A NEW ACIDIC PROTEASE IN HUMAN SEMINAL PLASMA

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SUMMARY

A new acidic protease with pH optimum of 3.0 was partially purified from human seminal plasma. It can hydrolyze hemoglobin and N, N-dimethyl casein. It was stable in acidic but labile in neutral and alkaline solutions. Its molecular weight was determined by gel filtration to be 40,000. Its chromatographic behavior on carboxy methyl Sephadex column was quite different from the pH-8.0 protease of human seminal plasma. It was present in seminal plasma of normal and vasectomized individuals. Its activity was not affected by cysteine, metal cations or ethylene diamine tetraacetic acid.

Protease, peptidase and fibrinolysin have been detected in human seminal plasma as early as 1955 (1). Syner and Moghissi has recently reported some properties of the partially purified human seminal plasma protease (2). The enzyme displays some properties similar to chymotrypsin: pH optimum 7.5-8.0; substrate preference of casein, hemoglobin and benzoyltyrosine ethyl ester but not benzoylarginine ethyl ester; molecular weight 33,000. However, it is unaffected by 1 mM di-isopropyl phosphofluoridate or 1 mM metal cations, and in this respect differs from chymotrypsin. Fritz et al showed later that chymotrypsin inhibitors of natural origin did not inhibit the human seminal plasma protease (3). In this paper, the enzyme will be called "pH-8.0 protease". The peptidase and the fibrinolysin which also manifest their activities at neutral pH have not been well studied (1).

During the study of the pH-8.0 protease of human

seminal plasma, we discovered a new acidic protease with a pH optimum of 3.0. This communication describes some properties of the new acidic protease which is different from the pH-8.0 protease.

METHODS

The acidic protease was partially purified as described below. All steps in purification were carried out at 4°. Pooled human seminal plasma was centrifuged at 20,000 x g for 30 min, and then dialysed overnight against double distilled water. The dialysed seminal plasma was fractionated with ammonium sulfate. The precipitate obtained at 40-70% saturation was dissolved in minimum volume of water and dialysed against double distilled water. It was then applied to a column of carboxy methyl Sephadex, CM 50, (4.5 x 10 cm), equilibrated with 0.02 M sodium phosphate buffer, pH 6.5. Void volume fractions containing the acidic protease were pooled, adjusted to pH 3 with 2 M HCl and concentrated by using aquacide. The concentrated fluid was further purified on a column of Sephadex G-100 (2.5 x 50 cm), equilibrated with 1 mM HCl, pH 3 containing 0.1 M NaCl. Further purification was obtained by passing the protease fraction through a Sephadex G-100 column of smaller size (1.5 x 60 cm), using the same buffer. The specific activity of the enzyme so obtained was 75 OD_{280}/45 min/mg protein which was 30-fold higher than that of the ammonium sulfate fraction and represented half of the activity of the latter. The activity was assayed in 0.1 M citric-phosphate buffer pH 3 using acid-denatured hemoglobin as substrate (4). The enzyme was stored in 1 mM HCl, pH 3 containing 0.1 M NaCl at 0°, and was stable for 3-4 weeks.

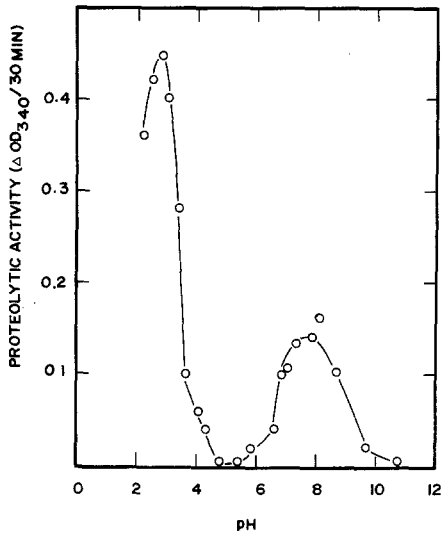

Fig. 1. pH profile of the proteolytic activity of 40-70% ammonium sulfate saturation fraction of seminal plasma. The activity was assayed at 37° in 0.1 M citrate-phosphate borate buffer, with N,N-dimethyl casein as substrate (5).

RESULTS

Figure 1 shows the pH profile of proteolytic activity of ammonium sulfate fraction of human seminal plasma. It should be noted that the peak of the proteolytic activity at pH 3.0 was three folds higher than that at pH 8.0. At pH 5.0, there was essentially no proteolytic activity. Upon further purification of the fraction by ion-exchange chromatography on carboxy methyl Sephadex and gel filtration on Sephadex G-100, the proteolytic activity at pH 8.0 was completely removed from that at pH 3.0. The pH profile of the partially purified acidic protease is shown in Figure 2. The partially purified acidic protease was quite stable at low pH and its activity decreases almost linearly as the pH increased from 2 to 7 (Figure 3). It was quite unstable in the neutral and alkaline solutions. The pH profile remained essentially unchanged whether hemoglobin or N,N-dimethyl casein

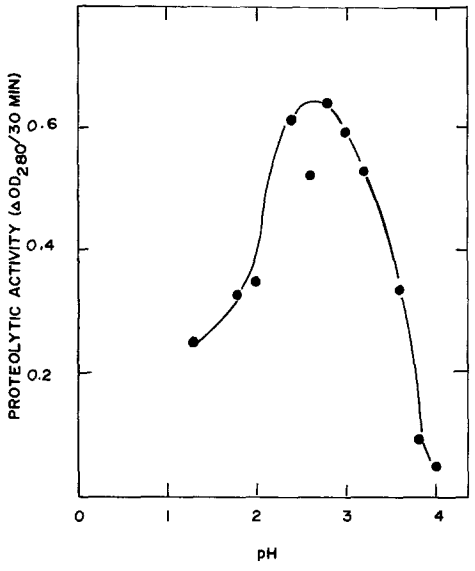

Fig. 2. pH dependence of the acidic protease activity of partially purified enzyme in 0.1 M citric-sodium phosphate buffer of various pH values using hemoglobin as substrate (4).

