PROTEOLYTIC ENZYMES OF THE FORMED ELEMENTS OF HUMAN BLOOD

I. ERYTHROCYTES*

BY WAYNE L. MORRISON AND HANS NEURATH

(From the Department of Biochemistry, University of Washington, Seattle, Washington)

(Received for publication, July 11, 1952)

A review of the literature conveys the impression that proteolytic enzymes capable of splitting protein substrates are largely associated with the plasma of mammalian blood, whereas enzymes acting on simple peptides are preponderantly associated with the formed elements. Thus, upon removal of inhibitory fractions from blood plasma, proteinases akin to trypsin and related to the process of blood coagulation have been recognized by several investigators (1, 2). Conversely, leucocytes and erythrocytes have been shown to have a high activity toward di-, tri-, and tetrapeptides of glycine, alanine, and leucine (3) for a review of the literature see Stern et al. (4)). The question of the occurrence of protein-splitting enzymes in suspensions or extracts of platelets, erythrocytes, or leucocytes is an important one in consideration of the stability and metabolism of these cells. As part of a program designed to characterize the enzymatic components of the formed elements of human blood, the proteinase activity of erythrocytes has, therefore, been investigated. The results of this work are presented in this communication, whereas, in a paper to follow, the proteinase activity of human leucocytes will be described.

EXPERIMENTAL

Materials

Blood—Fresh human blood was obtained from the King County Blood Bank, Seattle.¹ The blood was collected in the usual acid citrate-dextrose medium and centrifuged at 700 X g in a refrigerated centrifuge for 20 minutes. The plasma and the “buffy coat” were removed by suction, and the packed erythrocytes washed three times by resuspension in cold 0.15 M NaCl. The washed cells were finally resuspended to the physiological

* Part of a thesis submitted by Wayne L. Morrison to the Graduate School of the University of Washington in partial fulfilment of the requirements for the degree of Master of Science. This work has been supported by a grant from the United States Public Health Service.

¹ We are indebted to Dr. Richard Czajkowski of the King County Blood Bank, Seattle, for supplying the blood used in these experiments.
concentration (0.4 volume of packed erythrocytes plus 0.6 volume of cold 0.15 M NaCl).

*Buffers*—In the range of pH 6 to 9, 0.1 M phosphate buffers were used. In the more acid pH range, Veronal-acetate buffers and glycine-HCl buffers were used. The former were prepared according to Michaelis (5), and the latter by adjusting glycine solutions with 0.1 N HCl to the desired pH and a final concentration of 0.1 M glycine.

**Methods**

Proteolytic Activity—The method of Anson (6) with urea-denatured human hemoglobin as substrate was employed in most cases; in some instances, a casein substrate was used as well. The enzymatic reaction mixture contained 5 cc. of substrate solution, 1 to 3 cc. of activator or inhibitor, when used, 1 to 3 cc. of enzyme solution, and buffer of the desired pH to a final volume of 15 cc. Enzyme, buffer, and activator or inhibitor were incubated for 7 to 10 minutes in a Dubnoff constant temperature bath at 37° prior to the addition of the substrate. Blanks containing all materials except the enzyme were run with each experiment. After incubation for various time intervals, 2 cc. aliquots were added to 5 cc. of 5 per cent trichloroacetic acid and allowed to stand for 1 hour at room temperature prior to centrifugation. The clear supernatant was analyzed for hydrolysis products according to the method of Folin and Ciocalteu (7) by measuring the optical density at 760 m\(\mu\) in a Beckman model B spectrophotometer. In cases in which reagents used in the enzymatic system interfered with the color normally produced by the Folin reagent, the optical density of the clear supernatant was determined directly at 280 m\(\mu\) in a Beckman DU spectrophotometer. This latter method of analysis was also used under conditions of low proteolytic activity in order to maintain satisfactory accuracy.

Nitrogen determinations were carried out by the micro-Kjeldahl method with selenium oxychloride as catalyst.

