brief communication

Dynamical search for bis-penicillamine enkephalin conformations

B. M. Pettitt,* T. Matsunaga,‡ F. Al-Obeidi,‡ C. Gehrig,‡ V. J. Hruby,‡ and M. Karplus§
*Chemistry Department, University of Houston, Houston, Texas 77204-5641; ‡Department of Chemistry, University of Arizona, Tuscon, Arizona 85721; and §Department of Chemistry, Harvard University, Cambridge, Massachussetts 02135

ABSTRACT Quenched molecular dynamics is used as a conformational search technique for the constrained cyclic analog [*D*-Pen^2,*D*-Pen^5]enkephalin (DPDPE) in a continuum solvent. The results show a Gaussianlike distribution of conformations as a function of energy, unlike the distributions found for simple liquids which have sharp bands for different crystal forms and broad glasslike states are found. The lowest energy conformers have structural features in common with those obtained from constrained searches based on energy minimization. (Hruby, V. J., L.-F. Kao, B. M. Pettitt, and M. Karplus. 1988. *J. Am. Chem. Soc.* 110:3351–3359.) Many of the low energy configurations are amphiphilic with the carbonyl groups on one surface and the hydrophobic groups on the other. This supports the conclusions from the previous modeling study, which yielded amphiphilic structures as the most probable conformations of DPDPE when NOE data were included.

INTRODUCTION

In a recent study of the conformations of [*D*-Pen^2,*D*-Pen^5]enkephalin (Pen = penicillamine, or β,β-dimethylcysteine), molecular mechanics calculations were used as an aid in the interpretation of two-dimensional nuclear magnetic resonance (2D-NMR) measurements (1) of the compound in solution. Initial conformations were constructed using minimal constraints including ring closure and some of the NOE data. In addition to the NOE structures, a number of configurations, such as expected β-bend structures, were generated de novo. The resulting structures were minimized and compared with the complete 2D-NMR data set (1). Two pairs of structures, each pair differing in disulphide helicity, were found which satisfied the NMR data and were of low energy. Several of the structures were amphiphilic; one side had exposed polar groups, mostly carbonyls, whereas the other side had mainly aliphatic and aromatic groups exposed. The amphiphilicity of peptide compounds targeted to membrane bound receptors is recognized as an important characteristic (2–4).

In any such conformational search, there is the concern that the search is not complete. Because the number of conformations increases exponentially with the number of dihedrals, it is possible that one has missed one or more important or relevant conformers. Even in a molecule of only five amino acid residues with an internal macrocyclic constraint, there are many rotational degrees of freedom and relevant conformers could be overlooked. Several recent studies have demonstrated how time consuming a truly detailed search of a polypeptide or even hydrocarbon conformational space can be (5–11). Thus, it is important to have a method which establishes the confidence level of a search that is not exhaustive; this was the topic of a recent comparison (12).

In this paper we have employed a search methodology based on minimizing or quenching high temperature molecular dynamics configurations (13). In this quenched dynamics technique (14), a large number of individual configurations from the molecular dynamics trajectory are selected and thoroughly minimized. A similar technique has proved to be useful in the study of configurations obtained from protein (15) or liquid-state simulations. For simple liquids the various possible crystal states and the glasslike states (16–21) form distinct categories. This is different from the results found in the present conformational search.

In our implementation, a molecular dynamics simulation was run with the CHARMM program (22) for [*D*-Pen^2,*D*-Pen^5]enkephalin (DPDPE) in a continuum aqueous solvent, with a dielectric constant of 80; the same model potential function was used as in the earlier study (1). The temperature of the simulations was approximately 1,000° K. This elevated temperature allowed the molecule to explore a number of configurations that are separated by barriers which would be crossed very infrequently at room temperature. Thus, the dynamics is used to perform a Boltzmann weighted search for other conformers that might not have been found in previous model building. After an extensive heating and equilibration period of >20 ps, a 200-ps trajectory was determined. Two hundred configurations separated by 1-ps intervals were selected. Each of these coordinate sets was subjected to extensive ABNR mini-

0006-3495/91/12/1540/05 $2.00

mization (22, 23) (until the total RMS gradient for the calculation was $<.02$).

