THE PROTEOLYTIC ENZYME SYSTEM OF SKIN

I. EXTRACTION AND ACTIVATION*

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A proteinase, present in human, rat, rabbit, and guinea pig skin, has been reported by Beloff and Peters (1). The enzyme (or enzymes) digested casein and other proteins at a neutral pH and, because of the failure to digest benzoyl-L-argininamide and carbobenzyo-L-tyrosylglycinamide, the authors concluded that the specificity was not comparable to trypsin or chymotrypsin. The skin activity could not be extracted at room temperature with a 0.05 M phosphate buffer at pH 7.0, but could be partially extracted (to the extent of about 50 per cent relative to the activity obtained by incubation of skin slices with casein) if 5 per cent KCl (0.67 M) was added to the buffer. The authors also reported the existence of a peptidase that would hydrolyze L-leucylglycylglycine and which could be preferentially extracted from the proteinase activity in skin by using a 0.1 M Ringer-phosphate buffer at pH 7.3. Fruton (2) has described the presence of several peptidases in 2 per cent NaCl extracts of fresh rabbit skin. The “dermopeptidase” hydrolyzed L-leucylglycylglycine without the necessity for added manganese ions or cysteine and, in contrast to a leucine aminopeptidase also present, retained activity after dialysis against distilled water. A prolidase was also demonstrated, but activity comparable to trypsin, pepsin, or carboxypeptidase was not found. Neville-Jones and Peters (3) have compared the peptidase and proteinase activity obtainable from acetone-dried rat skin with the activity found in fresh skin extracts prepared according to Fruton (2). The acetone-dried skin extracts (5 per cent KCl or KNO₃) contained high proteinase but low “dermopeptidase” and aminopeptidase activity; in the fresh skin extracts the reverse was true.

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Wells and Babcock (4), in a more recent paper, separated the epidermis from the dermis of human skin and found that the greater amount of the proteinase activity was localized in the epidermis. Their extracts were prepared according to Beloff and Peters (1). A study of the increase in proteolysis of incubated guinea pig skin slices, after exposure to varying temperatures, has led Ungar and Damgaard (5) to postulate the presence of an inactive enzyme precursor in skin. Activation was mediated by heating tissue slices and this "secondary proteolysis" could be inhibited by a soy bean inhibitor or sodium salicylate.

The present paper is concerned with the conditions for the extraction and activation of the proteinase activity in rat skin. It will be shown that by extraction at 1°C with an extractant at 1.6 ionic strength considerably more activity can be obtained than was recognized by other workers (1, 3, 4, 6). The greater portion of the activity can be reversibly inactivated by exposure to an ionic strength much lower than that required for complete extraction. This reversible inactivation is postulated to occur by association of an enzyme with an inhibitor; the dissociation of this complex is obtained by an exposure to a high ionic strength (1.6). A preliminary statement of some of these results has been reported (7).

**Materials and Methods**

**Preparation of Skin Acetone Powder**—Male albino rats (400 to 500 gm.) of the Holtzman strain were decapitated and the hair was removed with an electric clipper. All of the skin, except that on the head, legs, and tail, was removed, and each skin was freed from underlying fatty and muscle tissue by scraping with a putty knife parallel to the fiber axis. Histological examination showed only the presence of dermis and epidermis in the collected skins. Each skin was then rolled into a tight ball, immersed in liquid nitrogen, and shattered in an iron mortar. The fragment size was further reduced by passage through a power-driven granulator and pulverizer at the temperature of liquid nitrogen. The Waring blender or Potter-Elvehjem homogenizer proved of little value in the disruption of an entire rat skin, although the latter apparatus can be successfully used to disintegrate the abdominal skin of young rats. The finely divided skin particles, admixed with dry ice snow, were placed in 4 liters of acetone and allowed to come to 3°C. Dehydration was continued with five changes of 4 liter quantities of acetone over a 24 hour period at 3°C with solvent removal accomplished by suction filtration. The skin powder was then air-dried in a hood until most of the acetone had volatilized. This was followed by drying in vacuo over P₂O₅ for 24 hours. The yield of skin acetone powder from 95 rats was 690 gm. (approximately one-third the weight of the skins before fragmentation). This type of preparation has been
kept at -30° for over a year and has shown no diminution in proteolytic activity.

