Accelerated Publications

Catalysis of a Rotational Transition in a Peptide by Crystal Forces†

David H. Kitson and Arnold T. Hagler*

The Agouron Institute, 505 Coast Boulevard South, La Jolla, California 92037

Received May 31, 1988; Revised Manuscript Received July 18, 1988

ABSTRACT: Detailed examination of the dynamics trajectories of the isolated cyclic peptide *cyclo*-(Ala-Pro-D-Phe)$_2$ and of the molecule in its crystalline environment led to the unexpected observation that the methyl groups of the alanine residues rotated more frequently during a simulation in the crystal environment than in a simulation of the isolated peptide. In effect, the crystal environment is "catalyzing" the rotational isomerization of the methyl groups. In order to understand how the crystal forces increase the rate of this rotation, and to explore any possible analogy to the inducing of strained conformations of ligands by enzymes, the barriers to rotation in the two environments were studied by using the torsion angle forcing method. The crystal forces induce a different, higher energy, conformation of the peptide than is found for the isolated molecule, and the different rates of rotation have been explained in terms of the resulting specific intramolecular interactions that, it turns out, give rise to the lower rotational barrier. Molecular dynamics simulations of the peptide were also run at higher temperatures in order to calculate the barriers to rotation through the use of Arrhenius plots. The barriers obtained in this way agree well with the barriers obtained through an adiabatic reaction path derived by rotating the methyl through the barrier while minimizing all remaining degrees of freedom. The rates of rotation calculated from these adiabatic barriers also agree well with the rates observed during the 300 K simulations.

The phenomena of catalysis and message transduction in biological systems are generally thought of as complex processes involving large molecular systems, including molecules such as enzymes or receptors. From analysis of the results of dynamics trajectories of a cyclic peptide in the isolated and crystalline states, we have observed a small system that models these processes in a simple way (Kitson & Hagler, 1988).

This model arose from a novel and unexpected result with regard to the effect of the environment on the rotations of the alanine side chains during a simulation of *cyclo*-(Ala-Pro-D-Phe)$_2$. This simulation was carried out as part of a series of simulations of small peptides, which are aimed both at evaluating the accuracy of the methods and force fields used for the simulations (Williams, 1965; Momany et al., 1974; Hagler et al., 1979; Dauber & Hagler, 1980; Hall & Pavitt, 1984; Hagler, 1985) and at exploring the effects of environment on the structure, energetics, and dynamics of peptides.

Methods

The 100-ps molecular dynamics trajectories were carried out on *cyclo*-(Ala-Pro-D-Phe)$_2$ both in vacuo and in the crystal environment (Kitson & Hagler, 1988). This peptide crystallizes in an orthorhombic unit cell with 2 peptide molecules and 16 water molecules per unit cell (Brown & Teller, 1976). For the crystal simulation, the two peptide molecules (and the 16 waters) in the cell were taken as the "asymmetric unit" and periodic boundary conditions were used to reproduce the crystal environment. A cutoff of 15 Å was used. The simulations were run using the DISCOVER simulation program (Biosym Technologies, Inc., San Diego, CA). Full details of these simulations have been reported (Kitson & Hagler, 1988). The rotational transitions of the alanine methyl groups were observed initially by plotting the value of the dihedral angle χ as a function of time. Adiabatic barriers to rotation around the alanine C^α–C^β bond were calculated by using the torsion angle forcing method. In this method, energy minimizations are performed in which the energy function is augmented by adding a harmonic term to the energy for the appropriate torsion angles to force them to the desired values. By performing a series of minimizations in which the target torsion angles are gradually changed from their values in the starting structure to their values in the final structure, one can obtain an estimate of the barrier to rotation. The barriers to rotation were also calculated from Arrhenius plots. The data for these plots were obtained by running 100-ps dynamics simulations of the peptide in both the crystal and isolated conformations at 300, 350, 400, 500, 600, and 800 K and counting the number of alanine rotations that took place at each temperature.

