IN VITRO EFFECTS OF POLYVINYLPYRROLIDONE ON THE ENZYMATIC ACTION OF PEPSIN

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1. Introduction

Pepsin is inhibited by a basic polypeptide, liberated from pepsinogen during the activation process and isolated in a crystalline form by Herriott [1], and also by certain synthetic polyamino acids – poly-L-lysine, polyornithine and poly-*p*-aminophenylalanine [2, 3]. Only basic high molecular compounds have so far been found to act as pepsin inhibitors; neutral or acidic polyamino acids, such as poly-DL-alanine and poly-aspartic and polyglutamic acids proved to be inactive Katchalski et al. [2] thus assumed that an inactive enzyme-inhibitor complex is formed by electrostatic attraction between the negatively charged pepsin molecule and the positively charged inhibitor compound.

The present communication describes the effect of polyvinylpyrrolidone [PVP], a neutral molecule [4], on enzymatic reactions catalyzed by pepsin.

2. Materials and methods

The reagents and enzymes were purchased from the commercial source indicated: polyvinylpyrrolidone, average M.W. 10,000; 40,000 and 360,000, Sigma Chemical Company, St. Louis Mo., U.S.A.; 1-vinyl-2-pyrrolidine, Eastman Kodak Co., Rochester, N.Y., U.S.A.; crystallized porcine pepsin and crystallized chymotrypsin, Armour Research Division, Chicago, Ill., U.S.A.; trypsin (20,000 U./g), ovalbumin and casein (Hammarsten), (E.Merck A.G., Darmstadt, Germany). The hemoglobin was prepared by crystallization from horse red blood cells.

Proteolytic activity was determined photometrically according to Anson [5] and expressed as micro-equivalents of tyrosine found in the trichloroacetic acid (3.1%) filtrate of the digest. Milk clotting activity was determined as by Herriott [1] using a 20% solution of dried milk ("Reina del Campo") in acetate buffer. A more detailed description of the experimental conditions is given in the legends to the figures.

3. Results and discussion

Herriott's naturally occurring polypeptide, whose basic nature has been confirmed by its amino acid composition, amide-N content and chromatographic properties [7], inhibits pepsin action between pH 5 and 6 as revealed by the milk clotting test [1]. At a higher hydrogen ion concentration, pepsin hydrolyses this peptide. Unlike the natural pepsin inhibitor, but like the other basic high polymers mentioned, PVP exerts within the lower pH-range an inhibition of pepsin action on hemoglobin (fig. 1). Double reciprocal plots of 1/activity versus 1/substrate (fig. 1) suggest a competitive inhibition with respect to the substrate.

The PVP inhibition of pepsin action is not due to

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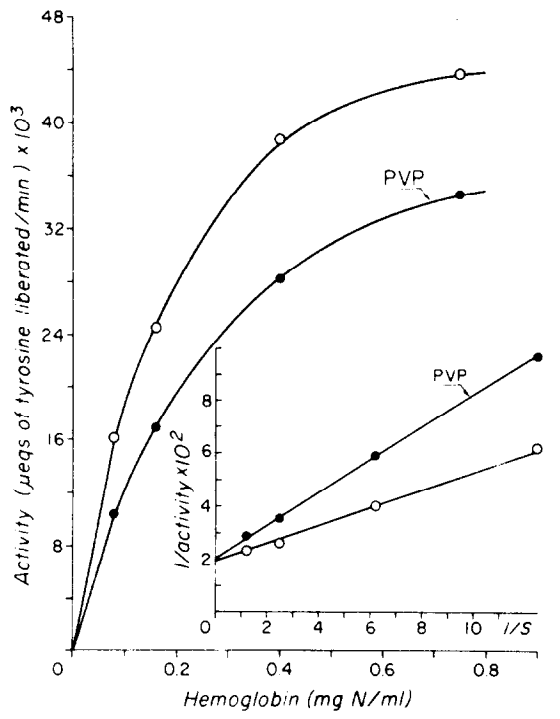

Fig. 1. Polyvinylpyrrolidone inhibition of pepsin proteolysis of hemoglobin. Substrate concentration was varied as indicated using 15 mg of PVP (average M.W. 40,000) as inhibitor. Incubation was at 37.5° for 10 min with 0.1 mg pepsin at pH 1.0 (HCl-KCl buffer) in a total volume of 6.0 ml. Pepsin activity was determined photometrically as by Anson [5] and expressed in microequivalents of tyrosine liberated per minute. Results are corrected for blank values. The same results are shown in the insert as a double reciprocal plot of 1/activity versus 1/substrate.