**Results**

Proteolytic Activity of Stroma Suspensions

In the initial phase of this work, stroma suspensions were prepared by hemolysis of the erythrocytes by 20-fold dilution with water, followed, after 16 hours standing in the cold, by centrifugation and repeated washings of the particulate fraction with 0.15 M NaCl. Whereas the clear solutions were free of demonstrable proteolytic activity, aliquots of the stroma, when resuspended at pH 3 or 8, were appreciably active. Several conventional methods of hemolysis (8) were similarly tested for proteolytic activity of the resulting stroma, but, with the single exception noted below, none of
them proved to be more suitable than the others. These included hemolysis by dilution followed by acidification to pH 5.5 with hydrochloric acid or with carbon dioxide, and hemolysis by the addition of saponin instead of by simple dilution.

The method of lysis, which produced the highest demonstrable proteolytic activity of the washed stroma and hence was used in all subsequent experiments, was that of freezing and thawing. The essential features of this method are the rupture of the erythrocytes by freezing and collection of the stroma after thawing, followed by repeated washings with cold saline.

In a typical preparation, 200 cc. of a suspension of washed erythrocytes in 0.15 M NaCl were quickly frozen in a methyl Cellosolve-dry ice mixture and thawed by immersion of the flask in lukewarm water. The process of freezing and thawing was repeated, and at no time was the temperature of the suspension allowed to rise above 5°. The suspension was then centrifuged in the Spinco model L preparative ultracentrifuge at 36,000 × g for 45 minutes in the cold. The clear hemolysate was carefully withdrawn, and the residual precipitate washed three times with 0.15 M NaCl and then resuspended in 0.15 M NaCl to a volume of 100 cc. This stock solution will be referred to as the "crude stroma suspension." The suspension retains proteolytic activity for several weeks when kept under refrigeration.

Fig. 1 shows the relation of the proteolytic activity of the crude stroma suspension to pH, with hemoglobin and casein as substrates, respectively.

---

2 This procedure was suggested to us by Dr. Eric Ponder.
In these experiments, aliquots of the suspension were adjusted to the desired pH by the addition of 0.1 m HCl and NaOH, respectively, care being taken to use as nearly as possible the same amount of stroma in each aliquot. On the acid side of neutrality, maximum proteolytic activity at pH 3 is indicated by casein substrate, precipitation of hemoglobin below this pH preventing the exact definition of the maximum with the latter substrate. On the alkaline side of neutrality, a maximum at pH 8 is indicated by measurements with hemoglobin substrate, whereas the optical density readings of the casein digests were too low to be of significance in this range.

*Extraction*

Simple extraction of the crude stroma suspension with glycerol, potassium iodide, lithium, magnesium, or potassium chloride (all salts in final concentrations of 1 or 0.5 m), or with hydrochloric acid (to pH 5.5) failed to yield appreciable proteolytic activity when the clear supernatant solution was tested at pH 3 or 8. Mechanical disintegration of the stroma with the aid of alumina (9) or in a Mickle vibrator3 likewise failed to yield detectable proteolytic activity in the clear aqueous phase.

*Extraction with Potassium Thiocyanate*—Proteolytic enzymes of the crude stroma suspension could be obtained in soluble form by the addition of 1 part of the stroma suspension to 3 parts of a freshly prepared, cold solution of 1 m potassium thiocyanate. The resulting suspension (pH 6.5) was allowed to stand overnight in the refrigerator and was then centrifuged at 60,000 X g in the cold for 1 hour. The faintly pink-colored supernatant solution, which appeared optically clear when placed in a beam of light in the dark room, was found to contain appreciable proteolytic activity. The results of activity measurements are shown in Fig. 2, in which the specific activity (A) is plotted against pH. The specific activity is arbitrarily defined as the optical density of the protein-free filtrate per mg. of “enzyme” nitrogen of the incubation mixture, observed after 40 minutes of proteolysis. Protein nitrogen in the presence of thiocyanate was determined on the washed coagulum obtained by precipitation with trichloroacetic acid. It is apparent from Fig. 2 that under the conditions chosen an enzyme was extracted which revealed optimum activity at about pH 7.5. The enzymatic nature of the reaction was demonstrated by the direct proportionality of the extent of hydrolysis, after 40 minutes of incubation, to protein nitrogen concentration of the solution serving as enzyme source. This is indicated by the plot of Fig. 3.