Contrary to the results of Beloff and Peters (1), the acetone powder yielded a much greater amount of proteinase activity than did an equivalent weight of a similarly fragmented fresh skin preparation.

Buffers—The pH of all buffer solutions was determined at room temperature with a glass electrode by using a Cambridge research model pH meter. In the preparation of buffers containing KCl an aliquot of a 0.1 M phosphate buffer (KH₂PO₄-Na₂HPO₄) was added to a weighed amount of the salt, made nearly to volume, adjusted to the desired pH, and made to volume. The ionic strength of the buffer solutions was calculated from the composition of the buffer salt at a given pH and from the molarity of the added salt. The salts were assumed to be completely dissociated.

Proteinase Assays—1 ml. of 2 per cent casein¹ and 1.0 ml. of buffer or water were incubated together at 35° for 5 minutes before the addition of 2.0 ml. of the enzyme solution.² The pH of all solutions was 7.5³ and the buffer concentration in the reaction mixture was 0.05 M. The ionic strength was 0.8. After incubation for 20 minutes at 35° with shaking, 5 ml. of 5 per cent trichloroacetic acid were added. Control flasks were not incubated and were identical with the reaction flasks, with the exception that the enzyme solutions were added after the addition of the trichloroacetic acid. The optical density increment in the absence of casein was negligible. After centrifugation, the optical density at 280 μm of the supernatant fluid from the reaction flask was measured by using the supernatant fluid from the control flask as the blank.

All assays were performed with casein as the substrate and with enzyme concentrations adjusted to be proportional to the optical density increment.

The activity of the various enzyme preparations is expressed as a "dermo-proteinase" (DP) unit which is defined as that amount of enzyme which will produce an optical density increment of 1.00 per minute at 280 μm under the above standard conditions. The activity obtainable from 1 gm. of skin acetone powder is designated [DP]ᵣ.ₚ.

¹ Casein, after Hammersten (Nutritional Biochemicals Corporation, Cleveland, Ohio), was denatured according to Beloff and Peters (1) and prepared as a 2 per cent suspension. It was freshly prepared for each day of use.

² The enzyme solutions, in buffer, were not incubated at 35.0°, owing to their great lability in the absence of substrate at this temperature. This thermal instability varied greatly with the type of enzyme pretreatment, and for comparative purposes all enzyme solutions were kept at 1° before addition to the reaction flasks. Otherwise, they were stored at -30°.

³ The pH optimum for the digestion of casein and urea-denatured hemoglobin was found to be in the range of pH 7 to 7.5. Phosphate, Veronal, and the universal buffer of Östling and Virtama (8) gave concordant results.
Results

Enhancement of Enzymatic Activity—Initial studies were directed toward the purification and further characterization of the proteinase activity of extracts prepared by extracting skin acetone powder at room temperature.

Table I

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beloff-Peters' type extract†</td>
<td>90‡ (440)$</td>
</tr>
<tr>
<td>&quot; &quot; frozen at -30°, thawed at 1°</td>
<td>385</td>
</tr>
<tr>
<td>Acetone ppt. (EP-1)¶</td>
<td>210 (295)$</td>
</tr>
<tr>
<td>Ionic strength 1.6 extract**</td>
<td>1 (415)$</td>
</tr>
</tbody>
</table>

