ENZYMATIC REACTIONS IN THE PRESENCE OF POLYMERS THE COMPETITIVE INHIBITION OF TRYPSIN BY λ-CARRAGEENAN

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1. Introduction

Enzymatic reactions procede *in vivo* in the presence of numerous (bio) polymers and, in order to understand the interactions between an enzyme and its environment, it is of importance to determine the effect of these substances upon enzyme kinetics *in vitro*.

Ceska has investigated the *in vitro*-effect of non-ionic polymers upon various enzymes. He was thus able to show that α-amylase was activated by the presence of dextrans [1] and, at a given dextran concentration, the enhancing effect was observed to be a function of the dextran molecular weight. He has also shown that the polymer, polyethylene glycol, caused a similar increase in the affinity between dextranase and the synthetic, crosslinked, blue dextran [2].

Similar studies with non-ionic polymers have shown that in the following systems, (a) degradation of hyaluronic acid by hyaluronate lyase in the presence of polyethylene glycol and (b) the lactate dehydrogenase reaction in dextran solution, the presence of the polymer produced a decrease of the apparent K_m value for the substrates [3]. This has been interpreted as being due to an exclusion of the compound from part of the solvent.

It appears that the interaction between enzymes and ionic polymers has not been greatly studied. This paper reports the results of some investigations concerning the effect of ionic polymers upon enzyme activities, in this case trypsin.

2. Experimental

2.1. *Materials*

Bovine trypsin (freeze dried; salt free) was obtained from Boehringer Mannheim as was the test combination which was used to determine its activity. The various polymers were obtained from the following firms: Lamda- and kappa-carrageenan from the Copenhagen Pectin Factory Ltd., protanal SF (alginate) from Protan and Fagertun A.S., Drammen, Norway and pectin N (sodium pectate, 92%) from Roth, Karlsruhe.

2.2. *Methods*

Trypsin activity was determined according to the Boehringer test combination method using 2.0 ml 0.2 M Tris buffer at pH 7.8; 0.2 ml 12 mM benzoyl arginine *p*-nitroanilide; 0.2 ml trypsin solution containing 100 γ per ml and 1.0 ml 1% polymer solution. The activities were expressed as percentages of the trypsin activity measured in the presence of 1 ml bidistilled water instead of the polymer solution. The viscosities of the polymer solutions were measured with an Epprecht-Rheomat 15 from Contraves AG, Zürich, Switzerland, and the polarities were expressed in arbitrary units based upon the average number of ionisable groups per C_6 unit of the polymer. This information was obtained from the producers. (Thus pectin N is 92% sodium pectate (degree of esterification, 8%) and thus has a polarity of 0.92).

3. Results and discussion

Fig. 1 shows that ionic polymers at the 1% level bring about trypsin inhibition, the degree of which is determined by two factors, the viscosity and the polarity of the polymer.

These facts may be satisfactorily explained by assuming i) the increase in viscosity causes a reduced enzyme–substrate contact due to diffusion factors and ii) the negatively charged ionic polymers interact electrostatically with the amphoteric enzyme molecule and thus either block the active site or bring about unfavourable conformational changes. It has been shown in other enzyme systems that an increase in the polymer viscosity always causes a decrease in the enzyme activity but in some cases an increase in polarity causes an increase in activity [4].

It was of interest to study this inhibition in more detail and for this purpose λ-carrageenan was chosen since it had the highest polarity of those polymers available. The hydrolysis of various amounts of benzoyl arginine-*p*-nitroanilide by a constant amount of trypsin, in the presence and absence of λ-carrageenan, is shown in a Lineweaver–Burk plot (fig. 2). The increase in the Michaelis constant in the presence of λ-carrageenan suggests the latter acts as a competitive inhibitor toward trypsin. In this way, λ-carrageenan and the other polymers tested here, fall within the general definition that competitive inhibitors are very often substances with acidic or basic groups [5]. The inhibitory effect of λ-carrageenan upon trypsin thus parallels the inhibitory effect of the sulphated polysaccharides, heparin and chondroitin upon pepsin [6], and dextran sulphate upon ribonuclease [7]. The electrostatic nature of the interaction between milk casein and phosphates has recently been demonstrated with the help of toluidine blue [8], in which the absorption maximum of the latter was displaced from 620 to 600 nm or to 550 nm depending upon the phosphate used, i.e. sodium phosphate or sodium hexametaphosphate, respectively. This procedure was adopted in order to investigate the possible electrostatic interaction between trypsin and λ-carrageenan. Upon addition of a solution of the latter to the trypsin–toluidine blue solution, an immediate shift in the absorption maximum was noticed in that the solution became violet and considerably less intensely coloured (fig. 3). The colour

