Baumstark, *J. Am. Chem. Soc.*, **98**, 1086 (1976), and references cited therein; (d) H. Basch, *Theor. Chim. Acta*, **28**, 151 (1973). We thank Professor C. Trindle (University of Virginia) for this reference.
(3) For reviews, see (a) G. L. Closs in *Top. Stereochem.*, **3**, 193 (1968); (b) P. P. Gaspar and G. S. Hammond in "Carbenes", Vol. II, M. Jones, Jr., and R. A. Moss, Ed., Wiley, New York, N.Y., 1974.
(4) (a) T. McConaghy and W. Lwowski, *J. Am. Chem. Soc.*, **89**, 2357, 4450 (1967); (b) W. Lwowski, "Nitrenes", Wiley-Interscience, New York, N.Y., 1970, p 170; (c) W. H. Richardson, F. C. Montgomery, M. B. Yelvington, and H. E. O'Neal, *J. Am. Chem. Soc.*, **96**, 7525 (1974).
(5) R. J. Crawford and D. M. Cameron, *J. Am. Chem. Soc.*, **88**, 2589 (1966).
(6) J. A. Berson, C. D. Duncan, and L. R. Corwin, *J. Am. Chem. Soc.*, **96**, 6175 (1974).
(7) J. A. Berson, L. R. Corwin, and J. H. Davis, *J. Am. Chem. Soc.*, **96**, 6177 (1974).
(8) R. J. Crawford and A. Mishra, *J. Am. Chem. Soc.*, **88**, 3963 (1966).
(9) S. G. Cohen, R. Zand, and C. Steel, *J. Am. Chem. Soc.*, **83**, 2895 (1961).
(10) J. A. Berson, R. J. Bushby, J. M. McBride, and M. Tremelling, *J. Am. Chem. Soc.*, **93**, 1544 (1971).
(11) M. S. Platz, J. M. McBride, R. D. Little, A. Shaw, J. J. Harrison, S. E. Potter, and J. A. Berson, paper in preparation.
(12) It must be recognized that the singlet may not actually be the diyl **4** but instead may be one of its unstable ring-closed isomers, 5-isopropylidenebicycyo[2.1.0]pentane or 6,6-dimethylbicyclo[3.1.0]hex-1-ene.
(13) (a)M. J. S. Dewar and J. S. Wasson, *J. Am. Chem. Soc.*, **93**, 3081 (1971); (b) W. W. Schoeller, *Tetrahedron Lett.*, 2043 (1973); (c) W. Hehre, L. Salem, and M. R. Willcott, *J. Am. Chem. Soc.*, **96**, 4328 (1974); (d) W. T. Borden, *ibid.*, **97**, 2906 (1975); (e) D. R. Yarkony and H. F. Schaeffer, *ibid.*, **96**, 3754 (1974); (f) J. H. Davis and W. A. Goddard, *J. Am. Chem. Soc.*, **98**, 303 (1976).

Jerome A. Berson,* Charles D. Duncan
Genevieve C. O'Connell, Matthew S. Platz
Department of Chemistry, Yale University
New Haven, Connecticut 06520
Received December 8, 1975

Temperature Dependence of Positron Lifetimes in Carbonic Anhydrase

Sir:

There are numerous reviews dealing with positron annihilation in matter,[1] yet biochemical applications are still in their early stages. The technique was applied to optical isomers[2] and amino acids[3] but positron annihilation in proteins was reported only recently.[4] Here we present preliminary results for the temperature dependence of the lifetime and intensity parameters in carbonic anhydrase and attempt to correlate the variation of free volume and the changes in conformation of this enzyme.