Results

During the dynamics simulations of the peptide in both the isolated and crystal environments, rapid rotations of the methyl groups in the alanine residues were seen to take place (see Figure 1). This same type of behavior has been deduced from experimental studies of proteins. NMR studies of proteins in solution have shown methyl groups to undergo rapid spinning (Wittebort et al., 1979). In a neutron diffraction study of the protein crambin (Kossiakoff & Shteyn, 1984), the conformational preferences of methyl groups on the side chains were studied by sampling the neutron density around the C_3 rotation axis of each methyl group at the appropriate distance for a

†This work was supported by Grant DMB 8510865 from the National Science Foundation and by Grant GM 30564 from the National Institutes of Health. Grants of computer time (on the Cray X-MP/48 at the San Diego Supercomputer Center) were provided by the National Science Foundation (Grant PCM 8421273) and by Cray Research Inc.

0006-2960/88/0427-7176$01.50/0 © 1988 American Chemical Society

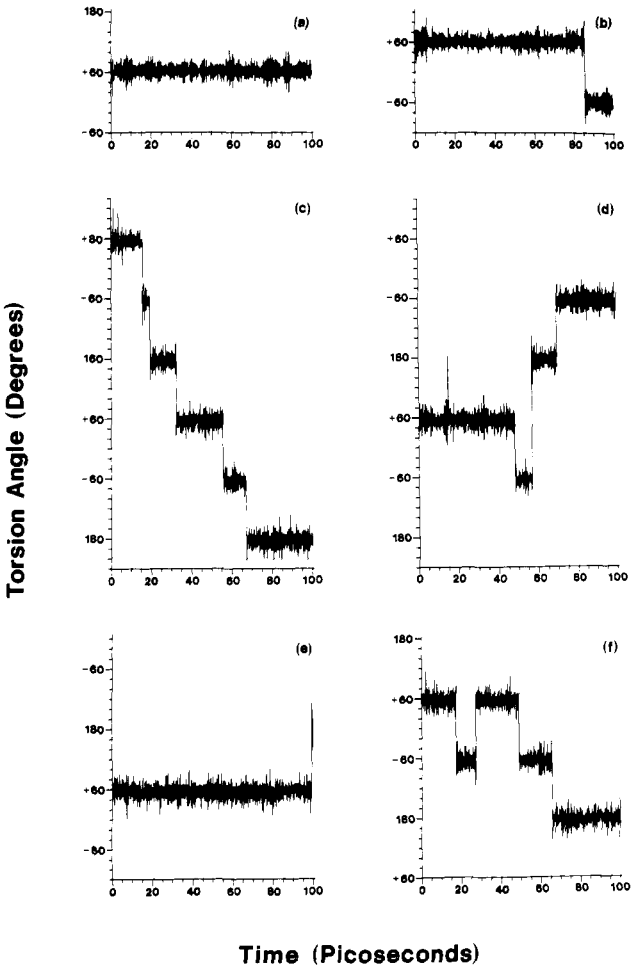

FIGURE 1: Behavior of the alanine side chains during the in vacuo (a and b) and crystal (c–f) simulations of *cyclo*-(Ala-Pro-D-Phe)$_2$. The rapid rotations of the methyl group that take place during the dynamics simulations are illustrated. These transitions occur more frequently during the simulation of the peptide in its crystal environment than during the in vacuo simulation, and this is consistent with the lower barrier to rotation calculated for the crystal system (see text).