Dialysis of the extracted enzyme against cold distilled water in a rapid

---

3 Kindly placed at our disposal by Dr. H. C. Douglas of the Department of Microbiology.
dialyzer until complete removal of thiocyanate resulted in partial precipitation of the enzyme and loss of enzymatic activity. The latter was not

restored by the subsequent addition of KCNS to the initial concentration (0.75 M). Removal of thiocyanate by dialysis against 0.15 M NaCl produced a decrease in enzymatic activity which was only partly restored by

![Graph of pH dependence of activity](Fig. 2)

**Fig. 2.** pH dependence of activity of thiocyanate-extracted enzyme (0.13 mg. of protein per cc.) of crude stroma suspension. All measurements in the presence of 0.26 M thiocyanate, with hemoglobin substrate. 40 minutes incubation at 37°. (A)\textsubscript{280} is the specific activity measured at 280 nm.

**Fig. 3.** Linear dependence of rate of hemoglobin hydrolysis on protein nitrogen content of thiocyanate-extracted enzyme of crude stroma suspension. Measurements in 0.1 M phosphate buffer, pH 7.4, containing 0.26 M thiocyanate.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>Concentration CNS\textsuperscript{−}</th>
<th>(A)\textsubscript{760}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undialyzed extract</td>
<td>0.75</td>
<td>2.36</td>
</tr>
<tr>
<td>Dialyzed against water</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>KCNS added after dialysis against water</td>
<td>0.38</td>
<td>0.60</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>0.76</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>1.52</td>
</tr>
<tr>
<td>Dialyzed against 0.15 M NaCl</td>
<td>0</td>
<td>0.52</td>
</tr>
<tr>
<td>KCNS added after dialysis against 0.15 M NaCl</td>
<td>0.75</td>
<td>0.77</td>
</tr>
</tbody>
</table>

\[(A)\textsubscript{760}\] is the specific activity measured at 760 nm (see the text).
the addition of KCNS to the original concentration. The results are given in Table I.

Effects of Activators and Inhibitors on Enzyme Extracted by Thiocyanate—Table II illustrates the effects of common enzyme activators or inhibitors on the proteolytic activity of the proteolytic enzyme at pH 7.4 rendered soluble by thiocyanate. In these experiments, the thiocyanate concentration was reduced to 0.26 M by dialysis of the enzyme extract against cold 0.15 M NaCl. Several enzyme preparations were used to obtain the results given in Table II. The marked activation by reducing agents and the complete inactivation by mercuric ions are indicative of the presence of essential sulfhydryl groups. A further characteristic demonstrated by the results of Table II is the strong activation by zinc and ferrous ions.

The present proteolytic enzyme is unaffected by specific inhibitors of trypsin and chymotrypsin, i.e. crystalline soy bean trypsin inhibitor (10) and 3-indolepropionic acid (11), respectively. The condition of the experiments in which these inhibitors were tested was analogous to those represented by Table II, except that the inhibitors were present in a concentration of 0.1 per cent.

Extraction with n-Butanol—Morton (12) has recently described a method for the extraction of a variety of enzymes from animal tissue by means of n-butanol, which is believed to be effective by inducing separation of lip-
oprotein complexes into the constituent components. This method appeared, therefore, to be particularly suited for the present system.

Application of Morton's method involved the dropwise addition of n-butanol to an aliquot of the crude stroma suspension immersed in a water bath at 37°, until a final ratio of alcohol to stroma suspension of 2:5 was reached. In a typical experiment, 20 cc. of 95 per cent n-butanol were added with stirring to 50 cc. of crude stroma suspension during a period of 45 minutes. Stirring was then continued for another 15 minutes and the suspension centrifuged at about 500 \( \times \) \( g \) for 15 minutes at room temperature. Three layers resulted, which were separated by careful decanting of the upper alcohol layer from the packed disk of denatured protein and other insoluble material that formed between the alcohol layer and the aqueous phase. A pipette was then inserted along the side of the centrifuge tube, past the packed disk, into the aqueous layer which was carefully withdrawn. The collected aqueous layers from various extractions were combined and dialyzed in a continuous flow dialyzer overnight against cold distilled water until the extract gave a negative ceric nitrate test for alcohol (13). The dialyzed solution was then centrifuged at 60,000 \( \times \) \( g \) for 15 minutes in the cold to remove any particulate matter that may have remained in suspension. The water-clear supernatant solution contained about 0.02 per cent protein and exhibited proteolytic activity at pH 3 and 7.5.

