-state analog inhibitors (including α-hydroxy β-amino acids and their peptide derivatives, fluoro ketone and phosphoramidate derivatives)[4] have led us to develop another class of compounds (see Table I, **6-8**) which have proven to be better inhibitors than those simply based on the amidase activity. These inhibitors contain a transition-state mimic of the enzyme-catalyzed amide cleavage as a "core" and additional complementarity components (the aromatic moieties) which resemble the hydrophobic nature of the conjugated polyene system of the natural substrate LTA_4, which binds to the enzyme more tightly than the amide substrates.[4] We chose α-keto esters instead of α-keto amides[8] for further development because the ester derivative **2** binds to the enzyme more tightly than the amide **1**. The α-keto amide with a free carboxyl group (**4**) is, however, a better inhibitor (IC_{50} = 0.5 μM) than the corresponding α-(*S*)-OH derivative (IC_{50} = 20 μM; the α-(*R*)-OH derivative is not an inhibitor).[4]

Further adjustment of the inhibitor structure at the P1′ and P2-P3 sites led to the development of an α-keto β-amino ester (**8**) with K_i = 0.1 μM. The NMR spectra indicate that both α-keto β-amino amide and α-keto β-amino esters are completely hydrated in water and 60% hydrated in DMSO containing 5% H_2O. We therefore propose that the inhibitor exists as a *gem*-diol bound in the enzyme active site;[9] the free amino group and one of the hydroxyl groups may coordinate to the Zn^{2+} (as *N*-Boc and *N*-Cbz derivatives are not inhibitors) and the other hydroxyl group interacts with the general base (CO_2^-) via H-bonding (Scheme III).

In summary, the α-keto β-amino esters developed in this study are a new class of selective[10] inhibitors of LTA_4 hydrolase. The bound inhibitor seems to resemble the transition-state structure of the enzymatic amide cleavage and LTA_4 binding.[12] Work is in progress to determine the structure of the inhibitor-enzyme complex for mechanistic investigation and to develop better inhibitors.

Acknowledgment. The work at Scripps was supported by the NSF and NIH and that at Karolinska was supported by Swedish Medical Research Council, Magnus Bergvalls, and the O.E. & Edla Johanssons Foundations.

Supplementary Material Available: Listings of experimental procedures and physical data (mass and NMR data) for all of the inhibitors (5 pages). Ordering information is given on any current masthead page.

(6) Evans, J. F.; Nathaniel, D. J.; Zamboni, R. J.; Ford-Hutchinson, A. W. *J. Biol. Chem.* **1985**, *260*, 10966-10970. Nathaniel, D. J.; Evans, J. F.; Leblanc, Y.; Leveille, C.; Fitzsimmons, B. J.; Ford-Hutchinson, A. W. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 827-835. Ohoshi, N.; Izumi, T.; Minami, M.; Kitamura, S.; Seyama, Y.; Ohkawa, S.; Terao, S.; Yotsumoto, H.; Takaku, F.; Shimizu, T. *J. Biol. Chem.* **1987**, *262*, 10200-10205.

(7) Orning, L.; Krivi, G.; Fitzpatrick, F. A. *J. Biol. Chem.* **1991**, *266*, 1375.

(8) α-Keto amide inhibitors of aminopeptidase were reported: Ocain, T. D.; Rich, D. H. *J. Med. Chem.* **1992**, *35*, 451. For other types of keto ester inhibitors, see: Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. In *Peptides: Structure and Function*, Proceedings of the 9th American Peptide Symposium; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 819. Angelastroc, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P.; Bey, P. *J. Med. Chem.* **1990**, *33*, 11. Hu, L. Y.; Abeles, R. H. *Arch. Biochem. Biophys.* **1990**, *281*, 271.

(9) All α-keto compounds were made as the HCl salt (**3**, **7**, and **8**) or trifluoroacetate salt (**4**). The chemical shifts of β-protons are ~3.8 ppm in D_2O for the hydrate forms and ~5.0 ppm in DMSO for the keto forms. The ^{13}C chemical shift for the α-carbonyl carbon of **8** is 188 ppm in DMSO and 92 ppm in D_2O. These active keto esters or keto amides are similar to fluoro ketones, which are easily hydrated in aqueous solution.[8]

(10) Compounds **6-8** are very weak inhibitors of aminopeptidases; the IC_{50} values are >100, 80, and >100 μM for aminopeptidase M, and 80, 50, and >100 μM for cytosolic leucine aminopeptidase, respectively.