The distribution of conformers versus energy showed a bell-shaped, Gaussianlike pattern (see Fig. 1). Such distributions have also been observed for a flexible crown ether by both an ellipsoid search and by quenching MD structures between 300 and 350 K (24). In the case of simpler liquids, the quenched dynamics procedure often finds various stable and metastable crystalline forms as well as the glassy states (16–21). The different structural states tend to be segregated by energy. In the case of this peptide and several others under study (25) there were no discernible preferred conformations of "crystal-like" stability, i.e., no isolated minimum far lower in energy than the other conformers was found. Most of the configurations minimized to relatively high energy conformations, i.e., they had energies >10 kcal/mol above the minimum (see Fig. 1) and, therefore, could be assumed to be improbable in solution. Those with lower energies were analyzed for the family of conformers (defined in terms of backbone and side chain torsion angles) to which they belong.

Examination of the 30 lowest energy structures (within 7 kcal/mol of the minimum) showed that few conformers had backbone torsion angles significantly different from those found in the previous search. However, all were lower in energy than the lowest energy structure found by the manual search done in the vicinity of the conformations allowed by NMR data (1). In several cases, the resulting structures found in the quenched MD search were related to previously described structures by rotations about side chain dihedrals, e.g., they involved $\pm 120°$ or $180°$ rotations of the phenylalanine and tyrosine side chains about the angle X_1. Many contained hydrogen bonds not in the conformations found on the basis of modeling from NMR data (1). A common element of the low energy conformations was the partitioning of the molecule into an interior with a preponderance of amide NH groups and an exterior with the carbonyls on one surface and the hydrophobic aromatic rings and penicillamine methyl groups on the other. Thus, many of these low energy configurations displayed the biochemically important (2–4) amphiphilic features found in the previous NMR-modeling study (1). Fig. 2 shows nine of the lowest energy conformers found in the quenched molecular dynamics run. They may be compared to the low energy DPDPE conformation found in the NMR-modeling study (see Fig. 2 of ref. 1).

The ϕ, ψ, χ_1, χ_2 and disulphide χ_3 torsion angles are listed in Table 1. The disulphide shows three of the conformers impose a dihedral angle of $\sim 160°$ whereas the remainder are closer to 110°. A review of the literature of crystallized proteins and peptides reveals that the disulphide resides primarily in the $90° \pm 20°$ range (26–29). However, one notable exception is insulin where the a6-a11 disulphide possesses a torsion angle of 32° (30). Thus, considering that quaternary substituted β-carbons are found in both D-pen residues, the disulfide torsion angles are reasonable.

During the process of heating the molecule to 1,000° K, two equilibrations were used. A 20-ps equilibration

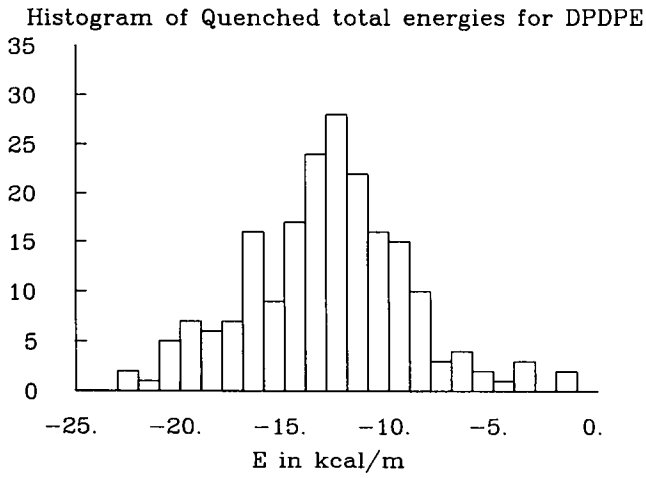

FIGURE 1 The energetic distribution of quenched structures for [*D*-Pen^2,*D*-Pen^5]enkephalin (DPDPE) in a continuum aqueous solvent.

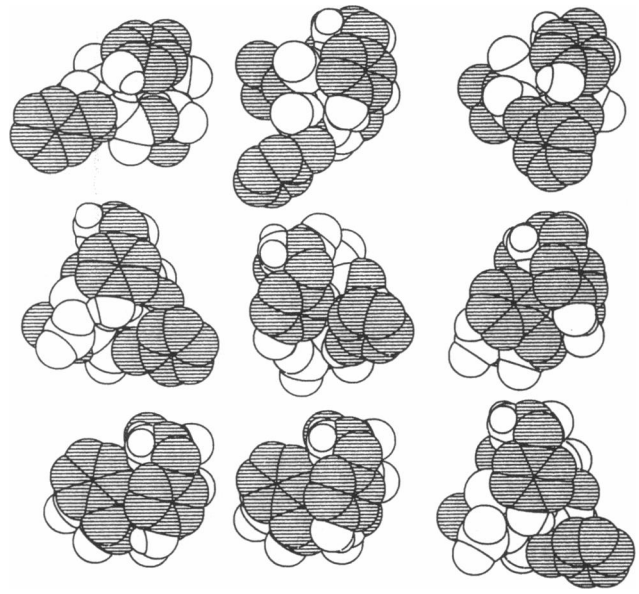

FIGURE 2 A space filling view of the lowest energy conformations. The nonpolar carbon atoms are cross-hatched for clarity. All of the structures are oriented with the hydrophobic face toward the viewer.

