The Esterase Activity of Bovine Erythrocyte Carbonic Anhydrase. Rapid Enzyme-catalysed Hydrolysis of 2-Hydroxy-5-nitro-α-toluenesulphonic Acid Sultone

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CARBONIC ANHYDRASE has been shown to possess esterase activity[1] on carboxylic acid esters in addition to its role as a catalyst for the reversible hydration of carbon dioxide[2] and other carbonyl systems.[3] Many sulphonamides are strongly bound inhibitors of the action of carbonic anhydrase,[1b,4] and this has prompted us to test the possibility that certain sulphonate esters might be substrates of the enzyme. We now report that carbonic anhydrase hydrolyses the cyclic sulphonate, 2-hydroxy-5-nitro-α-toluenesulphonic acid sultone (I), with extraordinary efficiency. Indeed, at the present time this sultone appears to be the most rapidly hydrolysed ester substrate of carbonic anhydrase known.

Direct nitration of (II), the parent sultone, by the method of Marckwald and Frahne, gave (I).[5,*] The hydrolysis of (I) is followed spectrophotometrically, with the appearance of the phenolate anion at 410 mμ (for pH > 7) or the corresponding phenol at 320 mμ (for pH 5—7). At pH 7·5 and 25·0°, k_{enz}† was found to be 1·27 × 10^6 l. $mole^{-1}$ $min.^{-1}$. A pH–rate profile was obtained for the pH range of 5 to 9. The curve has an inflection point between pH 7·2—7·4, which is very close to that obtained for the hydration of carbon dioxide (pK = 7·2[6]) and for the hydrolysis of *p*-nitrophenyl acetate (pK = 7·5[1d]). This could imply that the imidazole moiety of a histidine residue in the enzyme is involved in the catalysis, although we do not have any evidence to substantiate this at present.

The effect of the nitro-substituent in labilizing the sultone (I) to attack by carbonic anhydrase has been investigated. The value of k_{enz} for the unsubstituted ester (II) is 1·1 × 10^2 l. $mole^{-1}$ $min.^{-1}$ at pH 7·5, and thus, the nitro-group in (I) is responsible for a 10^4-fold increase in the rate constant for enzymic catalysis. This factor is considerably greater than the 30—40-fold acceleration observed for the hydroxide-ion catalysed hydrolysis of (I) compared to (II).

Similarities appear to exist between the behaviour of carbonic anhydrase in its catalysis of the hydration of carbon dioxide, of the hydrolysis of *p*-nitrophenyl acetate, and of the hydrolyses of the sulphonate esters. Thus, acetazoleamide, a strong inhibitor for the hydration of carbon dioxide, inhibits the enzymatic hydrolysis of (I) non-competitively with a K_I value of 1·45 × 10^{-7} mole $l.^{-1}$ Pocker[1d] had shown that K_I = 3·7 × 10^{-6} mole $l.^{-1}$ for the inhibition by acetazoleamide of the carbonic anhydrase-catalysed hydrolysis of *p*-nitrophenyl acetate at pH 8·45 and 25·0°.

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* The preparation of (II) is described by O. R. Zaborsky and E. T. Kaiser in *J. Amer. Chem. Soc.*, 1966, **88**, 3084. The position at which nitration occurred in (II) and hence the structure of (I) was established by an independent synthesis of (I) starting from *p*-nitrophenol.

† k_{enz} is defined as $(k_{obs} - {}_{spont})/E$, where k_{spont} is the spontaneous hydrolysis rate constant for the nitrosultone itself, and E is the enzyme concentration. k_{enz} was found to be independent of the substrate concentration for enzyme concentrations from 2·28 to 7·50 × 10^{-7} M and substrate concentrations up to about 10^{-3} M. At pH 7·5 and the enzyme and substrate concentrations used, the spontaneous hydrolysis accounted for about 8% minimally of the total rate and increased progressively to slightly over 50% maximally at pH 9·0 owing to OH^- catalysis (k_{OH^-} ~ 7 × 10^4 l. $mole^{-1}$ $min.^{-1}$). 0·01 M Tris-HCl or Tris-HOAc buffers were used, the ionic strength was kept at 0·09 with added NaCl, and solutions contained 3·3% acetonitrile.

[1] (a) F. Schneider and M. Lieflander, *Z. physiol. Chem.*, 1963, **334**, 279; (b) B. G. Malmstrom, P. O. Nyman, B. Strandberg, and B. Tilander, in "Structure and Activity of Enzymes", eds. T. W. Goodwin, J. I. Harris, and B. S. Hartley, Academic Press, New York, 1964, p. 121; (c) R. E. Tashian, D. P. Douglas, and Y.-S. L. Yu, *Biochem. Biophys. Res. Comm.*, 1964, **14**, 256; (d) Y. Pocker and J. T. Stone, *J. Amer. Chem. Soc.*, 1965, **87**, 5497.

[2] For a review see R. P. Davis in "The Enzymes", Vol. V, eds. P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, 1961, p. 545.

[3] Y. Pocker and J. E. Meany, *J. Amer. Chem. Soc.*, 1965, **87**, 1809; *Biochemistry*, 1965, **4**, 2535.

[4] H. Keller, *Z. Vitamin-, Hormon-, u. Ferment-forschung*, 1957–1958, **9**, 297.

[5] W. Marckwald and H. H. Frahne, *Ber.*, 1898, **31**, 1854.

[6] M. Kiese, *Biochem. Z.*, 1941, **307**, 400.