The sample was prepared by mixing 2.0 g of chromatographically pure bovine carbonic anhydrase (carbonate dehydratase, EC 4.2.1.1)[5] with 4.0 g of deionized distilled water and placing the homogeneous mixture into a glass sphere (22 mm i.d.; 1-mm wall thickness). The source was prepared by enclosing 15 μCi of carrier-free ^{22}Na (as aqueous NaCl) in a sealed thin-walled glass bead (1 mm o.d.; <0.1 mm wall thickness).[6] The bead was positioned at the center of the glass sphere containing the sample. The system was degassed and sealed under vacuum (10^{-5} Torr) and it was immersed in a jacketed cell connected to a circulating thermostat. Positron lifetimes were measured with a standard fast-slow coincidence system[7] utilizing constant fraction timing and Naton 136 scintillators (3.8 cm diameter; 1.3 cm thickness) mounted to RCA 8575 photomultipliers with a time resolution of 0.2 ns. The geometry of the system and the sample thickness (200 mg/cm^2) were chosen to optimize the solid angle between the source and detectors. The time spectra were analyzed on an IBM 360/50 computer using a computational method developed by Cumming.[8]

Positron annihilation intensities and lifetimes were measured over a temperature range −20 to 50°. The lifetime spectra could be resolved into two components. In all cases the lifetime (τ_1) of the short-lived component remained constant within experimental uncertainties. The temperature dependence of

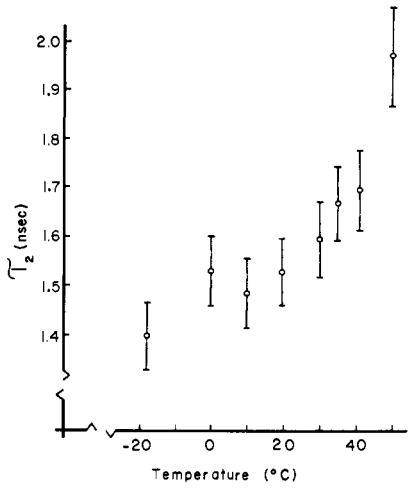

Figure 1. Temperature dependence of the lifetime of the long-lived component of positron annihilation in carbonic anhydrase.

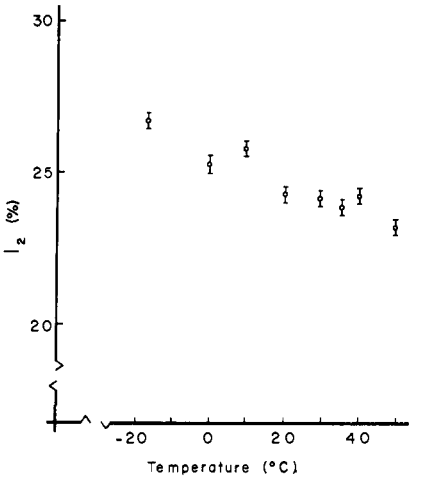

Figure 2. Temperature dependence of the intensity of the long-lived component of positron annihilation in carbonic anhydrase.

the lifetime (τ_2) and the intensity (I_2) of the long-lived component is shown in Figures 1 and 2. The lifetime τ_2 increases moderately between −20 and 40° and then rises steeply to 50°. The intensity I_2 decreases slightly from 27 ± 1 to 23 ± 1% in the temperature range −20 to 50°. Within experimental uncertainties the product $I_2\tau_2$ (Figure 3) is constant over the temperature range −20 to 30°. Above this temperature rapid increase of $I_2\tau_2$ is observed. Over the temperature range 0–50° the activity of carbonic anhydrase increases steadily[9] (Figure 4).

Changes in activity are associated with conformational changes of the molecule. At the present time, the nature of these changes is not well understood.[10] Qualitative evaluation of our data in terms of the free volume model[11] allows for the following speculative interpretation of I_2 and τ_2. Since pick-off annihilations are associated only with the long-lived component, the I_2 values represent the number of free volume sites where pick-off annihilations occur.[12] The decrease in the number of free volume sites at higher temperatures is interpreted as the redistribution and collection of free volumes into a system compatible with the new conformation at the elevated temperature. Increasing τ_2 values may correspond to an increase of the average size of the free volume sites which is a further indication of the collection of free volumes. In carbonic anhydrase the total free volume, $I_2\tau_2$ (Figure 3) increases with increasing temperature in the range of 30–50°. These findings, when correlated with enzyme activity measurements, indicate that the increase in activity involves conformational changes