* Calculated as [DP]_{700} X 10^2 (see under "Materials and methods"). All values represent the average of several determinations.
† 1 gm. of skin acetone powder was extracted at room temperature for 30 minutes with 10 ml. of 0.05 M phosphate buffer containing 0.67 M KCl at pH 7.5 (ionic strength 0.8). The use of this pH rather than 7.0, as used by Beloff and Peters (1), gave a slightly greater yield of activity.
‡ This value was increased to 125 when the Beloff and Peters' type extract was assayed at the ionic strength (0.4), used by Beloff and Peters (1) in their assays. However, if activity determinations at ionic strength 0.4 were corrected for the increase in the optical density at 280 mλ contributed by the enzyme preparation when incubated in the absence of casein, the activity value decreased to 70.
§ The values in parentheses represent activities determined subsequent to exposure of the enzyme solutions to an ionic strength of 1.6. In this type of determination it was imperative that the enzyme solution was not exposed to the ionic strength of assay (0.8) until contact with the substrate had been accomplished. This was conveniently achieved by adjusting the volume of the enzyme aliquot so that a final ionic strength of 0.8 was obtained upon mixing with the substrate.
¶ Assays performed immediately after thawing.
‖ The Beloff and Peters type extract was precipitated with 45 volume per cent acetone at -4° to -6°. After collection of the precipitate by centrifugation at 0° it was suspended in the original extraction medium (ionic strength 0.8).
** Prepared by extracting 1 gm. of skin acetone powder for 30 minutes at room temperature with 10 ml. of 0.1 M phosphate buffer containing 1.34 M KCl at pH 7.5 (ionic strength 1.6).

with 0.05 M phosphate buffer containing 0.67 M KCl at pH 7.5 (ionic strength 0.8). This procedure is essentially that of Beloff and Peters (1) and extracts prepared in this manner will be referred to as the Beloff and Peters' type extract. Early in these studies it became apparent that much of the proteinase activity in these extracts existed in an inactive state which could be activated by various procedures. When this type of enzyme preparation, containing 90 units per gm. of skin acetone powder, was
frozen at -30° and assayed immediately after thawing at 1°, a 4-fold increase in activity resulted (Table I). Activity potentiation was also obtained when this type of extract was treated with acetone (Table I). This procedure yielded approximately twice as much activity as was contained in the initial extract.

These two observations indicated that a reinvestigation of extraction conditions might lead to increased yields of activity. When an extract of skin powder was prepared at room temperature by extraction at ionic strength, 1.6, an apparent 5-fold increase in activity was obtained (Table I). However, when the Beloff and Peters' type extract was exposed to an ionic strength 1.6 (cf. Table I) before assay, the apparent increase in yield by extraction at ionic strength 1.6 disappeared. Only a slight increase in activity was observed when the fraction obtained by acetone precipitation was exposed to ionic strength 1.6 before assay.

**Effect of Ionic Strength on Proteinase Extraction at 1°**—Since it was observed that the activity in the extract of ionic strength 1.6 at room temperature was unstable at approximately 25°, an observation that did not pertain to the active and potentially active proteinase activity in the Beloff and Peters' type extract, the further investigations of extraction conditions were conducted at 1°. The extraction of skin powder with solutions of different ionic strengths is shown in Fig. 1, and it is apparent that the yield

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**Fig. 1.** The extraction of proteinase activity at 1° as a function of ionic strength. 1 gm. samples of skin acetone powder were extracted at pH 7.5 and 1° for 4 hours with 10 ml. of 0.1 M phosphate buffer containing KCl to give the indicated ionic strengths. Curve A, activity determinations were performed by exposing each extract to an ionic strength 1.6 before assay; Curve B, assays were performed in the usual manner at ionic strength 0.8 without prior exposure to ionic strength 1.6.
of proteinase activity is a marked function of the ionic strength of extraction (Curve A). Maximal extraction occurred at ionic strength 1.6. The activity values in this curve were obtained by prior exposure of the enzyme solutions to ionic strength 1.6 and therefore represent the total activity obtainable from the skin acetone powder. The maximal yield (850 to 880 units) obtained by extraction at 1°C with a buffer of ionic strength 1.6 was approximately 10 times that derived by the original procedure of Beloff and Peters (90 units; cf. Table I).

