Catalysed Hydrolysis of *S*-*p*-Nitrophenyl Thioacetate by Human Carbonic Anhydrase

By PATRICIA C. HARRINGTON and RALPH G. WILKINS*
(*Department of Chemistry, New Mexico State University, Las Cruces, New Mexico* 88003)

Summary Sigmoidal k_{enz}–pH profiles are observed for catalysis of the hydrolysis of *S*-*p*-nitrophenyl thioacetate by native, cobalt(II), and copper(II) forms of human carbonic anhydrase B and by native C.

THE catalysed hydrolysis of *p*-nitrophenyl acetate by carbonic anhydrase (EC 4.2.1.1) has been well studied[1] but no report appears available on the sulphur analogue as a potential substrate. We report here on the characterization of the catalysis of *S*-*p*-nitrophenyl thioacetate by the native, cobalt(II), and copper(II) forms of human carbonic anhydrase B and by native C. Manipulation of the enzyme was essentially as described previously,[2] except that the apo form was prepared by dialysis of the native enzyme against dipicolinic acid for 4—5 h.[3]

Base catalysed hydrolysis of *S*-*p*-nitrophenyl thioacetate (12 l mol^{-1} s^{-1}) was unimportant in determining k_{enz} based on equation (1). Values of k_{enz} as a function of pH for

$$\text{Rate} = k_{enz}[\text{substrate}][\text{enzyme}] \quad (1)$$

both *p*-nitrophenyl acetate and thioacetate as substrates toward human carbonic anhydrase B are shown in Figure 1. The full curve represents the expected k_{enz}–pH profile on the basis of an (unreactive) acidic (k_{AH}) and basic (k_A) form of the enzyme with pK_{AH} = 7·2. The apo form of the enzyme is inactive, but activity is regenerated by the addition of cobalt(II) and copper(II) ions. The second-order catalytic rate constants *vs.* pH profiles are again sigmoidal for both holoenzymes (Figure 2). The associated values of k_{AH} and pK_{AH} for both the thioacetate and acetate are shown in the Table. The order of activity $Co^{II} > Zn^{II} > Cu^{II}$ for the thioacetate parallels that observed with the acetate. The Table shows that the thioacetate is as suitable a substrate as the acetate for the native enzyme and a much better one with Co^{II} and Cu^{II}-human carbonic anhydrase B. In all cases *N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)acetamide (diamox)[4] inhibited the enzymic activity. Attempts to break down k_{enz} into K_m and k_{cat} values with the native enzyme were unsuccessful since K_m is larger than the highest concentrations of thiol ester which could be used (*ca.* 1 mM).

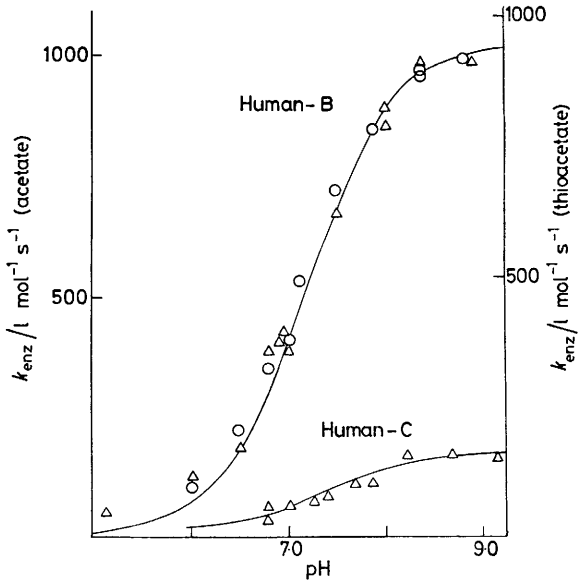

FIGURE 1. pH dependence of k_{enz} for catalysed hydrolysis of *p*-nitrophenyl acetate (○) and thioacetate (△) by human carbonic anhydrase B and C. The full lines represent the equation $k_{enz} = k_{AH}[H^+] + k_A K_{AH}/(K_{AH} + [H^+])$ using k_A and K_{AH} values indicated in the Table and k_{AH} = 10 l mol^{-1} s^{-1}.