(11) For transition-state structures of Zn^{2+} proteases, see: Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569. Tronrud, D. E.; Holden, H. M.; Matthews, B. W. *Science* **1987**, *235*, 571. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62. Breslow, R.; Chin, J.; Hilvert, D.; Trainor, G. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4585. Izquierdo-Martin, M.; Stein, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 325. Burley, S. K.; David, P. R.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6917.

(12) The procedures for the synthesis of compounds in Table I are essentially the same as described previously.[4] The keto esters or keto amides were prepared from the corresponding OH compounds via Swern oxidation.

Substituent Effects on Diazomethanes and Diazirines by ab Initio Molecular Orbital Calculations

Michael A. McAllister and Thomas T. Tidwell*

Department of Chemistry, University of Toronto
Toronto, Ontario, Canada M5S 1A1

Received March 4, 1992

Diazo compounds (**1**)[1] and the isomeric diazirines (**2**)[2] are important substrates in synthetic and mechanistic studies, particularly as precursors of carbenes,[3] and substituent effects on all these species is a topic of major current interest.[1-3] Although many substituted derivatives of these compounds have been prepared, there is considerable uncertainty[1,2] as to how substituents affect the ground-state stability of **1** and **2**.[4] The effect of substituents on the diazomethane/diazirine equilibrium[4b-e] and on reactions of these species such as carbene formation cannot be properly evaluated in the absence of an understanding of ground-state substituent effects on **1** and **2**.

We have recently reported ab initio molecular orbital studies on the effect of substituents on ketenes (**3**),[5a] which are isoelectronic to diazomethanes, and on diazocyclopolyenes such as diazocyclopentadiene (**4**),[5b] for which there is evidence for aromatic

(1) (a) Regitz, M.; Maas, G. *Diazo Compounds. Properties and Synthesis*; Academic Press: New York, 1986. (b) *The Chemistry of Diazonium and Diazo Groups*; Patai, S., Ed.; Wiley: New York, 1978.

(2) (a) *Chemistry of Diazirines*; Liu, M. T. H., Ed.; CRC Press, Inc.: Boca Raton, FL, 1987; Vols. I and II. (b) Creary, X. *Acc. Chem. Res.* **1992**, *25*, 31-38. (c) Moss, R. A. *Acc. Chem. Res.* **1989**, *22*, 15-12. (d) Liu, M. T. H. *Chem. Soc. Rev.* **1982**, *11*, 127-140. (e) Cameron, T. S.; Bakshi, P. K.; Borecka, B.; Liu, M. T. H. *J. Am. Chem. Soc.* **1992**, *114*, 1889-1890.

(3) (a) Li, J.; Jones, M., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 1094-1095. (b) Moss, R. A.; Ho, G.; Liu, W. *J. Am. Chem. Soc.* **1992**, *114*, 959-963. (c) Burke, B. T.; Pople, J. A.; Krogh-Jespersen, M.; Apeloig, Y.; Karni, M.; Chandrasekhar, J.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1986**, *108*, 270-284. (d) Wang, F.; Winnik, M. A.; Peterson, M. R.; Csizmadia, I. G. *J. Mol. Struct.* **1991**, *232*, 203-210.

(4) (a) The thermal stability order for $RCHN_2$ has been summarized:[1a] Me_3Si > EtO_2C > $Ph_2P(O)$ > CH_3CO > $ArSO_2$ > PhCO > Ph > *n*-Pr. (b) Meier, H. Vol. 2, Chapter 6 in ref 2a. (c) Boldyrev, A. I.; Schleyer, P. v. R.; Higgins, D.; Thomson, C.; Kramarenko, S. S. *J. Comput. Chem.* **1992**, *13*, in press. (d) Kramarenko, S. S.; Boldyrev, A. I.; Stepanov, B. I.; Korolev, M. V.; Redchenko, V. V. *Zhur. Fiz. Khim.* **1990**, *64*, 2413-2416; *Chem. Abstr.* **1991**, *114*, 41799h. (e) Cheng, H.; Tang, A. *Sci. Sin., Ser. B. (Engl. Ed)* **1988**, *31*, 385-392; *Chem. Abstr.* **1989**, *110*, 94154t.