At this stage, other methods of lysis were investigated in order to establish conditions for maximum yield of soluble enzyme. None was found to be superior to that of freezing and thawing, nor did direct extraction of a suspension of erythrocytes with n-butanol prove to be advantageous.

Properties of Proteolytic Enzymes Rendered Soluble by n-Butanol—By use of the method of freezing and thawing for preparation of the crude stroma suspension and of n-butanol extraction, as described above, the enzymatic properties of the water-soluble proteinases were determined. The pH dependence of activity is shown in Fig. 4. In these determinations, 2 cc. of the dialyzed aqueous extract, containing 0.048 mg. of protein N per cc., were incubated at 37° for 40 minutes. Two pH maxima, at pH 3.2 and 7.5, are evident from Fig. 4. As shown in Fig. 5, the velocity of hydrolysis, measured after 40 minutes of incubation, is proportional to protein nitrogen concentration, both at pH 3.2 and 7.4.

The enzyme solutions lose their activity at pH 7.4 within about 2 to 3 days at refrigeration temperature, but the activity at pH 3.2 is retained. Heating of the dialyzed extract for 3 minutes to 60° destroys about 80 per cent of the activity at pH 7.4 but the activity at pH 3.2 is fully retained. After heating for 3 minutes to 98° both activities are reduced to about 20 per cent.

Effects of Activators and Inhibitors—The effects of reducing agents and
ions on the activity of the proteinases at pH 7.4 and 3.2, respectively, are shown in Table III.

At pH 7.4, the proteolytic enzyme rendered soluble by extraction with n-butanol differs markedly from the enzyme rendered soluble with thiocyanate in that it fails to be activated by reducing agents or to be inactivated by mercuric ions. In both cases, however, zinc and ferrous ions produced marked activation. In order to ascertain whether the differences noted between these two enzymes were related to the presence of thiocyanate, n-butanol-extracted, aqueous enzyme preparations were adjusted to 0.26 M potassium thiocyanate and subjected to activity measurements in the presence of 0.6 mM of ascorbic acid and 0.8 mM of cysteine hydrochloride, respectively. The failure to observe any activation strengthens the supposition that different enzymes are extracted by n-butanol and thiocyanate, respectively.

The specific inhibitors of trypsin and chymotrypsin, i.e. soy bean trypsin inhibitor (0.05 per cent) and 3-indolepropionic acid (2.6 mM), had no significant effect on the proteolytic activity of the n-butanol-extracted material at pH 7.4, when tested under the conditions described in Table III. The n-butanol-extracted proteolytic enzyme differs at pH 3.2 from that at pH 7.4 in that it fails to be activated by zinc or ferrous ions.
Activation by Zinc and Ferrous Ions—The activating effect of zinc and ferrous ions on the n-butanol-extracted enzyme at pH 7.4 can be largely reversed by the addition of disodium ethylenediamine tetraacetate\(^4\) (Sequestrene). This chelating reagent appears to have no effect of its own on the enzyme, whereas, in the presence of 0.001 M Zn\(^{++}\) or 0.001 M Fe\(^{++}\), 0.002 M Sequestrene causes a marked reduction in proteolytic activity, presumably due to competitive binding. These results are given in Table IV. Metal activation can also be largely reversed by dialysis against running distilled water in the cold (Table IV). When to the activated enzyme Sequestrene was added and the solution then dialyzed in the cold for several hours to remove a large part of the complex-forming agent, the enzyme could be fully reactivated by the subsequent addition of the metal ions (Fe\(^{++}\) or Zn\(^{++}\)).