If the extracts obtained at ionic strengths 1.2, 1.35, and 1.47 were assayed without prior exposure to ionic strength 1.6, it can be seen (Curve B, Fig. 1) that the activity was suppressed. Thus, the differences in the ordinate values of Curves A and B represent the additional activation obtained by exposure of these extracts to an ionic strength 1.6 before assay. For maximal activation, the ionic strength of exposure must be 1.4 or greater since Curves A and B become identical at this abscissa value.

**Table II**

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-1.6 extract†</td>
<td>(800)‡</td>
</tr>
<tr>
<td>Fraction EDP-1§</td>
<td>265 (765)‡</td>
</tr>
<tr>
<td>Inactivated E-1.6 extract$</td>
<td>275 (800)‡</td>
</tr>
</tbody>
</table>

* The values represent the average of a number of experiments and are expressed as [DP] TV X 10³.
† 1 gm. of skin acetone powder, extracted for 15 hours at 1°C with 10 ml. of 0.1 M phosphate buffer containing 1.34 M KCl at pH 7.5 (ionic strength 1.6).
‡ The values in parentheses were obtained by exposure of the enzyme solution to ionic strength 1.6 before assay at 0.8 (cf. § footnote, Table I).
§ Prepared by dialysis of the E-1.6 extract at 3°C against several changes of distilled water until essentially all salt had been removed. The resulting precipitate was collected by centrifugation at 1°C and suspended in 0.8 M KCl.
$ The E-1.6 extract was diluted with an equal volume of distilled water to give an ionic strength of 0.8 and kept at 1°C for 20 hours.

Demonstration of Reversible Inactivation—Experiments presented in Table II demonstrated that a fully activated enzyme extract (E-1.6) could be inactivated by exposure to a lower ionic strength and that the activity was suppressed. Thus, the differences in the ordinate values of Curves A and B represent the additional activation obtained by exposure of these extracts to an ionic strength 1.6 before assay. For maximal activation, the ionic strength of exposure must be 1.4 or greater since Curves A and B become identical at this abscissa value.

Enzyme extracts prepared by extraction of skin acetone powder (1 gm.) at 1°C with 10 ml. of 0.1 M phosphate buffer containing 1.34 M KCl at pH 7.5 (ionic strength 1.6) or with 1.6 M KCl will be referred to as E-1.6. The extracts prepared with either extractant were apparently equivalent, and, although the yield was maximal in a 4 hour extraction period, it proved convenient to extract overnight for approximately 15 hours.
inactivated extract could be fully reactivated by raising the ionic strength to the original value of 1.6.

Dialysis of the E-1.6 extract for 20 hours against distilled water yielded a precipitate which, when suspended in 0.8 M KCl (fraction EDP-1), possessed only one-third of the original activity. The supernatant fluid from the dialysis procedure was inactive. Original activity was restored by raising the ionic strength of fraction EDP-1 to 1.6 before assay. This experiment showed that all of the activity initially in E-1.6 was contained in the EDP-1 fraction but in a partially inactive state. Thus, the apparent loss in activity after dialysis of E-1.6 was due to a reversible inactivation process and suggested that storage of the enzyme extract at 1° and ionic strength 0.8 would produce a similar reduction in activity. The expected result was obtained and, again, full activity could be regenerated by recycling the inactivated extract through an ionic strength of 1.6 (Table II).

