PRESSURE DEPENDENCE OF AROMATIC RING ROTATIONS IN PROTEINS: A COLLISIONAL INTERPRETATION

Martin KARPLUS* and J. Andrew McCAMMON+

*Department of Chemistry, Harvard University, Cambridge, MA 02138, USA, *Laboratoire D'Enzymologie Physicochimique et Moléculaire, Université de Paris-Sud, 91405 Orsay, France and +Department of Chemistry, University of Houston, Houston, TX 77004, USA*

Received 16 June 1981

Activated processes are of central importance to many biochemical phenomena, including ligand binding and enzyme catalysis [1,2]. A simple model for such processes, provided by the rotation ('flipping') of aromatic amino acid sidechains in the interior of globular protein has been studied intensively by experimental [3–8] and theoretical techniques [9–12]. Energy minimization [9,10] and activated trajectory [11,12] calculations have demonstrated that the nature of the rotational transition and its effective barrier are determined by the positions and fluctuations of the protein matrix atoms surrounding the aromatic ring. The importance of frictional effects for the ring motion and for other processes involving fluctuations in the protein interior has been pointed out [11–15].

Recently, Wagner [16,17] has determined the hydrostatic pressure dependence of the aromatic ring rotation rates in the bovine pancreatic trypsin inhibitor (PTI). Over the measured range (1 -1200 atm), interpretation of the rate data for two of the rings (Phe 45 and Tyr 35) in terms of transition state theory [18]:

$$\left(\frac{\partial \ln k}{\partial p}\right)_T = -\frac{\Delta V^\dagger}{k_B T} \qquad (1)$$

yielded activation volumes, $\Delta V^\dagger$, of about 50 Å^3; the positive sign of $\Delta V^\dagger$ corresponds to a decrease of the rate with increasing pressure.

The observed magnitude of the activation volume, on the order of that associated with protein denaturation [19,20], provides an important test for the theoretical interpretation of the ring rotation process given previously [9–12]. For motion in the interior of a protein, as for solution reactions [21] in general, the pressure dependence of the rate constant is not related directly to a physical volume change between the reactant and transition state. Instead, it can be dominated by the interactions between the reacting species and the solvent environment, which in the case of the ring rotations is provided by the surrounding protein atoms. To analyze the factors involved, we make use of the Kramers formulation for an activated process in the diffusive limit [21–24]; this is an approximation since dynamical calculations [11,12] suggest that the ring motion is in the intermediate damping regime [22,23]. Considering the ring flipping as a one-dimensional problem defined by the ring rotation angle, we can write the rate constant as [24]:

$$k = \frac{\omega_i\, \omega_{ts}}{2\pi}\left(\frac{I_r}{f_r}\right) e^{-\Delta H^\dagger / k_B T} \qquad (2)$$

where $\Delta H^\dagger$ is the effective activation enthalpy, ω_i and ω_{ts} are the vibrational frequencies in the initial well and at the top of the inverted barrier, respectively, and f_r and I_r are the rotational friction coefficient and moment of inertia of the aromatic ring. Previous studies have shown that except for the moment of inertia, all of the parameters in eqn 2 are determined by interactions between the aromatic ring and the surrounding protein matrix; the intrinsic torsional potential of the ring is negligible [9–12]. Since compression of the protein will tend to decrease the distances between the ring and the matrix atoms, a pressure dependence for the rate constant is expected from eqn 2. The changes in the activation enthalpy, $\Delta H^\dagger$, and the frictional coefficient, f_r, are expected to be most important; we have:

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50 © 1981 Federation of European Biochemical Societies

$$\left(\frac{\partial \ln k}{\partial p}\right)_T \cong -\left(\frac{\partial \ln f_r}{\partial p}\right)_T - \frac{1}{k_B T}\left(\frac{\partial \Delta H^\dagger}{\partial p}\right)_T \quad (3)$$

To estimate the order of magnitude of the two terms in eqn 3, we make use of the known properties of liquid hydrocarbons because the aromatic rings are located in the hydrophobic regions of PTI; of interest are the pressure dependence between 1 and 1000 atm of the viscosity, η [25]:

$$\left(\frac{\partial \ln \eta}{\partial p}\right)_{T=25°C} \cong 8 \times 10^{-4}\ \text{atm}^{-1}$$

and the isothermal compressibility, β_T [26]:

$$\beta_T = -\left(\frac{\partial \ln V}{\partial p}\right)_{T=25°C} \cong 1 \times 10^{-4}\ \text{atm}^{-1}$$