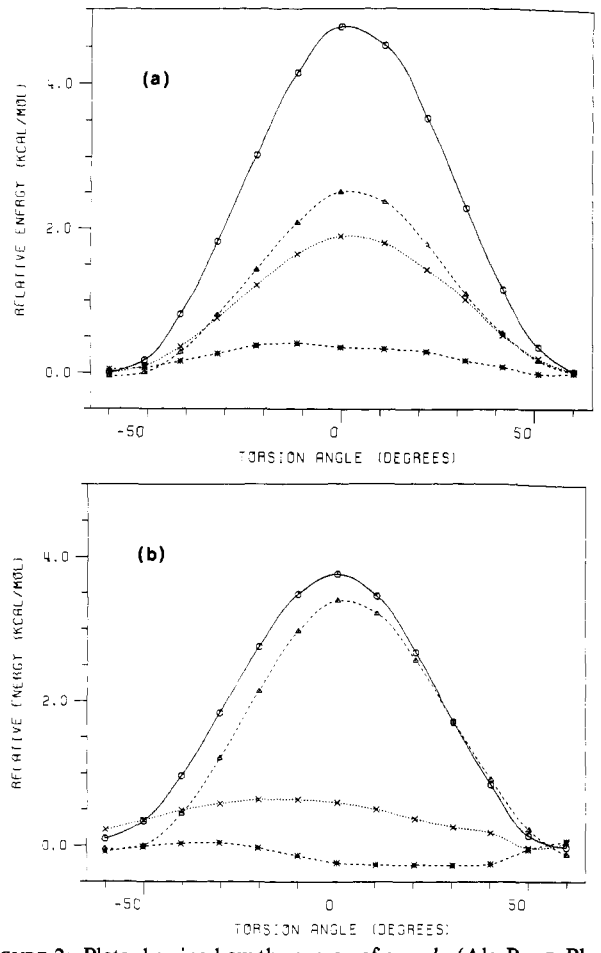

FIGURE 2: Plots showing how the energy of a *cyclo*-(Ala-Pro-D-Phe)$_2$ molecule varies as a function of the χ torsion angle of one of the alanine residues. The data were obtained by using the torsion angle forcing method (see text). Plot a is for the lowest energy conformation found for the peptide in vacuo, while plot b is for the peptide in its crystal environment. The solid lines marked with circles represent the total intramolecular energy for the peptide, the dashed lines marked with triangles represent the torsional energy, the dotted lines marked with crosses represent the total nonbonded (van der Waals plus Coulombic) intramolecular energy, and the dashed lines marked with asterisks represent the total of the other components of the intramolecular energy. The forcings were started at $\chi \approx 60°$, and the first forcing was to a target torsion angle of 60°. Subsequent forcings were to target torsion angles in 10° steps from 50° to −60°. These plots show that the barrier is higher for the isolated peptide and that the barriers break down into their components in quite different ways for the two environments.

hydrogen atom. This study showed that the methyl groups have a strong perference to adopt a staggered conformation. Thus the NMR and neutron diffraction data together suggest that the methyl groups undergo a rapid jump from one staggered conformation to the next and then oscillate about this configuration for a period until the next jump event, as observed in the dynamics simulations (Figure 1).

One would intuitively expect the rate of rotation of the alanine side chains to be lower for the peptide in its crystal environment than in the isolated state, due to the presence of other peptide and solvent molecules in the crystal that might interfere sterically with the rotation. It was, therefore, surprising to see, as Figure 1 shows, that the rotations occurred ≈7.5 times more frequently during the crystal dynamics (1 rotation for the two alanine residues during the isolated simulation compared with 15 rotations for the four alanine residues during the crystal simulation). We have investigated this phenomenon in more detail, in order to explain this unexpected finding, and the results of this analysis are presented below.

Barrier to Rotation. The more rapid rate of rotation in the crystal system suggests that the crystal forces, unexpectedly, lower the barrier to rotation of the methyl group. In order to confirm this, since the sample is small, the barrier to rotation of the methyl group was calculated for both the isolated and crystal systems by using the torsion angle forcing method (see Methods). For the isolated peptide, the barrier turned out to be ≈4.8 kcal/mol, while for the peptide in the crystal environment, the barrier is indeed lower, ≈3.8 kcal/mol, with the contribution from intermolecular interactions being only ≈0.1 kcal/mol. [Since the conformation of the isolated molecule is asymmetric (Kitson & Hagler, 1988), the barrier was calculated for both alanine residues. The value turned out to be the same in both cases. The peptides in the simulated crystal are very close to being C_2 symmetric, and so the barrier was calculated for only one of the alanines.] The more rapid rate of rotation for the crystal system seen during the dynamics is, therefore, consistent with the lower energy barrier calculated for the peptide in the crystal environment.