**Digestion of Casein and Inactivation of Proteinase Activity As Function of Ionic Strength**—When aliquots of an E-1.6 extract were exposed to

![Graph](image-url)
various ionic strengths at 1° for 0, 1, 4, and 10 hours, the results shown in Fig. 2 were obtained. In contrast to the usual assays conducted at an ionic strength of 0.8, assays in this experiment were performed at the ionic strengths of exposure (0.2 to 1.6). Since the proteinase activity in E-1.6 is

![Graph](http://www.jbc.org/)

**Fig. 3.** The inactivation of proteinase activity in E-1.6 as a function of pH at 1° and ionic strength 0.6. E-1.6 was prepared by extraction of skin powder at 1° with 1.6 M KCl and aliquots exposed to pH 5.9 (●), 7.2 (△), and 9.1 (○) at ionic strength 0.6 for various times before assay (solid lines). The extent of reactivation was determined by recycling 360-minute inactivated aliquots through an ionic strength 1.6 before assay (dash lines). The ionic strength in all reaction flasks was 0.6 and the pH, 7.5. From the results of a titration curve of E-1.6, varying quantities of HCl or NaOH were added to *incubation* tubes at 1°, to which were added, at staggered intervals, identical aliquots of E-1.6 at zero time (0.47 mg. of protein N-ml⁻¹). The final ionic strength in all tubes was 0.6, and the pH was determined on aliquots with the glass electrode. Immediately after mixing and at various time intervals, aliquots from the *incubation* tubes were added to *dilution* tubes at 1° containing the appropriate phosphate-KCl buffer to give a final ionic strength of 0.6 and a pH of 7.5. Aliquots were then removed from the *dilution* tubes to the *reaction* flasks containing casein and buffer. It took approximately 1 minute after zero time before the enzyme solution was in contact with the substrate.

in a fully activated state, the zero time curve (t₀) defines the ionic strength requirement for the maximal rate of digestion of casein. This ionic strength is 0.6 and is distinct from the ionic strength requirement for maximal activation (1.4 or greater).

With increasing exposure to the ionic strengths indicated in Fig. 2, the rate of inactivation was greater at ionic strength 0.6 than at ionic strengths above or below this value when the activity remaining at any time was compared to the zero time value.
Additional Observations on Reversible Inactivation of Proteinase Activity—
The rate of inactivation of an E-1.6 extract as a function of pH was determined at 1° and ionic strength 0.6. The results obtained at pH values of 5.9, 7.2, and 9.1 are shown in Fig. 3. Below pH 5.5 and above a pH of approximately 9 the rate studies were obscured by the marked instability of the enzyme (or enzymes). After standing for 360 minutes at the indicated pH values, the extracts were reactivated by raising the ionic strength to 1.6. As seen in Fig. 3, full activity could be restored in the extracts which had been inactivated at pH 5.9 and 7.2. Under these conditions, therefore, no irreversible acid or base-catalyzed denaturation had occurred. Only 80 per cent of the total activity could be regained in extracts exposed to pH 9.1, while exposure to pH 3.4 destroyed all activity.

The effects of variations in enzyme concentration and temperature upon the rate of inactivation at an ionic strength of 0.6 and pH 7.5 have been investigated. An increase in either of these two variables led to an increased inactivation rate. For example, the inactivation rates shown in

### Table III

Relative Thermal Inactivation of Proteinase Activity at Ionic Strengths of 1.6 and 0.8

<table>
<thead>
<tr>
<th>Temperature (°C.)</th>
<th>Time of heating (min.)</th>
<th>Per cent inactivation, heated at ionic strength 1.6</th>
<th>Per cent inactivation, heated at ionic strength 0.8</th>
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<tr>
<td>25</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td></td>
<td>30</td>
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<td>0</td>
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<td>35</td>
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<td>50</td>
<td>2</td>
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Fig. 3 were greater than those plotted in Fig. 2, where studies were conducted with an extract containing a considerably lower concentration of enzyme.