TABLE 1 **Torsion values for the lowest energy conformations found by quenched dynamics**

	58 ps	59 ps	133 ps	134 ps	135 ps	139 ps	140 ps	141 ps	142 ps
ϕ_1	103.47	105.34	99.17	95.71	95.45	77.15	67.18	77.02	104.99
ϕ_2	−139.27	−135.53	−162.71	−158.8	−170.63	−114.09	−150.37	−114.57	−142.59
ϕ_3	−117.7	−121.45	−71.26	−69.96	−155.16	−118.90	−82.11	−119.03	−107.18
ϕ_4	82.25	82.71	82.92	82.20	83.55	131.55	88.59	130.75	88.76
ψ_1	−29.59	−30.3	−21.4	−22.17	−21.96	−46.85	−59.51	−46.85	−28.75
ψ_2	50.11	49.72	53.30	52.00	54.16	58.58	51.15	58.67	50.49
ψ_3	−81.78	−81.76	−29.12	−29.67	37.76	−55.79	−43.27	−55.99	−86.50
ψ_4	68.39	70.63	−34.96	−36.34	−34.59	−49.32	−26.96	−48.67	55.56
χ_{11}	58.5	59.33	60.89	53.91	55.09	67.73	48.26	67.97	62.13
χ_{12}	−69.82	−67.89	−60.35	−62.76	−60.25	−64.96	−59.71	−64.73	−71.55
χ_{13}	—	—	—	—	—	—	—	—	—
χ_{14}	−168.53	−164.4	67.7	64.28	66.49	−55.83	−60.28	−55.20	−58.86
χ_{15}	−80.66	−78.55	−68.57	−67.39	−63.00	−53.86	−74.32	−53.97	−83.64
C_β-S_γ-S_γ-C_β	−161.49	−163.04	112.75	116.87	115.66	109.82	114.08	110.21	−160.97
χ_{21}	94.05	94.17	−79.37	−88.66	−88.44	−63.48	98.83	−63.23	−81.83
χ_{22}	113.85	110.72	−158.01	−156.69	171.34	132.91	−167.6	133.24	112.08
χ_{24}	−116.11	−112.13	110.02	107.22	117.71	−76.84	−74.25	−76.33	−75.46
χ_{25}	57.04	55.85	76.46	77.25	76.68	77.87	75.60	77.81	62.23

The simulated time of the sampled configuration is given at the top.

run near 300° K was performed. During that run a highly correlated motion of the Phe and Tyr side chains was noticed. In the most NMR-consistent minimum energy structure previously reported (1), the two aromatic rings were placed such that they were situated "on top of" or roughly parallel to the macrocycle. During the dynamics, the rings often were found to move in concert (in a positively correlated fashion) so as to present a similar orientation of the rings as the interior NH moieties correspondingly changed solvent exposure. At higher temperatures (1,000° K), a crankshaft motion was observed about the disulphide dihedral in concert with these side chain motions. This motion is hindered by the presence of the pair of geminal dimethyls from the two pencillamine residues, so that it passes through the geometry with the disulphide torsion angle equal to 180° rather than 0° as it would in hydrocarbons (31).

None of the structures in Fig. 2 individually satisfies all of the NOE and NMR data, although most do so approximately. Comparing the interresidue NOE constraints (500 MHz at pH = 3.8, 90% H_2O, 10% D_2O, unpublished data) with the structures found in this study, we find that the constraints are qualitatively consistent with the proposed models, with the exception of the NH-NH distances between D-Pen^2-Gly^3 and Gly^3-Phe^4. Temperature-dependent chemical shift studies in DMSO (Prakash and Hruby, unpublished results) suggest that only the D-Pen^5 NH may be solvent shielded, whereas the Gly^3 and Phe^4 amide protons are less so. Although annealing with the use of constraints from NMR data (32) could have been introduced at this point, we did not do so because our purpose here was not to refine the experimental data (25, 32) but rather to generate other probable structures.