No measurements of the internal protein viscosity or of its pressure dependence are available; analysis of the calculated picosecond dynamics [13] of the tyrosine rings in PTI yields a viscosity about a factor of two smaller than that of liquid hydrocarbons. Measurements of the adiabatic compressibility of proteins yield values in the range $1-5 \times 10^{-5}$ atm^{-1} for the entire molecule [27,28]. We employ a slightly higher value to take account of the fact that proteins are inhomogeneous systems and that larger compressibilities are expected for the hydrophobic regions, relative to the more rigid portions that involve secondary structure.

By use of Stokes' law, we have $[(\partial \ln f_r)/(\partial p)]_T = [(\partial \ln \eta)/(\partial p)]_T$; with the pressure dependence of η, given above, we find that the rate constant decreases by a factor of 2.46 for an increase in pressure from 1 to 1000 atm; this corresponds by eqns 1 and 3 to an effective activation volume of about 35 Å^3. A more physical picture of the viscosity effect can be obtained by use of a collisional model employed previously [13]. From the compressibility, the relative change $\Delta\ell/\ell$ in nonbonded interatomic distances in the protein due to a change Δp in pressure is:

$$3\Delta\ell/\ell = \Delta V/V = -\beta_T\, \Delta p \quad (4)$$

For $\Delta p = 10^3$ atm, we have $\Delta\ell/\ell = -0.033$. Since this decrease in interatomic distances occurs in the small gaps between the atoms, it leads to a substantial reduction of the mean free path between collision partners. To estimate the resulting change in collision frequency, we consider a specific arrangement of atoms. A matrix atom is assumed to be located on a line which passes through the center of a ring δ or ϵ carbon atom and which is normal to the plane of the ring. For the hard-sphere radii of the matrix and ring atoms, we use the values $R_m = 1.35$ Å (e.g., an O or N atom) and $R_r = 1.70$ Å, respectively [16]; the gap between the surfaces of these atoms at $p = 1$ atm is taken equal to the mean free path derived elsewhere [13], $R_g = 0.15$ Å. Thus, the total distance between atomic centers at $p = 1$ atm is $R_t = R_m + R_r + R_g = 3.20$ Å. At 1000 atm, R_t is reduced to 3.094 Å according to eqn 4; this corresponds to a decrease of R_g to 0.044 Å. The collision frequency, τ^{-1}, which varies inversely with the mean free path [13], thus increases by a factor of 3.4 between 1 and 1000 atm. Since the frictional coefficient in proportional to the collision frequency [21–24], this model calculation is in accord with the pressure dependence obtained above for the pre-exponential factor in eqn 2. More generally, it can be shown that for $p << \beta_T^{-1}$, the collisional model yields a rate constant that has an exponential dependence on p with an apparent activation volume $\Delta V^\dagger \approx \beta_T k_B T(R_t/3R_g)$.

The pressure dependence of the energy barrier contribution to the rate constant can be estimated by a similar argument. In earlier studies [9–12] it was shown that the dominant contribution to the rotation barrier comes from a small number of nonbonded contacts between the ring and the surrounding protein matrix atoms and that relaxation of the protein relative to the rotating ring leads to an important reduction of the static barrier. Since the relaxation is expected to be less effective in the compressed protein, an increased barrier will exist at higher pressures. To indicate the magnitude of this effect, we use the mean nonbonded contact distance found for Tyr 35 in the transition state geometry (2.90 Å) and eqn 4 with the appropriate van der Waals parameters [9,13]; the resulting increase in the barrier height is ~1 kcal, which corresponds to a rate constant decrease by a factor of 5.3 and an effective activation volume of 75 Å^3.

The present analysis, although illustrative rather than quantitative, indicates that the pressure dependence of the aromatic ring rotation rates in the interior of proteins is accounted for by the interactions between the ring and the protein matrix atoms.