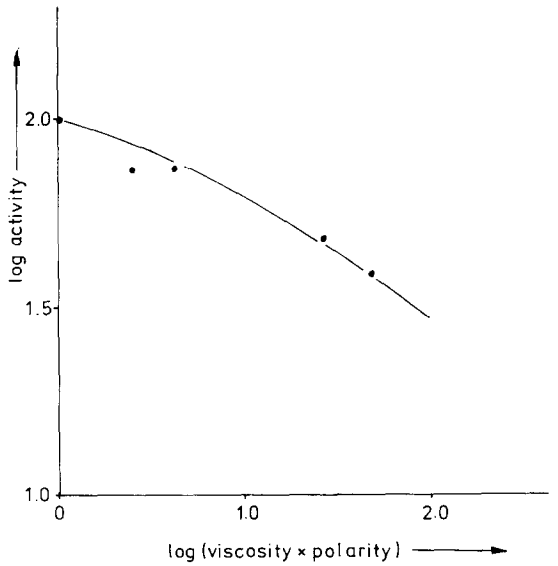

Fig. 1. Relationship between the viscosity and polarity of ionic polymers (1%) and their inhibitory effect upon trypsin.

intensity seems to depend upon the concentration of the added λ-carrageenan (correlation coefficient, *r*: −0.96) until above a certain trypsin: λ-carrageenan ratio whereupon the toluidine blue is quantitatively precipitated. As a result of these experiments, it seems certain that an electrostatic interaction does occur between trypsin and λ-carrageenan. This interaction probably accounts for part, if not all, of the inhibitory effect of the latter, due to steric factors. Interactions between macromolecules and enzymes leading to enzyme inactivation, are of utmost importance in enzyme-containing systems of plant and animal origin e.g. meat, vegetables or food in general.

It may be tentatively concluded from the results presented here that such interactions could be exploited by adding well-defined macromolecules to food systems in order to block certain enzymes responsible for spoilage.

Acknowledgement

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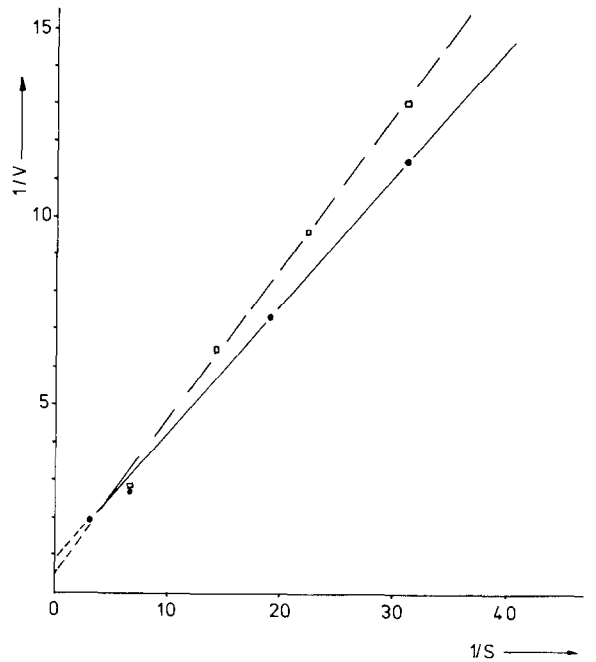

Fig. 2. Lineweaver–Burk plot of trypsin activity throughout a 10-fold increase of substrate concentration (from 0.3 to 0.03 mg/ml of reaction mixture). (□—□—□) In the presence of 1% λ-carrageenan; (●—●—●) without λ-carrageenan.

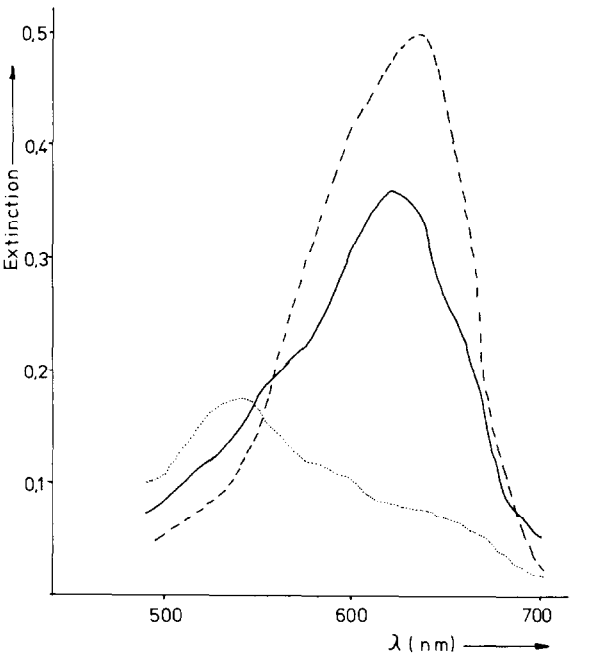

Fig. 3. Visible spectra showing the interaction between λ-carrageenan and the trypsin–toluidine blue complex. (——) Toluidine blue; (- - - -) toluidine blue + trypsin; (·····) toluidine blue + trypsin + λ-carrageenan.

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