Other conformationally restricted analogs of Met-enkephalin have been synthesized and characterized pharmacologically. In particular, the highly restricted tetrahydroisoquinoline carboxylate (Tic) analog [*D*-Pen^2,*Tic*^{4},*D*-Pen^5]enkephalin has been shown to have good specificity but less potency than [*D*-Pen^2,*D*-Pen^5]enkephalin (33). The Tic compound was examined by traditional molecular mechanics for energy minima. The lowest energy conformer was found to be very similar in overall shape to those found for the bis-penicillamine compound with the tetrahydroisoquinoline ring and the tyrosine ring in a similar position to that of the Phe and Tyr in [*D*-Pen^2,*D*-Pen^5]enkephalin. Thus, the minimization study suggests that the compounds should have similar pharmacological profiles. To test this conclusion, a 10-ps dynamical simulation was made of the Tic compound. A disulphide crankshaft motion similar to that found (see above) in the [*D*-Pen^2,*D*-Pen^5]enkephalin compound occurred. Interestingly, this motion disturbs the relative orientation of the Tic aromatic ring and the tyrosine ring so that the ring conformations do not correspond to that of the Phe and Tyr residues in [*D*-Pen^2,*D*-Pen^5]enkephalin. In particular, they are pushed off the macrocycle and into solution. This suggests the possibility that a dynamical difference in the two peptides may be responsible, in part, for the difference in biological activity. There may be an entropic component of the conformational free energy that stabilizes the conformation with the conformationally restrictive Tic residue away from the macrocyclic ring.

Although this dynamical or entropic hypothesis is attractive, more work remains to be performed to confirm its validity. The previous energy minimization studies showed that the lowest energy conformers often came in pairs that differed only in their chirality about the disulphide bridge. Thus, the static calculations suggested the possibility of these conformations in the [*D*-Pen^2,*D*-Pen^5] compounds.

The use of quenched MD without the use of experimental constraints in this study confirmed the results of a previous modeling effort (1) with NOE constraints. In addition, from a dynamical analysis of related compounds, new insights were obtained that may prove to be of direct biochemical significance.

Financial support is acknowledged from the R. A. Welch Foundation (B. M. Pettitt) and the National Institutes of Health (B. M. Pettitt, V. J. Hruby, and M. Karplus). B. M. Pettitt is an Alfred P. Sloan Fellow. T. Matsunaga acknowledges the National Institute on Drug Abuse for their generous support in the form of a NIDA fellowship (DA 05371-02). B. M. Pettitt thanks the San Diego Supercomputer Center for computational support.

Received for publication 13 September 1990 and in final form 3 June 1991.