Thus in some way the crystal environment serves to lower the rotational barrier for the methyl group. An obvious question that now arises is "what interactions lower the barrier in the crystal" or "how do crystal forces increase the rate of rotational transitions"?

Table I: Torsional and Nonbond Contributions to the Barriers to Rotation of the Alanine Methyl Groups in the Isolated and Crystal Conformations of *cyclo*-(Ala-Pro-D-Phe)$_2$[a]

Torsional Contributions		
	ΔE[c] (kcal/mol)	
bond[b]	isolated	crystal
Ala C^α–Ala C^β	+2.81	+2.73
Ala C′–Pro N	−0.23	+0.22
Ala′ C′–Pro′ N	−0.06	+0.12
Pro′ C^α–Pro′ C^β	+0.01	+0.18
other bonds	−0.03	+0.12
total	+2.50	+3.37

Nonbond Contributions		
	ΔE[c] (kcal/mol)	
interacting groups[d]	isolated	crystal
Ala cb, Ala can	+1.03	+0.85
Ala cb, Ala co	+0.31	+0.46
Ala cb, Pro can	+0.13	−0.20
Ala cb, Ala′ cb	+0.03	−0.19
Ala cb, Ala′ co	+0.14	0.00
Ala cb, Phe′ co	−0.01	−0.12
others	+0.27	−0.19
total	+1.90	+0.61

[a] In both the isolated and crystal systems the torsion angle forcing was applied to the methyl group of the residue labeled Ala. The following two residues are labeled Pro and Phe, while the last three residues are labeled Ala′, Pro′, and Phe′. [b] This is the central bond of the torsion angle. The ΔE values for all torsion angles around the bond (a total of nine for the C^α–C^β bonds and four for the C′–N bonds) are included in the ΔE value listed. [c] The ΔE value is the difference in energy between the systems at the maximum point of the barrier ($\chi \approx 0°$) and the starting system. [d] The molecules are divided into small groups of atoms that have a neutral net charge. The atoms in the groups included here are as follows: (Ala cb) C^β, $H^{\beta 1}$, $H^{\beta 2}$, $H^{\beta 3}$; (Ala, Phe co) C′, O′; (Ala can) C^α, H^α, N, H; (Pro can) C^α, H^α, N, C^δ, $H^{\delta 1}$, $H^{\delta 2}$.

The total intramolecular, nonbond (van der Waals and Coulombic), and torsion energy components for the peptide in the two environments as a function of the χ torsion angle are given in Figure 2. This analysis shows not only the higher barrier for the isolated system [4.8 vs 3.7 kcal/mol (this is the intramolecular component of the barrier in the crystal)], which was discussed above, but also that these barriers break down into their components in different ways in the two environments. For the isolated peptide, the torsion and nonbond components are ≈2.5 and 1.9 kcal/mol, respectively. The other components contribute ≈+0.4 kcal/mol to the barrier. For the peptide in the crystal environment, the torsional component of the barrier is higher, 3.4 kcal/mol, but this is compensated by a much lower intramolecular nonbond component, 0.6 kcal/mol. The other components are considerably lower as well, ≈−0.2 kcal/mol (i.e., they are actually more favorable at the "transition state").