(5) (a) Gong, L.; McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1991**, *113*, 6021-6028. (b) McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1992**, *114*, 5362-5368.

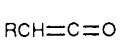
0002-7863/92/1514-6553$03.00/0

Table I. Energies (6-31G*//6-31G*) for Diazomethanes $RCHN_2$ (**1**) and Diazirines $R(CHN_2)$ (**2**), ΔE for the Isodesmic Stabilization of **1** (eq 1), and ΔE_{isom} [E(**2**) - E(**1**)]

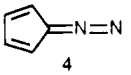
	$-E$- ($RCHN_2$) (**1**), hartrees	$-E$- [$R(CHN_2)$] (**2**), hartrees	ΔE,[a] kcal/mol	ΔE_{isom},[a] kcal/mol	χ_{BE}[b]
H	147.8438	147.8361	1.9	4.8	2.20
Li	154.7018	154.6674	19.7	21.6	1.00
BeH	162.4905	162.4581	14.3	20.3	1.47
BH_2	173.1285	173.0931	18.7	22.2	1.93
CH_3	186.8805	186.8775	0.0	1.9	2.56
NH_2	202.8637[c]	202.8659	-4.6	-1.4	3.10
OH	222.6820[d]	222.6937	-10.0	-7.3	3.64
F	246.6716	246.6913	-12.2	-12.4	4.00
Na	309.0862	309.0532	19.8	20.7	1.00
MgH	347.4304	347.3974	15.4	20.7	1.33
AlH_2	390.3414	390.3062	15.6	22.1	1.62
SiH_3	437.9337	437.9110	7.7	14.2	1.91
PH_2	489.1448[e]	489.1279	6.2	10.6	2.17
SH	545.3535[f]	545.3420	1.6	7.2	2.63
Cl	606.7326	606.7357	-6.3	-1.9	3.05
CF_3	483.4642	483.4564	-1.0	4.9	2.68
$CH{=}CH_2$	224.7308[g]	224.7218	1.3	5.6	2.61
CH=O	260.5829[h]	260.5628	7.2	12.6	2.60
C≡CH	223.5187	223.5074	1.1	7.1	2.66
CN	239.5778	239.5626	0.5	9.5	2.69

[a] Converted by the relation 1 hartree = 627.5 kcal. E(RCH=CH_2) from ref 5a. [b] Group electronegativity from ref 6f, except Pauling electronegativity for H. [c] 202.8472 (coplanar). [d] 222.6761 (coplanar). [e] 489.0661 (coplanar). [f] 545.3418 (coplanar). [g] Anti (syn, 224.7294). [h] Syn (anti, 260.5810).

stabilization in the 5-membered ring case and anti-aromatic destabilization which is strong in diazocyclopropene and marginal in diazocycloheptatriene. We have now extended these studies to **1** and **2**.



3



4

The energies and geometries of **1** and **2** for a representative group of substituents were calculated using the Hartree–Fock method with geometry optimization and energy calculations using the 6-31G* basis set[6a] using the Monstergauss[6b] program implementing the GAUSSIAN 88 package[6c] by standard procedures,[6d] as we have done previously.[5,6e] The energies are presented in Table I, along with values of ΔE (kcal/mol) for the isodesmic reaction of eq 1, group electronegativities χ_{BE} for the substituents,[6f,g] and values of ΔE_{isom} for E(**2**) - E(**1**). Calculated geometries, dipole

$$RCHN_2 + CH_3CH{=}CH_2 \xrightarrow{\Delta E} CH_3CHN_2 + RCH{=}CH_2 \quad (1)$$

moments, and atomic charges for **1** and **2** are presented in the supplementary material. These agree with the limited amount of experimental[7] and previous theoretical[8] data on these compounds within the limits we have observed for related compounds.[5] Isodesmic energy exchanges as in eq 1 are a valuable measure of energy differences due to electronic changes, since the number and types of bonds are unchanged.[6d]