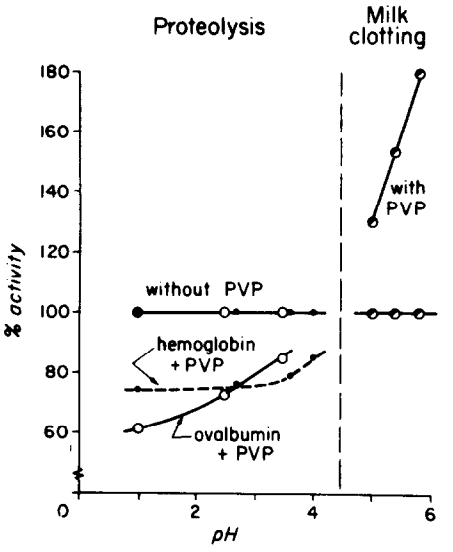

Fig. 2. Influence of hydrogen ion concentration on the effect of polyvinylpyrrolidone on pepsin-catalyzed proteolysis and milk clotting. Proteolysis: acetate buffers (0.2 M), previously adjusted to the indicated pH with HCl or NaOH, respectively. Ovalbumin (20 mg) and hemoglobin (2.4 mg N) were the substrates in a total volume of 6.0 ml containing 0.2 mg pepsin. Milk clotting: activity was determined according to Herriott [6] using 5.0 ml of a 20% solution of milk powder (Reina del Campo) in acetate buffer (1 M) of the indicated pH in a total volume of 6.0 ml containing 6.25 mg of pepsin. PVP (15 mg) effector activity in both experimental groups is expressed as percentage of the values obtained without the effector at the same pH.

changes in viscosity of the digestion medium, since PVP samples of average molecular weight 10,000 to 360,000 in corresponding experiments produced the same loss of enzymatic activity when added in the amount of 3.5 mg/ml to each incubation medium. When PVP was treated with hot hydrochloric acid (8 M, 45 hr at 110°), a treatment which gives a slightly hydrolyzed product (3.0 to 7.7%) as revealed by alcoholic basic titration [8], the action of the inhibitor was not enhanced. No inhibition was found when hemoglobin, under the conditions described above (fig. 1), was incubated with pepsin at pH 1 in the presence of 6 to 21 mg of the monomer, 1-vinyl-2-pyrrolidone.

The inhibition of proteolysis was observed over the whole "physiological pH-range" of pepsin action (fig. 2). At higher pH values, a less degree of inhibition was found.

By use of the milk clotting test between pH 5 and 6, a striking difference is observed between the effects on pepsin action of the basic natural pepsin inhibitor and PVP. As shown in fig. 2, PVP apparently enhances, whereas under the same experimental conditions the natural inhibitor decreases, the milk clotting activity of the enzyme [1]. The effector function of PVP seems to be restricted to pepsin action. At least, trypsin and chymotrypsin are not influenced by PVP in their proteolytic actions at pH 7.6 and 8.0, respectively, at concentrations at which the pepsin activity at pH 1.0 is evidently reduced (fig. 3). The mecha-

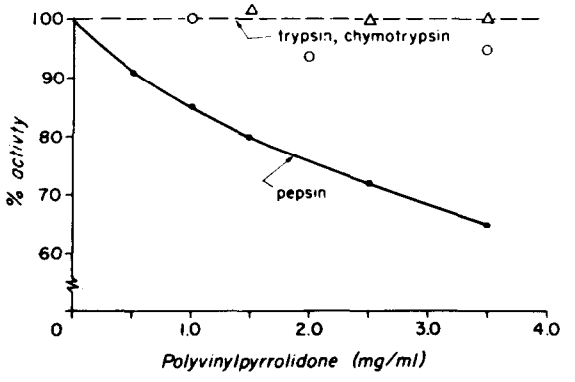

Fig. 3. Effects of polyvinylpyrrolidone on pepsin, chymotrypsin and trypsin-catalyzed proteolysis. The enzymes used in 6.0 ml of test volume were: pepsin 0.1 mg, chymotrypsin 0.2 mg, trypsin 0.2 mg. Incubation was for 10 min at 37°. Pepsin (●—●) acted on native hemoglobin (2.4 mg N) at pH 1.0 in HCl-KCl buffer, trypsin (○—○) on urea-denatured hemoglobin (2.4 mg N) in phosphate buffer, pH 7.5, and chymotrypsin (△—△) on casein (20 mg) in borate buffer, pH 8.0. Enzymatic activity was determined photometrically according to Anson [5]. The action of PVP in the indicated amount was expressed as percentage of the activity obtained in control experiments without PVP.

nism by which PVP exhibits inhibition of the proteolytic action of pepsin on ovalbumin and hemoglobin, but activates the milk clotting action, obviously must be different from that involved in the pepsin interaction with basic polymers, since the vinyl polymer is a neutral molecule and has no charged groups. Furthermore, the PVP molecule cannot act as a hydrogen donor in the formation of hydrogen bonds. However, the $\geq$CO groups of its alternating cyclic lactam side chains may serve as acceptors of hydrogen supplied by an appropiate donor molecule. By this means, and assisted by hydrophobic bonding, an inactive PVP-pepsin complex can be formed. It is assumed that tyrosyl residues are involved in this type of interaction, and especially those which are associated with the pepsin activity [1, 0]. Support for such assumption comes from binding studies with synthetic dyes, the results of which indicate that certain of these compounds are bound to the PVP molecule by establishing hydrogen bonds between their phenolic hydroxyls and the carbonyl groups of the polymer [10].

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