One of the significant advantages of theoretical techniques is that features such as barriers to conformational change can be broken down and analyzed in terms of the specific atom–atom interactions and internal coordinate changes that give rise to the feature. In the case of these methyl rotations, we can look at the specific torsion angles and nonbond interactions that are involved in the barriers. The results of this analysis are given in Table I. As this table shows, the increase in torsional strain is largest in both cases for the torsion angles around the C^α–C^β bond of the alanine residue that is being forced (2.81 kcal/mol, isolated, and 2.73 kcal/mol, crystal). However, other correlated changes in torsion angles driven by this torsion take place during the forcings that are quite different in the two environments. In the isolated system, both of the Ala–Pro amide bonds become slightly less strained, leading to a decrease in torsional energy of 0.29 kcal/mol. In the crystal environment, there is an increase in strain for the corresponding amide torsion angles, which increases the barrier by 0.34 kcal/mol. Torsional strain around one of the Pro C^α–C^β bonds also increases the barrier in the crystal system by 0.18 kcal/mol. Thus we see significant differences in the distribution of strain during rotation due to the induced conformational changes in the peptide resulting from the forces applied by neighboring molecules. Taking into account only these torsional differences yields a difference in the barrier of 0.87 kcal/mol in favor of the isolated molecule.

The nonbond component of the barrier can be analyzed in terms of the interactions of the alanine methyl group with the rest of the peptide. It is in the nonbond interactions that the biggest differences between the two systems are found. In both environments, the contribution from interactions with other atoms in the alanine to which the methyl belongs is very similar (1.34 kcal/mol, isolated, and 1.31 kcal/mol, crystal). As Table I shows, however, there are significant differences in the interactions with groups of atoms in other residues, which lead to an increase in the barrier for the isolated system but a decrease in the barrier for the crystal system. For example, the methyl group interacts less favorably with the C^α, H^α, N, C^δ, $H^{\delta 1}$, $H^{\delta 2}$ group of atoms in the adjacent proline as it is rotated in the isolated molecule, but it interacts more favorably in the molecule in the crystal. Again, it interacts more favorably with the methyl group of the opposite alanine in the transition state for the crystal system, but slightly less favorably for the isolated system. Thus the torsional strain is higher for the molecule in the crystal, but this is more than compensated for by the lower nonbonded energy in this environment.

Thus, the torsional and nonbond components together account for 0.42 kcal/mol of the difference in the barrier, in favor of the crystal system. The other ~0.6 kcal/mol of the difference arises from several different sources. Approximately 0.24 kcal/mol comes from differences in bond strain. In the crystal system many bonds in different parts of the molecule are actually less strained at the transition state than in the equilibrium crystal conformation, which serves to reduce the barrier, unlike the isolated system where bond strain adds to the barrier. Similarly, changes in angle strain in different parts of the molecule account for a further difference of 0.29 kcal/mol in the barrier. Thus these additional interactions in the molecules, which give rise to the different barriers, are spread among many different internal coordinates of the peptide.

In order to confirm that the adiabatic barriers correspond to a rate of rotation that is consistent with that observed during dynamics, approximate rates of rotation, at 298 K, were calculated from these barriers. This was done by using the simplified rate equation (Atkins, 1978a)

$$k_2 = (kT/h) \exp(-\Delta H^\ddagger / RT) \qquad (1)$$

where k_2 is the rate of rotation, k is Boltzmann's constant, T is the temperature, h is Planck's constant, $\Delta H^\ddagger$ is the adiabatic barrier to rotation, and R is the gas constant. Using this equation to calculate the approximate rate of rotation of the methyl groups, at 298 K, which would be expected from the calculated barriers (4.8 kcal/mol, isolated, and 3.8 kcal/mol, crystal) yields 0.2 and 1.0 rotation per alanine side chain for the 100-ps trajectory of the isolated and crystal systems, respectively. This is in rough agreement with the 0.5 and 3.8 rotations per side chain observed in the dynamics. The ratio of the rates calculated from these barriers to rotation, 1:5.4 (isolated:crystal), is also in rough agreement with the ratio

Table II: Energy Barriers for Rotation of Alanine Methyl Groups in *cyclo*-(Ala-Pro-D-Phe)$_2$

system	torsion forcing barrier (kcal/mol)	Arrhenius barrier (kcal/mol)
isolated	4.8	4.2
crystal	3.7[a]	3.0

[a] This is the intramolecular component of the barrier to rotation for the peptide in its crystal environment. Intermolecular interactions contribute only 0.1 kcal/mol to the barrier.

seen in the trajectory, 1:7.5, indicating that the behavior observed in the trajectory is representative.