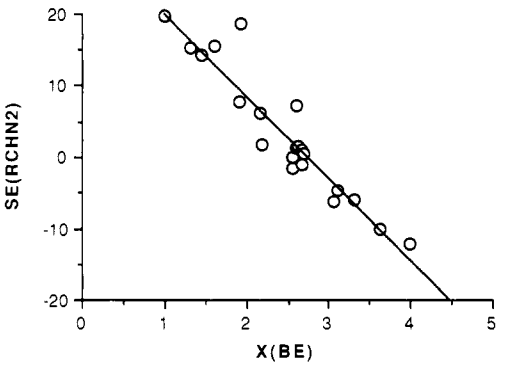
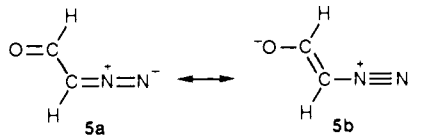


Figure 1. Isodesmic stabilization energy (kcal/mol) by eq 1 for RCH=N=N (**1**) versus group electronegativities χ_{BE}.

Just as for ketenes **3**, there is a correlation[6h] (Figure 1) between the isodesmic stabilization energies for diazomethanes **1** (eq 1) and the substituent group electronegativities[6f,g] (eq 2). Thus,

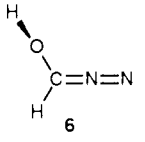
$$\Delta E = -11.5\chi_{BE} + 31.7 \qquad (r = 0.95) \qquad (2)$$

diazomethanes are stabilized by electropositive groups and destabilized by electronegative groups. There are positive deviations from the correlation of eq 2 for π-acceptor groups, particularly for R = CH=O and BH_2, of 5.4 and 9.2 kcal/mol, respectively, indicating stabilization of these species by resonance as shown in **5**. This is in accord with the well-known stability of diazo ketones.[1]

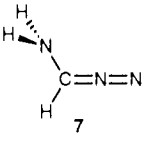


5a **5b**

For the substituents HO and H_2N the geometries with coplanar substituents are less stable than the twisted conformations **6** and **7**, which minimize lone pair donation to the diazomethane function, by 3.7 and 10.3 kcal/mol, respectively. Twisted conformations are also favored for the H_2P and HS substituents. Such destabilization by π-donation was also observed for ketenes[5a] and is attributed to repulsion between the lone pairs of π-donors and the π-systems of ketenes and **1**, which have negative charge on the substituted carbon.



6



7

The values of ΔE_{isom} for the isomerization of diazomethanes **1** to diazirines **2** range from 22.2 (for BH_2) to -12.4 kcal/mol (for F) and are related to χ_{BE} by $\Delta E_{isom} = -11.8\chi_{BE} + 36.9$ (r = 0.93). Furthermore, diazomethanes substituted with the groups $CH{=}CH_2$, CH=O, C≡CH, CN, and CH_3 are more stable than

(6) (a) Francl, M. M.; Pietro, W. J.; Hehre, W. J.; Binkley, J. S.; Gordon, M. S.; DeFrees, D. J.; Pople, J. A. *J. Chem. Phys.* **1982**, *77*, 3654–3665. (b) Peterson, M. R.; Poirier, R. A. Department of Chemistry, University of Toronto. (c) Frisch, M. J.; Head-Gordon, M.; Schlegel, H. B.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; DeFrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R.; Kahn, L. R.; Steward, J. J. P.; Fluder, E. M.; Topiol, S.; Pople, J. A. Gaussian Inc., Pittsburgh, PA, 1988. (d) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley-Interscience: New York, 1986. (e) Critical points were determined for all structures at 6-31G* or 3-21G by numerical differentiation of the first derivatives of the restricted Hartree–Fock wave functions, and for the structures reported here they were found to equal zero indicating these are true energy minima, with the exception of the planar structures fixed for the substituents OH, NH_2, SH, and PH_2. (f) Boyd, R. J.; Edgecombe, K. E. *J. Am. Chem. Soc.* **1988**, *110*, 4182–4186. (g) Boyd, R. J.; Boyd, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 1652–1655. (h) Schleyer, P. v. R. *Pure Appl. Chem.* **1987**, *59*, 1647–1660.