Tests for Enzymatic Activities of Known Specificities The n-butanol-extracted proteolytic enzyme was tested at pH 7.4 for activities toward specific substrates for certain proteinases and peptidases (14). (a) The enzyme was inactive toward synthetic substrates for trypsin, i.e. benzoyl-

\(^4\) Obtained from the Alrose Chemical Company, Providence, Rhode Island, under the name Sequestrene.
L-arginine ethyl ester and benzoyl-L-argininamide. (b) The enzyme was likewise inactive toward the chymotrypsin substrates, acetyl-L-tyrosine ester and acetyl-L-tyrosinamide. (c) Negative results were also obtained toward the carboxypeptidase substrate, carbobenzoxyglycyl-L-phenylalanine. (d) In view of previous reports on the enzymatic hydrolysis of triglycine and L-leucylglycylglycine by erythrocytes (3, 4), both of these tripeptides were carefully tested in a system containing 0.045 mg. of protein nitrogen in 0.04 M phosphate buffer at pH 7.3 and 0.02 M substrate. When the alcohol titration method of Grassmann and Heyde, as described by Putnam and Neurath (15), was employed, no measurable hydrolysis was observed with or without added Zn++, Fe++, or Co++.

**Comparison of Activities to Trypsin and Pepsin**

The relatively low protein concentration prevailing in relatively active solutions of the proteolytic enzyme extracted either with potassium thio-

---

**Table IV**

Reversal of Metal Activation of Proteinase at pH 7.4 by Sequestrene (SEQ) and by Dialysis

All enzymatic measurements in 0.1 M phosphate buffer, pH 7.4; 40 minutes incubation with hemoglobin substrate at 37°. The variations in specific activities, (A)_{280}, recorded for comparable test solutions are due to different enzyme preparations used in these experiments.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>(A)_{280}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td></td>
</tr>
<tr>
<td>&quot; + 0.001 M Fe++</td>
<td>3.0</td>
</tr>
<tr>
<td>Same + 0.002 M SEQ</td>
<td>10.8</td>
</tr>
<tr>
<td>Enzyme + 0.001 M Zn++</td>
<td>4.6</td>
</tr>
<tr>
<td>Same + 0.002 M SEQ</td>
<td>11.4</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
</tr>
<tr>
<td>&quot; + 0.001 M Fe++</td>
<td>3.2</td>
</tr>
<tr>
<td>Same after dialysis</td>
<td>9.7</td>
</tr>
<tr>
<td>Enzyme + 0.001 M Zn++</td>
<td>8.6</td>
</tr>
<tr>
<td>Same after dialysis</td>
<td>4.5</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
</tr>
<tr>
<td>&quot; + 0.001 M Fe++</td>
<td>5.4</td>
</tr>
<tr>
<td>Same + 0.002 M SEQ and dialyzed</td>
<td>22.9</td>
</tr>
<tr>
<td>Enzyme + 0.002 M SEQ + 0.001 M Fe++ and dialyzed</td>
<td>13.5</td>
</tr>
<tr>
<td>&quot; + 0.001 M Zn++</td>
<td>24.4</td>
</tr>
<tr>
<td>Same + 0.002 M SEQ and dialyzed</td>
<td>18.1</td>
</tr>
<tr>
<td>Enzyme + 0.002 M SEQ + 0.001 M Zn++ and dialyzed</td>
<td>10.6</td>
</tr>
<tr>
<td>Enzyme + 0.002 M SEQ + 0.001 M Zn++ and dialyzed</td>
<td>20.0</td>
</tr>
</tbody>
</table>
cyanate or with n-butanol warranted a comparison of the specific activities of these preparations with those of trypsin and pepsin. In these experiments, the initial hydrolysis rate of trypsin (at pH 7.4) and of pepsin (at pH 2.0) was determined as a function of enzyme concentration, and compared to the specific activity (initial hydrolysis rate per mg. of protein nitrogen) of the proteolytic enzymes from erythrocyte stroma. The results are presented in Table V, from which it is apparent that at pH 7.4 the n-butanol-extracted enzyme is about one-tenth as active toward hemoglobin as trypsin, and the thiocyanate-extracted enzyme about one-hundredth as active. While at pH 3.2 the specific activity of the n-butanol-extracted enzyme is also one to two orders of magnitude lower than that of pepsin at pH 2, these activities are, none the less, of significant magnitude if it is considered that these enzymes have not been subjected to isolation procedures other than fractional extraction.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Method of extraction</th>
<th>pH</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin*</td>
<td></td>
<td>7.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Erythrocyte proteinase</td>
<td>n-Butanol</td>
<td>7.4</td>
<td>0.56</td>
</tr>
<tr>
<td>&quot;</td>
<td>KCNS</td>
<td>7.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Pepsin†</td>
<td></td>
<td>2.0</td>
<td>9.85</td>
</tr>
<tr>
<td>Erythrocyte proteinase</td>
<td>n-Butanol</td>
<td>3.2</td>
<td>0.64</td>
</tr>
</tbody>
</table>

\( k \) denotes the initial hydrolysis rate per mg. of protein N.