An Arrhenius Experiment: Estimation of the Barrier to Rotation from the Effect of Temperature. A standard experimental method of obtaining the activation energy for a chemical process is to measure the rate of the process as a function of temperature (Atkins, 1978b). From these data, the activation energy can be obtained through the use of an Arrhenius plot. We can carry out the same experiment computationally.

In order to do this, 100-ps molecular dynamics simulations were run of the peptide at approximately 300, 350, 400, 500, 600, and 800 K. Two sets of simulations were run. In the first set, the peptide was simulated in vacuo, while in the second set, the peptide was simulated in the crystal conformation. Since the barrier to rotation in the crystal arises primarily from intramolecular interactions (see above), this simulation was performed on an isolated peptide, with all residues except for one of the alanines tethered to the crystal conformation during the dynamics. The process of tethering works by constraining each of the tethered atoms to its initial position by using a harmonic potential function. In this case, all atoms of every residue except for one of the alanines were tethered in the crystal conformation by using a force constant of 25 kcal mol^{-1} Å^{-1}. Running the experiment in this way gives rise to considerable savings in computer time compared with running simulations including the full crystal environment.

The relationship between the number of rotations of the methyl group (reaction rate) and the temperature is given by

$$k_2 = A \exp(-E_a/RT) \quad (2)$$

where k_2 is the rate of rotation, T is the temperature, A is a preexponential factor, E_a is the activation energy, and R is the gas constant. From this equation, it follows that

$$\ln k_2 = \ln A - E_a/RT \quad (3)$$

A and E_a can therefore be determined from a plot of $\ln k_2$ against $1/T$ (an Arrhenius plot), in which the slope is $-E_a/R$ and the intercept is $\ln A$.

Figure 3 shows the Arrhenius plots for the isolated and crystal systems. The activation energies calculated from these plots are compared in Table II with the barriers to rotation obtained from the torsion angle forcing experiments. Again this reveals that the barrier calculated from the Arrhenius plot is lower for the crystal conformation than for the isolated peptide, consistent with the results from the torsion angle forcing and from the 300 K dynamics simulations (see Figure 1). It also shows that the barriers from the Arrhenius plots are lower than from the torsion angle forcings, by 0.6 kcal/mol for the isolated peptide and by 0.7 kcal/mol for the crystal conformation. This is reasonable, since the torsion forcing experiment yields an upper bound to the barrier and does not allow for dynamic fluctuations in the molecule, which can serve to lower the barriers. This result is also consistent with the too low rates of rotation calculated from the barriers obtained by torsion angle forcing (see above).

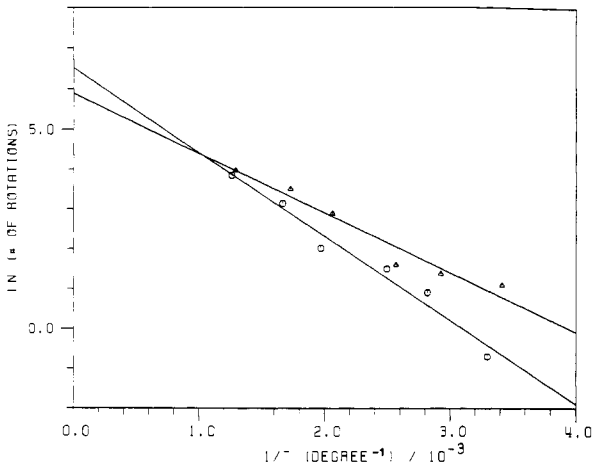

FIGURE 3: Arrhenius plots showing how the rate of rotation of the alanine methyl groups in *cyclo*-(Ala-Pro-D-Phe)$_2$ varies as a function of temperature during molecular dynamics simulations of the peptide. The circles represent the data for the isolated peptide, and the triangles represent the data for the simulation in which all of the residues except for one of the alanines were tethered to the crystal conformation. The best fitting straight lines have been drawn through each of the sets of points. The slope of the lines is equal to $-E_a/R$, where E_a is the activation energy (the barrier to rotation) and R is the gas constant. The steeper slope for the isolated peptide indicates that this system has a higher barrier than the crystal peptide.