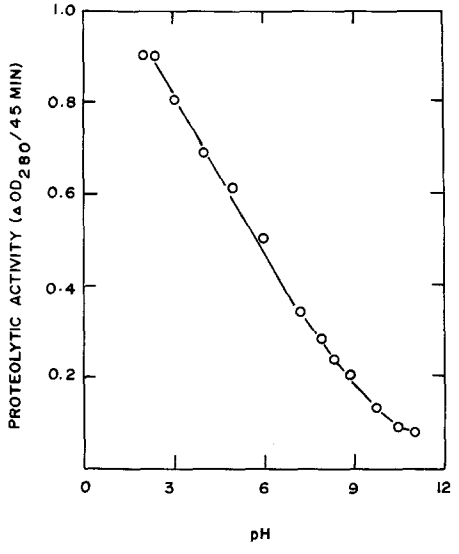

Fig. 3. Stability of the acidic protease at different pH values. Aliquots of partially purified enzyme were incubated in 0.1 M citrate-phosphate-borate buffer of the pH values indicated for 3 hrs at 37°. At the end of the incubation, the proteolytic activity was determined at pH 3 using hemoglobin as substrate (4).

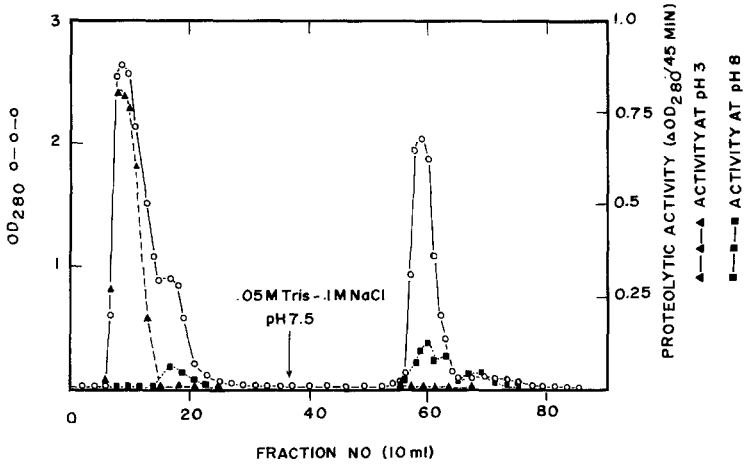

Fig. 4. Chromatography of 40-70% ammonium sulfate saturation fraction on Sephadex CM 50 (10 x 4.5 cm). Starting buffer, 0.02 M sodium phosphate buffer, pH 6.5, eluting buffer, 0.05 M Tris-HCl 0.1 M NaCl, pH 7.5

was used as the substrate of the enzyme.

On a Sephadex G-200 column, calibrated with standard proteins of known molecular weights, the acidic protease behaved like a protein with molecular weight of 40,000. The acidic protease was not retained in a carboxy methyl Sephadex column at pH 7.5 while the pH-8.0 protease was retained as reported previously (2). Thus, the two proteolytic activities could be easily separated by the ion-exchange chromatography (Figure 4).

The activity of partially purified acidic protease at pH 3.0 was not affected by 1 mM of $CaCl_2$, $MgCl_2$, KCl, $PbCl_2$, $HgCl_2$, $ZnCl_2$, or 10 mM of ethylene diamine tetraacetic acid or cysteine. It was also observed that seminal plasma from normal and vasectomized individuals possessed similar amounts of the acidic protease.

DISCUSSION

The properties of the acidic protease of human seminal plasma described in this communication suggest that the enzyme is distinct from the pH-8.0 protease reported previously (2). Its acidic pH optimum makes it unlikely to be a fibrinolysin or a peptidase. Whether there is other hydrolytic activity associated in the acidic protease requires further experimentation. Its acidic pH optimum, its stability in acidic solution and its molecular weight seem to be quite similar to those of pepsin (6).

The presence of the acidic protease in the seminal plasma of vasectomized individuals suggested that the enzyme was not of testicular origin. The pH-8.0 protease was implicated to be produced by the prostate glands (1). These and other auxilliary glands of male reproductive system may be the origin of the acidic protease. It was unlikely that the enzyme came from microbial contamination in the semen samples, since all samples used were free of microorganisms as judged by microscopic inspection.

It is presently difficult to envisage any function of the acidic protease in human reproduction. The pH-8.0 protease has been suggested to be involved in the hydrolysis of proteins in seminal-plasma as well as those in cervical mucus which will result in a decrease in the viscosity of the fluids and hence an increase of sperm motility (2,7). The acidic protease may participate in similar function except that it would require an acidic environment which has not been shown to exist under the physiological conditions.

Studies are in progress to probe further into the enzymic properties and the physiological function of the new acidic protease.

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