(7) (a) Cox, A. P.; Thomas, L. F.; Sheridan, J. *Nature* **1958**, *181*, 1000–1001. (b) Sheridan, J. *Adv. Mol. Spectrosc., Proc. Int. Meet., 4th, 1959* **1962**, *1*, 139. (c) Costain, C. C.; Yarwood, J. *J. Chem. Phys.* **1966**, *45*, 1961–1965. (d) Pierce, L.; Dobyns, V. *J. Am. Chem. Soc.* **1962**, *84*, 2651–2652. (e) Verma, U. P.; Moller, K.; Vogt, J.; Winnewisser, M.; Christiansen, J. J. *Can. J. Phys.* **1985**, *63*, 1173–1183. (f) Scharpen, L. H.; Wollrab, J. E.; Ames, D. P.; Merritt, J. A. *J. Chem. Phys.* **1969**, *50*, 2063–2069. (g) Bell, J. A. *J. Chem. Phys.* **1964**, *41*, 2556–2557. (h) Paulett, G. S.; Ettinger, R. *J. Chem. Phys.* **1964**, *41*, 2557.

(8) (a) Moffat, J. B. *J. Phys. Chem.* **1978**, 82, 1083–1087. (b) Moffat, J. B. *J. Mol. Struct.* **1979**, *52*, 277–280. (c) Thomson, C.; Glidewell, C. *J. Comput. Chem.* **1983**, *4*, 1–8. (d) Guimon, C.; Khayar, S.; Gracian, F.; Begtrup, M.; Pfister-Guillouzo, G. *Chem. Phys.* **1989**, *138*, 157–171. (e) Liang, C.; Allen, L. C. *J. Am. Chem. Soc.* **1991**, *113*, 1878–1884.

the corresponding diazirines by 5.6, 12.6, 7.1, 9.5, and 1.9 kcal/mol, respectively, even though all of these groups have similar χ_{BE} values. The larger effect for the first four groups is attributed to π-acceptor stabilization of the diazomethanes. Thus there are major effects of the substituents on which isomer is more stable that are largely attributable to the influences of electronegativity and conjugation on the diazomethane stabilities.[9]

Acknowledgment. Financial support by the Natural Sciences and Engineering Research Council of Canada and computer time from the Computer Mathematics Laboratory of the Scarborough Campus of the University of Toronto are gratefully acknowledged.

Supplementary Material Available: Tables of geometries, dipole moments, and Mulliken atomic charges of diazomethanes and diazirines (Tables 2–6) (9 pages). Ordering information is given on any current masthead page.

(9) **Note Added in Proof.** Prof. Schleyer has kindly sent us a preprint of ref 4c, which reports calculations on **1** and **2** for R = H and F at a higher level of theory than reported here. In agreement with their results we also find diazomethane to be 6.4 kcal/mol more stable than diazirine at the MP4/6-31G*//MP2/6-31G* level. Thus these results suggest that our essential conclusions are unchanged at higher levels of theory.

New Results on Protein Folding from Simulated Annealing

David G. Garrett and Keith Kastella

PARAMAX Systems Corporation, P.O. Box 64525
St. Paul, Minnesota 55164-0525

David M. Ferguson*

Department of Medicinal Chemistry and Minnesota Supercomputer Institute, University of Minnesota
Minneapolis, Minnesota 55455

Received January 17, 1992

The prediction of the native states of proteins from their primary sequences entails the solution of two basic types of problems. First, one must model the interaction energies of the peptides and the solvent with adequate fidelity; second, one must locate the low-lying energy states that dominate at biological temperatures. Model potentials that are capable of reproducing the low-energy states of peptide fragments with ideal structural motifs have been described recently by Rey and Skolnick[1] and by Honeycutt and Thirumalai.[2,3] Studies by these groups have mainly focused on simulating and understanding folding pathways of protein models. Although the pathways and mechanics of folding are of great interest and represent yet another basic problem, our goals are to rigorously address the first two problems to ensure that the folded state can be reliably located. The purpose of the present work is to describe the application of a new simulated annealing method to locate the global minimum conformation and analyze the thermodynamic properties of a 22-residue model of a peptide structure. The ideal structural motif for the model chosen is an α-helical hairpin.