Both the pre-exponential factor (increased viscosity) and the barrier height (increased potential of mean force) contribute. Thus, the large effective activation volume deduced from the observed pressure dependence does not imply a physical volume change (free volume model) on the order of that determined experimentally. Instead, it is the pressure dependence of small packing defects, which have been shown to play a role in initiating the ring rotation [11,12], that are the essential elements in the apparent activation volumes.

Acknowledgements

We thank Dr G. Wagner for sending copies of his paper prior to publication. This work has been supported in part by grants from the NSF, the NIH, the Robert A. Welch Foundation, and the Petroleum Research Fund as administered by the American Chemical Society. J. A. McCammon is an Alfred P. Sloan Fellow and is the recipient of an NIH Research Career Development Award.

References

[1] Karplus, M. and McCammon, J. A. (1981) CRC Crit. Rev. Biochem. 9, 293–349.
[2] McCammon, J. A. and Karplus, M. (1980) Annu. Rev. Phys. Chem. 31, 29–45.
[3] Campbell, I. D., Dobson, C. M. and Williams, R. J. P. (1978) Adv. Chem. Phys. 39, 55–107.
[4] Gurd, F. R. N. and Rothgeb, T. M. (1979) Adv. Protein Chem. 33, 73–165.
[5] Snyder, G. H., Rowan, R., Karplus, S. and Sykes, B. D. (1975) Biochemistry 14, 3765–3777.
[6] Wagner, G., DeMarco, A. and Wüthrich, K. (1976) Biophys. Struct. Mech. 2, 139–158.
[7] Campbell, I. D., Dobson, C. M., Moore, G. R., Perkins, S. J. and Williams, R. J. P. (1976) FEBS Lett. 70, 96–100.
[8] Moore, G. R. and Williams, R. J. P. (1980) Eur. J. Biochem. 103, 513–521.
[9] Gelin, B. R. and Karplus, M. (1975) Proc. Natl. Acad. Sci. USA 72, 2002–2006.
[10] Hetzel, R., Wüthrich, K., Deisenhofer, J. and Huber, R. (1976) Biophys. Struc. Mech. 2, 159–180.
[11] McCammon, J. A. and Karplus, M. (1979) Proc. Natl. Acad. Sci. USA 76, 3585–3589.
[12] McCammon, J. A. and Karplus, M. (1980) Biopolymers 19, 1375–1405.
[13] McCammon, J. A., Wolynes, P. G. and Karplus, M. (1979) Biochemistry 18, 927–942.
[14] Gavish, B. and Werber, M. M. (1979) Biochemistry 18, 1269–1275.
[15] Beece, D., Eisenstein, L., Frauenfelder, H., Good, D., Marden, M. C., Reinich, L., Reynolds, A. H., Sorensen, L. B. and Yue, K. T. (1980) Biochemistry 19, 5147–5157.
[16] Wagner, G. (1980) FEBS Lett. 112, 280–284.
[17] Wüthrich, K., Wagner, G., Richarz, R. and Braun, W. (1980) Biophys. J. 32, 549–558.
[18] Glasstone, S., Laidler, K. and Eyring, H. (1941) Theory of Rate Processes, McGraw-Hill New York.
[19] Brandts, J. F., Oliveira, R. J. and Westart, C. (1970) Biochemistry 9, 1038–1047.
[20] Zipp, A. and Kauzmann, W. (1973) Biochemistry 12, 4217–4228.
[21] Montgomery, J. A., Chandler, D. and Berne, B. J. (1979) J. Chem. Phys. 70, 4056–4066.
[22] Kramers, H. A. (1940) Physica 7, 284–304.
[23] Skinner, J. L. and Wolynes, P. G. (1978) J. Chem. Phys. 69, 2143–2150.
[24] Levy, R. M., Karplus, M. and McCammon, J. A. (1979) Chem. Phys. Lett. 65, 4–11.
[25] Landholt-Börnstein (1969) Zahlenwerte and Functionen, Transportphänomene I, p. 153, Springer Verlag, Berlin.
[26] Weast, R. C. (ed) (1966) CRC Handbook of Chemistry and Physics, 47th edition pp. F-9 to F-11.
[27] Gavish, B., Gratton, E. and Hardy, C. (1981) Biophys. J. 33, 261a.
[28] Millero, F. J., Ward, G. K. and Chetirkin, P. (1976) J. Biol. Chem. 251, 4001–4004.