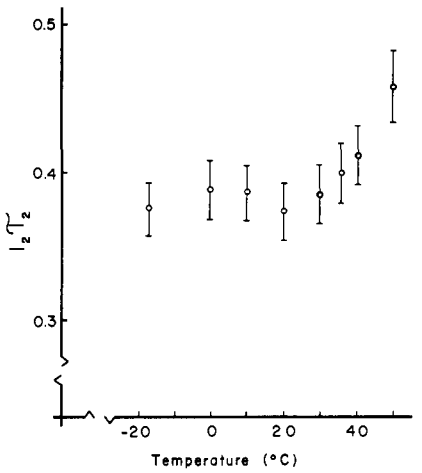

Figure 3. The intensity-lifetime product as function of temperature for the long-lived component of positron annihilation in carbonic anhydrase.

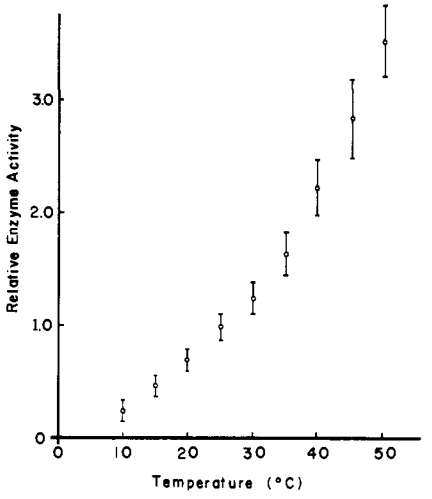

Figure 4. The relative esterase activity of carbonic anhydrase at various temperatures using *p*-nitrophenyl acetate as substrate. The measurements were made using the stopped-flow technique. The reaction mixture contained bovine carbonic anhydrase, 1.0×10^{-4} M; *p*-nitrophenyl acetate, 2.0×10^{-4} M; Tris-HCl, pH 8.0, 0.009 M; at ionic strength, 0.09 M (NaCl).

in which free volume regions rearrange or combine.[13] Thermal denaturation may be associated with the overall expansion of the molecule.[9] It appears to us that over a certain temperature range the positron is a selective probe of protein conformation. Further work is in progress to expand this correlation into a quantitative model by studying the effect of temperature, inhibitor binding, concentration, and pH on the positron annihilation parameters in a variety of polypeptides and proteins of well-defined conformation.

Acknowledgment. Support for this work by the North Dakota Agricultural Experiment Station, Fargo, N.D. 58102, is gratefully acknowledged.

References and Notes

(1) (a) J. Green and J. Lee, "Positronium Chemistry", Academic Press, New York, N.Y., 1964; (b) A. T. Stewart and L. O. Roellig, Ed., "Positron Annihilation", Academic Press, New York, N.Y., 1967; (c) J. A. Merrigan, J. H. Green, and S. J. Tao in "Physical Methods of Chemistry", Part IIID, Vol. I, A. Weissberger and B. W. Rossiter, Ed., Wiley-Interscience, New York, N.Y., 1972, pp 501–586; (d) H. J. Ache, *Angew. Chem., Int. Ed. Engl.*, 11, 179 (1972).

(2) A. S. Garay, L. Keszthelyi, T. Demeter, and P. Hrasko, *Chem. Phys. Lett.*, **23**, 549 (1973).

(3) I. Dezsi, D. Horvath, and Zs. Kajcsos, *Chem. Phys. Lett.*, **24**, 514 (1974).

(4) (a) P. C. Jain, S. C. Pancholi, and M. M. Gupta, *Indian J. Biochem. Biophys.*, **10**, 85 (1973); (b) S. Y. Chuang and S. J. Tao, *J. Phys. Chem.*, **78**, 1261 (1974); (c) P. C. Jain, R. R. Jain, P. Joshi, and M. M. Gupta, *Indian J. Biochem. Biophys.*, 11, 340 (1974).