Our method uses the continuum potential and peptide model described by Rey and Skolnick for their Brownian Dynamics (BD) simulations of protein folding.[1] We have combined simulated annealing[4] with the optimal histogram method of Ferrenberg–Swendsen[5] to analyze the density of states and specific heat of this continuous space model.[6] Unlike molecular dynamics (MD) based methods that require substantial computing resources, we found the energy minimization problem tractable on a modest desktop personal computer. Our implementation reproduces the low-energy helical hairpin states previously observed[1] and also reveals the existence of a lower, qualitatively different family of states not generated by the other methods (see Figure 1). Our results also indicate that the development of structure is associated with a peak in the specific heat vs temperature plot (see Figure 2), supporting the notion that appreciable structure forms before folding to the final state.

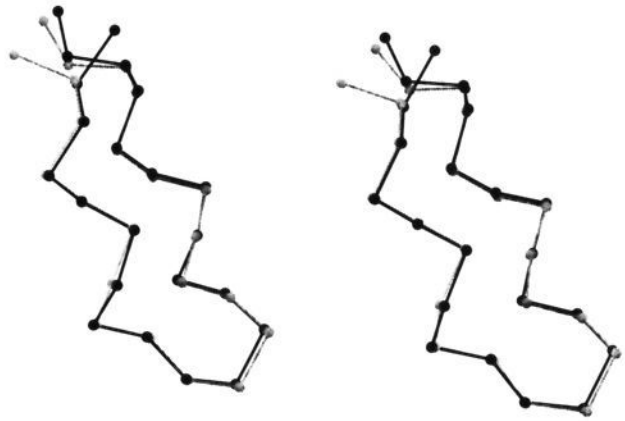


Figure 1. Stereoview of the minimum-energy scrunch state (black) and the higher energy α-hairpin state (gray) for a 22-bead protein model.

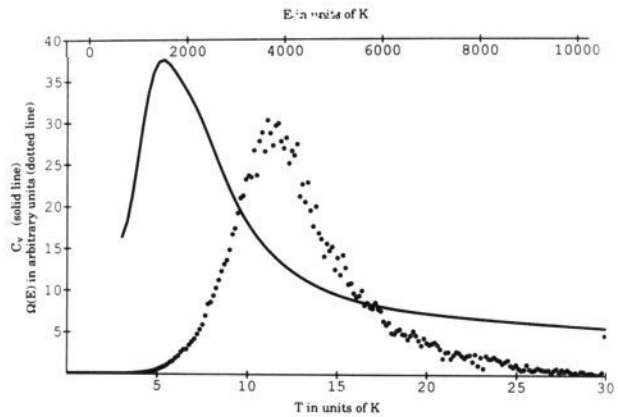


Figure 2. Density of states Ω vs E and specific heat C_v vs T for a schematic 22-bead α-hairpin protein.

We refer to the new states as "scrunch" configurations. Although the minimum-energy configuration obtained in all runs was the scrunch form, the scrunch and helical hairpin forms do overlap in energy, suggesting that a mixture of these configurations contributes to the free energy of the model at nonzero temperatures. At low temperatures the system is dominated exclusively by small fluctuations of the bead positions about the scrunch state. Low-energy states with structural anomalies have also been reported by Honeycutt and Thirumalai for a larger system.[2,3] However, that study was not specific about the relationship of such states to the global minimum configuration nor was it clear that these states have physical significance. It is argued that these states are "metastable" and would presumably interconvert to the expected conformational states given sufficient time. These states, and many others, are proposed to exist within the framework of the metastability hypothesis.[2] Our results, on the other hand, are consistent with the system being thermally equilibrated and indicate that at moderate temperatures (0.5–2 K) a population of structures exists that has minor fluctuations about the ideal α-helical hairpin and scrunch states. These should not be confused with states regarded as being metastable.

(1) Rey, J.; Skolnick, J. *Chem. Phys.* **1991**, *158*, 199–219.
(2) Honeycutt, J. D.; Thirumalai, D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3526–3529.
(3) Honeycutt, J. D.; Thirumalai, D. *Biopolymers* **1992**, *32*, 695–709.
(4) Kirkpatrick, S.; Gelatt, C. D., Jr.; Vecchi, M. P. *Science* **1983**, *220*, 671–680.
(5) Ferrenberg, A. M.; Swendsen, R. H. *Comp. Phys.* **1989**, Sept/Oct, 101–104.

(6) Specific details of the method can be obtained from the authors.

0002-7863/92/1514-6555$03.00/0