Discussion

Comparisons of the conformations adopted by *cyclo*-(Ala-Pro-D-Phe)$_2$ in the crystal and in vacuo (Kitson & Hagler, 1988) have shown that the peptide can adopt a conformation in vacuo that is ≈8.5 kcal/mol lower in energy than the conformation found in the crystal. The crystal is, therefore, providing an environment that is inducing a strained conformation of the peptide through favorable intermolecular interactions.

One can, therefore, look on the role that the crystal environment plays in determining the rate of rotation of the methyl groups as a form of catalysis. A conventional catalyst operates by lowering the activation energy (i.e., the barrier) for a chemical process, thereby allowing the process to take place more rapidly. Similarly, in this case, the crystal environment is inducing the peptide to adopt a conformation in which the barrier to rotation of the methyl groups is lowered with respect to the isolated molecule, resulting in a more rapid rate of rotation.

We can also consider this system to be a simple model for the process of message transduction that takes place when a molecule such as a hormone binds to a receptor. In this process, the binding of the hormone to the receptor leads to a change in the complex that causes a "message" to be sent to the next step in the system. In a similar way, the "binding" of *cyclo*-(Ala-Pro-D-Phe)$_2$ to its crystal environment (i.e., the incorporation of the peptide into its crystal lattice) leads to a conformational change in the peptide, which in turn leads to an increased rate of rotation of the alanine groups. This increased rate of rotation could be considered analogous to the sending of the message in the biological system described above (although in this case it is hard to conceive of the receiver).

Thus, we have shown that an unexpected catalytic effect of the crystal environment on the rate of rotation of the methyl groups in *cyclo*-(Ala-Pro-D-Phe)$_2$ can be accounted for in terms of the underlying interatomic interactions. This illustrates the

power of theoretical techniques such as energy minimization and molecular dynamics to investigate the structural, energetic, and dynamic properties of biomolecules. It also suggests further experiments to probe the fundamental dynamics of peptides and verify the predictions. Thus, low-temperature NMR of *cyclo*-(Ala-Pro-D-Phe)$_2$ deuteriated in the alanine methyl should be able to confirm the relative rotation rates (S. Opella, private communication) and will be attempted.

ACKNOWLEDGMENTS

We thank Dr. F. Avbelj for helpful discussions and advice.

REFERENCES

Atkins, P. W. (1978a) *Physical Chemistry*, pp 906–914, W. H. Freeman, San Francisco, CA.

Atkins, P. W. (1978b) *Physical Chemistry*, pp 864–866, W. H. Freeman, San Francisco, CA.

Brown, J. N., & Teller, R. G. (1976) *J. Am. Chem. Soc. 98*, 7565–7569.

Dauber, P., & Hagler, A. T. (1980) *Acc. Chem. Res. 13*, 105–112.

Hagler, A. T. (1985) *Peptides (N.Y.) 7*, 213–299.

Hagler, A. T., Lifson, S., & Dauber, P. (1979) *J. Am. Chem. Soc. 101*, 5122–5130.

Hall, D., & Pavitt, N. (1984) *J. Comput. Chem. 5*, 441–450.

Kitson, D. H., & Hagler, A. T. (1988) *Biochemistry 27*, 5246–5257.

Kossiakoff, A. A., & Shteyn, S. (1984) *Nature (London) 311*, 582–583.

Momany, F. A., Corruthers, L. M., McGuire, R. F., & Scheraga, H. A. (1974) *J. Phys. Chem. 78*, 1595–1620.

Williams, D. E. (1965) *J. Chem. Phys. 43*, 4424–4426.

