A PH EFFECT ON THE MACROCATIONIC INHIBITION OF PEPSIN

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pH and ionic strength are important factors in macrocationic - macroanionic interaction and such interaction may be the basis of control of enzymes which are usually macroions. The macrocation polylysine (PL) inhibits pepsin (Katchalski & others 1954; Anderson, 1970) which is strongly anionic, but the variation in inhibition with pH is not yet fully understood.

Twice crystallized pepsin (Sigma), 0.05 mg, was allowed to interact (0.5 h; 20°) with polylysine HBr, (PL), m.wt. 43870, 0.002 - 0.008 mg in 0.025M acetate buffer at pH 3.6, 4.0, 4.6, 5.0 followed by addition to Azocoll (Calbiochem) substrate (15 mg) in buffer; final volume, 3 ml; incubation (20 min; 39°) in a shaking waterbath was terminated by filtration and the spectrophotometric absorbance at 520 nm of the filtrate determined. Inhibition (means of 4 determinations) was the difference between control (without PL) and test expressed as a percentage of the control.

Table 1. Effect of concentration and pH on polylysine inhibition of pepsin

[I] mg/3 ml	Inhibition, % pH			
	3.6	4.0	4.6	5.0
0.002	29	28	18	7
0.004	52	48	39	29
0.005	60	56	49	39
0.006	62	61	57	53
0.0076*	68	68	68	68
0.008	70	71	74	80

[I] = inhibitor concentration; *, 0.0076 determined graphically.

For [I] = 0.0076 mg PL/3 ml, inhibition was constant (68% $\pm$ 0.8 S.E.M.) at all pH values. This concentration was determined graphically from the region of intersection of the inhibition vs [I] plots. 0.0076 mg/3 ml is designated the critical [I].

The ionic link formed in the pepsin-polylysine interaction, on which inhibition depends, will be sundered by ionic excess, for example excess of one of the interactants. On either side of the critical [I] at which pH has no effect (Table 1), inhibition may vary with pH depending on which macroion is in relative excess. At sub-critical [I] the macroanion pepsin is concluded to be in relative excess and increasing pepsin ionisation will accompany increasing pH and provide ionic excess which will result in less pepsin-polylysine interaction and less inhibition. At critical [I] the ionization promoting effect of pH and the complex dissociating effect of excess macroanion apparently balance complex formation over the pH range used, hence the constant inhibition.

At supra-critical [I] whilst inhibition is generally higher than at critical [I], increasing pH, which increases pepsin ionization, promotes increased complex formation and inhibition, at the same time reducing excess ionized polylysine and its complex dissociating effect. The results show how macrocationic control of pepsin could operate in vivo and the principle will guide development of peptide inhibitors of pepsin.

Anderson, W. (1970). J. Pharm. Pharmac., 22, 795-797.
Katchalski, E., Berger, A. & Neumann, H. (1954). Nature, 173, 998.