TABLE. Second-order rate constants and pK's for catalysed hydrolysis of esters by carbonic anhydrase at 25 °C, I = 0·1 M, Na_2SO_4.[a]

Enzyme	*p*-Nitrophenyl acetate		*S*-*p*-Nitrophenyl thioacetate	
	k_A/ l mol^{-1} s^{-1}	pK_{AH}	k_A/ l mol^{-1} s^{-1}	pK_{AH}
Human B	$1·0 \times 10^3$	7·2	$9·4 \times 10^2$	7·2
	$8·0 \times 10^2$ [b]	7·3[b]		
	$3·3 \times 10^2$ [c]	8·1[c]		
Co Human B	$5·0 \times 10^2$ [c]	8·1[c]	$2·5 \times 10^3$	7·4
Cu Human B	*ca.* 40	—	$5·6 \times 10^2$	8·1
Human C	$2·3 \times 10^3$ [b]	6·8[b]	$1·6 \times 10^2$	7·4

[a] Generally, product inhibition (J. Olander and E. T. Kaiser, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1083, and ref. 2) was avoided by using 2—10 μM substrate and 20—200 μM enzyme. Initial rates, with substrate in excess, gave similar values when either method could be used. Rate constants are generally ±5%. [b] Ref. 5. [c] J. E. Coleman, *J. Biol. Chem.*, 1967, **242**, 5212.

The study of human carbonic anhydrase C was less detailed, since it was a much less effective catalyst. The k_{enz}–pH profile for human C shown in Figure 1 corresponds reasonably well with the theoretical one with pK_a = 7·4 which is, however, significantly higher than that employed (6·8) for the catalysed hydrolysis of the acetate.[5] Generally, human carbonic anhydrase C has been found to be more active than the B isozyme towards most substrates.[5–7] Obviously, the opposite situation applies when the thioacetate is the substrate (Table). This may be because the B isoenzyme has a smaller K_m value since we have observed that the hydrolysis product, *S*-*p*-nitrothiophenolate ion, binds stronger with human B (K 10^6 l mol^{-1} at pH 7·5[2]) than with human C (K 2×10^5 l mol^{-1}).

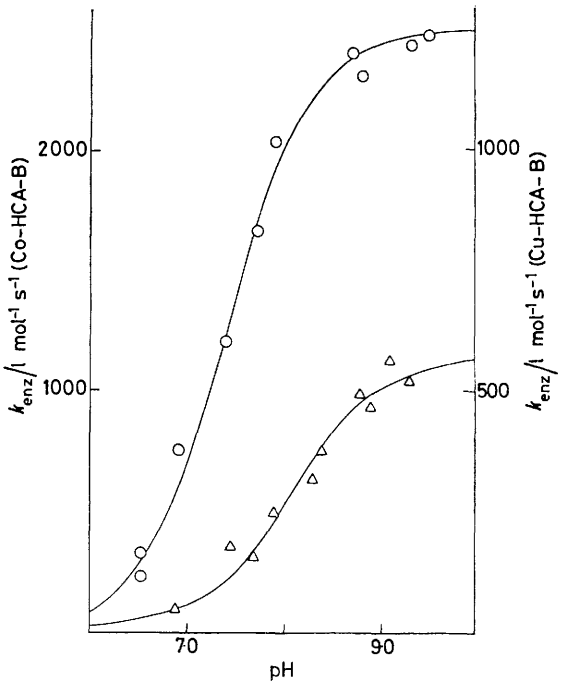

FIGURE 2. pH dependence of k_{enz} for catalysed hydrolysis of *S*-p-nitrophenyl thioacetate by cobalt (○) and copper (△) forms of human carbonic anhydrase B (HCA-B). Full lines constructed as indicated in Figure 1.

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