REFERENCES

1. Hruby, V. J., L.-F. Kao, B. M. Pettitt, and M. Karplus. 1988. The conformational properties of the Delta Opioid Peptide [*D*-Pen^2,*D*-Pen^5]. *J. Am. Chem. Soc.* 110:3351–3359.
2. Kaiser, E. T., and F. J. Kézdy. 1984. Amphiphilic secondary structure: design of peptide hormones. *Science (Wash. DC).* 223:244–255.
3. Kaiser, E. T. 1986. The design of peptides and proteins. *Ann. N.Y. Acad. Sci.* 471:233–238.
4. Hruby, V. J., F. Al-Obeidi, and W. Kazmierski. 1990. Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations. *Biochem. J.* 268:249–262.
5. Scheraga, H. A. 1989. Some computational problems in the conformational analysis of polypeptides and proteins. *In* Computer Assisted Modeling of Receptor-Ligand Interactions. Alan R. Liss, New York, 240–352.
6. Nemethy, G. and H. A. Scheraga. 1977. Protein folding. *Q. Rev. Biophys.* 3–77.
7. Ripoll, D. R., and H. A. Scheraga. 1988. On the multiple-minima problem in the conformational analysis of polypeptides. *Biopolymers.* 27:1283–1303.
8. Bruccoleri, R. E., and M. Karplus. 1987. Prediction of folding of short polypeptide segments by uniform conformational sampling. *Biopolymers.* 26:137–168.
9. Bruccoleri, R. E., and M. Karplus. 1990. Conformational sampling using high temperature molecular dynamics. *Biopolymers.* 29: 1847-1862.
10. Motoc, I., R. A. Dammkoehler, and G. R. Marshall. 1986. Three-dimensional structure-activity relationship and biological receptor mapping. *In* Mathematics and Computational Concepts. N. Trinajstic, editor. Ellis Howard, Chichester, England, 222 pp.
11. Dammkoehler, R. A., S. F. Karasek, B. Shands, and G. R. Marshall. 1989. Constrained search of conformational hyperspace. *J. Comp. Aided Mol. Design* 3:3–21.
12. Saunders, M., K. N. Houk, Y.-D. Wu, W. C. Still, M. Lipton, G. Chang, and W. C. Guida. 1990. Conformations of cycloheptadecane. *J. Am. Chem. Soc.* 112:1419–1427.
13. Brooks, C. L., M. Karplus, and B. M. Pettitt. 1988. Proteins: A Theoretical Perspective of structure, dynamics and thermodynamics. John Wiley and Sons, Inc., New York. 259 pp.
14. Kirkpatrick, S., C. D. Gelatt, and M. P. Vecchi. 1983. Optimization by simulated annealing. *Science (Wash. DC).* 220:671–680.
15. Elber, R., and M. Karplus. 1987. Multiple conformational states of proteins: a molecular dynamics analysis of myoglobin. *Science (Wash. DC).* 235:318–321.
16. Stillinger, F. H., and T. A. Weber. 1982. Hidden structure in liquids. *Phys. Rev. A* 25:978–989.
17. Rahman, A., M. J. Mandell, and J. P. McTague. 1976. Molecular dynamics study of an amorphous Lennard-Jones system at low temperature. *J. Chem. Phys.* 64:1564–1568.
18. Woodcock, L. V., C. A. Angell, and P. Cheeseman. 1976. Molecular dynamics studies of the vitreous state: simple ionic systems and silica *J. Chem. Phys.* 65:1565–1577.
19. Grabow, M. H., and H. C. Andersen. 1985. A comparison of the molecular dynamics method and energy minimization method for modeling the structure of amorphous metals. *J. Non-Cryst. Solids* 75:225–236.
20. Ding, K., and H. C. Andersen. 1987. Phase diagram of silicon by molecular dynamics. *Phys. Rev. B.*35:9120–9127.
21. Fox, J. R., and H. C. Andersen. 1984. Molecular dynamics simulations of supercooled monoatomic liquid and glass. *J. Phys. Chem.* 88:4019–4036.
22. Brooks, B. R., R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy, minimization and dynamics calculations. *J. Comput. Chem.* 4:187–217.
23. States, D. J. 1982. Ph.D. dissertation. Harvard University, Cambridge, MA.
24. Billeter, M., A. E. Howard, I. D. Kuntz, and P. A. Kollman. 1988. A new technique to calculate low-energy conformations of cyclic molecules utilizing the ellipsoid algorithm and molecular dynamics: application to 18-crown-6. *J. Am. Chem. Soc.* 110:8385–8391.
25. O'Conner, S. 1991. Senior honors thesis. University of Houston, Houston, TX.
26. Quiocho, F. A., and W. N. Lipscomb. 1971. Carboxypeptidase A: a protein and an enzyme. *Advanc. Protein Chem.* 25:1–78.
27. Birktoft, J. J., and D. M. Blow. 1972. The structure of Crystalline α-Chymotrypsin. *J. Mol. Biol.* 68:187–217.
28. Wyckoff, H. W., D. Tsernoglou, A. W. Hanson, J. R. Know, B. Lee, and F. M. Richards. 1970. The three-dimensional structure of Ribonuclease-S. *J. Biol. Chem.* 245:305–328.
29. Wood, S. P., I. J. Tickle, A. M. Treharne, J. E. Pitts, Y. Mascarenhas, J. Y. Li, J. Husain, S. Cooper, T. L. Blundell, V. J. Hruby, A. Buko, A. J. Fischman, and H. R. Wyssbrod. 1986. Crystal Structure Analysis of Deamino-Oxytocin: Conformational flexibility and receptor binding *Science (Wash. DC).* 232:633–636.

30. Blundell, T. L., G. G. Dodson, E. Dodson, D. C. Hodgkin, and M. Vijayan. 1971. X-ray analysis and the structure of insulin. *Recent Prog. Horm. Res.* 27:1–40.

31. Hefand, E., Z. R. Wasserman, and T. A. Weber. 1981. Kinetics of conformational transitions in bulk polymers. *Poly. Prep. (Am. Chem. Soc. Div. Polym. Chem.).* 22:279–280.

32. Nilges, M., G. M. Clore, and A. M. Gronenborn. 1988. Determination of three-dimensional structures of proteins from interproton distance data by dynamical annealing from a random array of atoms. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 239:129–136.

33. Hruby, V. J., L.-F. Kao, L. D. Hirning, and T. F. Burks. 1985. Design and synthesis of receptor selective peptide neurotransmitters, *In* Peptides: structure and function. Proceedings of the Ninth American Peptide Symposium. C. M. Deber, V. J. Hruby, and K. D. Kopple, editors. Pierce Chemical Company, Rockford, MD. 487–490.