Wittebort, R. J., Rothgeb, T. M., Szabo, A., & Gurd, F. R. N. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1059–1063.

Preparation and Characterization of Singly Labeled Ruthenium Polypyridine Cytochrome *c* Derivatives†

Lian Ping Pan, Bill Durham,* Justyna Wolinska, and Francis Millett

Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas 72701

Received June 23, 1988; Revised Manuscript Received July 22, 1988

ABSTRACT: A novel two-step procedure has been developed to prepare cytochrome *c* derivatives labeled at specific lysine amino groups with ruthenium bis(bipyridine) dicarboxybipyridine [$Ru^{II}(bpy)_2(dcbpy)$]. In the first step, cytochrome *c* was treated with the mono-*N*-hydroxysuccinimide ester of 4,4′-dicarboxy-2,2′-bipyridine (dcbpy) to convert positively charged lysine amino groups to negatively charged dcbpy-lysine groups. Singly labeled dcbpy-cytochrome *c* derivatives were then separated and purified by ion-exchange chromatography. In the second step, the individual dcbpy-cytochrome *c* derivatives were treated with $Ru^{II}(bpy)_2CO_3$ to form singly labeled $Ru^{II}(bpy)_2$(dcbpy-cytochrome *c*) derivatives. The specific lysine labeled in each derivative was determined by reverse-phase chromatography of a tryptic digest. All of the derivatives had a strong luminescence emission centered at 662 nm, but the luminescence decay rates were increased relative to that of a non-heme protein control, $Ru^{II}(bpy)_2$(dcbpy-lysozyme), which was 1.8×10^6 s^{-1}. The luminescence decay rates were found to be 21, 16, 7.2, 5.7, 4.3, 4.3, and 3.5×10^6 s^{-1} for derivatives singly labeled at lysines 13, 72, 25, 7, 39, 86, and 87, respectively. There was an inverse relationship between the luminescence decay rates and the distances between the ruthenium labels and the heme group. The increased luminescence decay rates observed in the cytochrome *c* derivatives might be due to electron transfer from the excited triplet state of ruthenium to the ferric heme group. However, it is also possible that an energy-transfer mechanism might contribute to the luminescence quenching. The luminescent decay rates of the ferrocytochrome *c* derivatives were nearly as large as those of the corresponding ferricytochrome *c* derivatives, suggesting the possibility of electron transfer from ferrous heme to the excited triplet state of ruthenium.

Our understanding of the factors involved in biological electron transfer has been significantly enhanced by the use of metalloproteins specifically labeled with ruthenium complexes. In the pioneering work of Winkler et al. (1982) and Isied et al. (1982), intramolecular electron transfer was found to take place between a $Ru^{II}(NH_3)_5$ group attached to histidine 33 of cytochrome *c* and the ferric heme group with a rate constant of about 30 s^{-1}. This long-range electron-transfer reaction has a driving force of 0.11 eV and a separation of about 12 Å between the ruthenium and the heme group. Paradoxically, Bectold et al. (1986) found that the rate of electron transfer from the ferrous heme group of cytochrome *c* to $Ru^{III}(NH_3)_4$(isonicotinamide)(histidine 33) was over 10^5 slower than the above rate, even though the driving force was larger (0.18 eV). Elias et al. (1988) have recently prepared a zinc-substituted derivative of cytochrome *c* containing $Ru^{III}(NH_3)_5$(histidine 33) and found that the rate of electron transfer from the triplet excited zinc porphyrin to ruthenium was 7.7×10^5 s^{-1}, while the rate of the thermal back-reaction was 1.6×10^6. The much larger rate constants for these reactions relative to those involving the native iron are presumably due to the large driving force of the reactions (0.88 V for the reaction involving triplet zinc). Intramolecular electron-transfer studies have also been carried out on ru-

† This work was supported by NIH Grants GM20488 and RR07101.

0006-2960/88/0427-7180$01.50/0 © 1988 American Chemical Society