(5) S. Lindskog, L. E. Henderson, K. K. Kannan, A. Liljas, P. O. Nyman, and B. Strandberg in "The Enzymes", Vol. V, 3d ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, pp 587–665.

(6) The positron annihilation in the source holder was negligible. Our data in Figures 1 and 2 show a small effect near 0° which might be attributed to residual bulk water. The overall temperature effect, however, is quite different from that observed in bulk water. Studies of bulk water have been discussed in ref 1b.

(7) G. Graf, J. C. Glass, and L. L. Richer in "Protein-Metal Interactions", M. Friedman, Ed., Plenum Publishing, New York, N.Y., 1974, pp 639–679.

(8) J. B. Cumming, BNL Report 6470. The authors are grateful for H. J. Ache and R. Wild for supplying their version of this program.

(9) E. D. Handel, unpublished results for esterase activity. (For CO_2 hydration see J. C. Kernohan, *Adv. Exp. Med. Biol.*, **28**, 189 (1972)). Prolonged incubation at 40° and at higher temperatures produces inactivation. In the irreversibly denatured protein τ_2 and $I_2\tau_2$ remained high even after returning to lower temperatures.

(10) Interactions with water are essential for conformational stability. Under conditions of our experiments protein concentrations are probably too high to allow direct comparison with thermal unfolding in dilute carbonic anhydrase solutions. However, experimental studies of the protein–water system are possible if the results are compared to a reproducible standard such as biological activity. The effect of protein concentration on τ_2 and I_2 is under investigation in our laboratory.

(11) (a) W. Brandt, S. Berko, and W. W. Walker, *Phys. Rev.*, **120**, 1289 (1960); (b) B. V. Thosar, R. G. Lagu, V. G. Kulkarni, and G. Chandra, *Phys. Status Solidi B.*, **55**, 415 (1973).

(12) The average lifetime of the o-Ps is reduced by various interactions with the substrate. Our lifetime measurements indicate pick-off annihilation to be the major process. In pick-off annihilation the positron is removed from the o-Ps under the influence of the electron cloud of the surrounding and undergoes free annihilation. In the free volume model τ_2 is related to the size of the cavities in which the o-Ps atoms are confined, and I_2 represents the number of o-Ps atoms which are formed and subsequently quenched at these sites. In our discussion, I_2 depends primarily on the number of free volume sites associated with the temperature dependent structural characteristics of the protein. The concept is being further tested in our laboratory.

(13) See R. Lumry and S. Rosenberg, "Proceedings of the Conference on Water at Roscoff, France", A. Alfsen and A. Berteaud, Ed., French National Research Center, Paris, France (in press); accordingly in folded proteins there are poorly H-bonded regions called defects providing a reservoir for potential energy. Some defects will be mobile due to the easy translocation of defect situations linked to conformation.

Edward D. Handel, G. Graf*

Department of Biochemistry, North Dakota State University
Fargo, North Dakota 58102

James C. Glass

Department of Physics, North Dakota State University
Fargo, North Dakota 58102

Received December 1, 1975

A Comparison of Translational Energies Released during Metastable Decomposition Following Electron Impact and Field Ionization. A Test of the QET Model and Mechanistic Probe

Sir:

We report here the first comparison between translational (kinetic) energies released during the decomposition of metastable ions formed by electron impact at 70 eV (EI) and field ionization (FI). We suggest that this is important for the following two reasons. The central tenet of the quasi-equilibrium theory (QET) is that of energy equilibration.[1] Failure to randomize vibrational energy[2] and/or nonrecognition of isolated electronic states[3] would complicate or undermine the QET approach. Rosenstock et al.[4] have outlined the underlying argument which predicts that if a particular molecule is ionized by two different methods, provided the internal energy of the molecular ion is equilibrated, the decomposition characteristics at times $>10^{-7}$ s in both cases should be the same. The two methods, EI at 70 eV and FI, provide a particularly stringent test of this point. Only the very lowest electronic states are expected to be reached by FI whereas EI at 70 eV should also yield more highly excited states. Further, even where the distributions of the total excitation energy overlap, the initial