* Twice crystallized trypsin, prepared by Dr. Frank Tietze.
† Armour Laboratories, 1:24,000 pepsin, lot No. 14465.

**DISCUSSION**

The results of the present work indicate that in addition to a high peptidase activity, previously reported (3, 4), erythrocytes of normal human blood also contain proteolytic activity toward protein substrates. This latter activity is a constituent of the hemoglobin-free stroma.

On the basis of the pH-activity relation of the crude stroma suspension, two enzymes can be discerned, one of these being maximally active at pH 3 and the other one at pH 8. However, if, in addition, the response of the soluble proteolytic enzymes to various cofactors is taken into account, three proteolytic entities can be recognized for which the generic name of *erythrocyte proteinases* is proposed. *Erythrocyte proteinase* I is obtained by extraction of the crude stroma suspension with potassium thiocyanate. It has a maximum activity at pH 7.4 and is activated by reducing agents, such as ascorbic acid and sulfhydryl compounds. It is completely inhibited by mercuric ions. These properties suggest the presence of essential sulf-
hydryl groups and it is of interest that other intracellular proteinases (cathepsins) have these characteristics also (16-18). The mechanism of activation has been considered by many authors (17, 19) and it has been suggested that a reversible equilibrium between disulfide and sulfhydryl groups is involved. In addition to activation by reducing agents, erythrocyte proteinase I also shows strong activation by ferrous and zinc ions. It is worthy of note that this latter characteristic is shared by a proteolytic enzyme recently found in striated muscle tissue (20).

Two enzymes are obtained in solution by extraction of the crude stroma suspension with n-butanol, presumably by cleavage of a lipide-protein complex. One of these enzymes, erythrocyte proteinase II, has the same pH maximum as enzyme I at pH 7.4, but it differs by being insensitive to reducing agents and to mercuric ions. However, like erythrocyte proteinase I, enzyme II is strongly activated by ferrous and zinc ions. The other of the n-butanol-extracted enzymes, erythrocyte proteinase III, shows a pH maximum at pH 3.2 and fails to be activated by reducing agents or metal ions. The lack of activation by zinc or ferrous ions may be tentatively correlated with the general lack of binding of heavy metals by proteins in pH regions in which they carry a strongly positive charge.

It is of interest that erythrocyte proteinase II fails to show activity toward any of the synthetic substrates which have been tested. The latter include those for trypsin, chymotrypsin, and for triglycine and leucylglycylglycine tripeptidase. In this respect, this enzyme compares with the proteolytic enzymes from muscle (20) and from the anterior pituitary gland (21), which have been recently described.

It is premature at the present time to speculate on the rôle of these erythrocyte proteinases in red cell production, maturation, and destruction. Further purification and characterization of these enzymes as proteins have to precede an elucidation of their metabolic rôle.

**SUMMARY**

The presence of protein-splitting enzymes in crude stroma suspensions of lysed human erythrocytes has been established. Three such enzymes have been obtained in soluble form by extraction with potassium thiocyanate and n-butanol, respectively. *Erythrocyte proteinase I*, extracted with potassium thiocyanate, has maximum activity at pH 7.4 and is activated by reducing agents, as well as by zinc and ferrous ions. *Erythrocyte proteinase II*, extracted by n-butanol, is also maximally active at pH 7.4 and activated by zinc and ferrous ions but not by reducing agents. *Erythrocyte proteinase III*, also extracted by n-butanol, has maximum activity at pH 3.2 and fails to be activated by reducing agents or metal ions. The specific activities of the present enzymes have been compared with those of trypsin and pepsin.
PROTEOLYTIC ENZYMES OF THE FORMED ELEMENTS OF HUMAN BLOOD: I. ERYTHROCYTES
Wayne L. Morrison and Hans Neurath


Access the most updated version of this article at http://www.jbc.org/content/200/1/39.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/200/1/39.citation.full.html#ref-list-1