CONDUCTIVITY CHANGES DURING ENZYME INHIBITOR INTERACTION

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Sulphated polysaccharides occur in the gastrointestinal tract (Glass & Slomiany, 1977) and inhibit several proteases certain of which participate in the development of inflammation. Protease control in vivo is not well understood. One sulphated polysaccharide, degraded i-carrageenan (DC) inhibits pepsin and trypsin and may elicit caecal ulceration in certain experimental conditions (Watt & Marcus, 1970). It is important to elucidate the mechanism and significance of the enzyme modifying effects of sulphated polysaccharides but study of mechanism through the usual types of kinetic plot yields equivocal results doubtless reflecting the chemical complexity of the system which places strain on the assumptions underlying the validity of the kinetic approach. For this reason the interaction of trypsin and DC has been studied conductimetrically and the amount of macroanion yielding minimum conductivity in trypsin solution over a pH range determined. Minimum conductivity is interpreted as an indication of maximum macrocation - macroanion interaction.

With constant stirring DC solution in 7 x 10^{-3}M HCl was added at 0.5ml min^{-1} to each of 1, 2, 3, 4 and 5 mg of bovine crystallized trypsin in 70ml of 7 x 10^{-3}M HCl (pH 3.8; 25°) and conductivity measured in an automatically balanced bridge (Wayne-Kerr) with a 1 cm Pt electrode cell. pH 2.8, 4.8, 5.2 obtained by adjusting HCl, were also used. DC (M.Wt.30,000) was obtained by mild acid hydrolysis of i-carrageenan from Eucheuma spinosium. Addition of DC to trypsin caused conductivity to fall and the weight (W) of DC giving minimum conductivity (κmin) was determined.

Table 1. Weights of macroanion giving minimum condictivity in trypsin solutions.

Weight of trypsin, mg	W, mg pH			
	2.8	3.8	4.8	5.2
1	-	0.28	0.24	0.30
2	-	0.58	0.39	0.34
3	-	0.91	0.60	0.58
4	-	1.19	0.80	0.73
5	1.60	1.40	0.99	0.88

Between pH 2.8 - 5.2 trypsin is net positively charged and electrostatic interaction with a macroanion is possible. With increasing pH in this range decreasing amounts of the macroanion DC are required to produce minimum conductivity suggesting that the number of ionized, interacting negative centres in the macroanion is increasing with pH. Also, the basic nature of trypsin will be diminishing. Increasing pH will tend to cause extension of the macroanion through increasing mutual repulsion of anionic centres and possibly also some contraction of the trypsin resulting in more extensive coverage of the trypsin molecule by a given amount of the macroanion. Extrapolation of the regression of W with pH to pH 8 at which inhibitory studies are normally conducted gives W= 0.05mg DC/mg trypsin which corresponds to the weight ratio of 1:20 for inhibition. The general electrostatic nature of the trypsin-DC interaction together with the knowledge that histidine and aspartic acid feature in the enzymically active site suggests that competitive inhibitory mechanism is highly unlikely.

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Watt, J. & Marcus, R., (1970). Gastroenterology, 59, 760-768.