It appears reasonable to propose, on the basis of the results obtained, that there exists an enzyme-inhibitor complex whose association and dissociation reactions are functions of the ionic strength environment, the dissociation being favored at an ionic strength of 1.4 or greater and the association reaction at lower ionic strengths.6

Relative Thermal Instabilities at Ionic Strengths 1.6 and 0.8—During the course of these studies, it became evident that the enzyme extracts kept at room temperatures were less stable at ionic strength 1.6 than at ionic strength 0.8. In the light of the hypothesis stated in the preceding section, it would appear that the free enzyme was less thermostable than the postulated proteinase-inhibitor complex. The results of a study, in which a range of temperatures was employed (Table III), clearly demonstrated that extracts at an ionic strength of 1.6 were considerably less thermostable than extracts of ionic strength 0.8. It should be emphasized that the latter extracts were kept at an ionic strength of 0.8 for at least 6 hours prior to thermal exposure. This procedure insured a maximal formation of the suggested proteinase-inhibitor complex.

DISCUSSION

The greater proportion of the proteinase activity in extracts from skin acetone powder, active at a neutral pH, has been found to undergo a reversible transformation between an active and inactive state which is determined by the ionic strength of the enzyme solution. Exposure to an ionic strength 1.6 effects an apparently instantaneous activation of the proteinase activity, whereas exposure of the activated enzyme to a lower ionic strength (0.6 to 0.8) induces a slower conversion to the inactive state. Exposure to ionic strengths much above or below 0.6 produces less inactivation. The rate of inactivation is probably greatest at or near this same value, although the actual ionic strength cannot be positively stated since the data in Fig. 2 were obtained with the ionic strength both of exposure and of assay as variables. With relatively concentrated enzyme preparations, inactivation approaches an asymptotic limit in approximately 6 hours at 1° and, at any stage of inactivation, full reactivation can be obtained by recycling the enzyme through an ionic environment of strength 1.6 before assay.

The inactivation process is pH-independent within the range of enzyme stability (pH 5.5 to 8) at 1°; above or below these values irreversible inacti-

6 Extracts possessing similar properties have also been obtained from fresh rat skin that had not been frozen or treated with acetone. Extractions were performed with scissor-minced skin that had been ground with sand.
vation occurs. As would be expected, reversible inactivation occurs at a faster rate when the temperature is increased, but rate determinations are obscured by the great thermal lability of the activity in the fully activated state.

A study of the digestion of casein by the fully activated enzyme preparation as a function of the ionic strength of assay demonstrated that maximal activity was obtained at 0.6. Thus, the ionic strength at which the fully activated enzyme is most active against casein is approximately the same ionic strength at which the greatest extent and rate of conversion to the inactive state occur in the absence of substrate. This is much lower than that required for activation, which is at ionic strength 1.4 or greater.

Since it appears unlikely that more than one enzyme in skin extracts could have such properties as herein discussed, the designation of Proteinase C has been applied to the activity capable of being reversibly inactivated. It is also postulated that the inactivation of Proteinase C occurs by association with an inhibitor (CIn), activation occurring upon the dissociation of the Proteinase C-CIn complex.

Other workers have been concerned with the effect of ionic strength on proteinase activity. Lumry, Smith, and Glantz (9) have reported that carboxypeptidase activity with carbobenzyoxgycyl-L-tryptophan as substrate increased as the ionic strength of assay increased. An ionic strength of approximately 0.3 produced a maximal rate of hydrolysis. This effect was also demonstrated with the substrates carbobenzyoxgycyl-L-phenylalanine and carbobenzyoxgycyl-L-leucine. Hess, Campbell, and Herranen (10), in an investigation of the proteinase activity from bovine palatine tonsils, showed that the activity was increased as the ionic strength was increased. However, the highest ionic strength that they studied was 0.1. It has also been reported that the α-chymotrypsin-catalyzed hydrolysis of N-acetyl-L-tyrosine ethyl ester (11) or of chloroacetyl-L-tyrosinamide (12) can be potentiated by the addition of MgSO₄. Shine and Niemann have studied the hydrolysis of the latter substrate as a function of the salt concentration and found it to be increased by the addition of NaCl or KCl (13). None of these systems has been shown to have a specific ionic strength requirement for maximal activity in a manner similar to that necessary for optimal Proteinase C activity.

The designation Proteinase A is reserved for another proteinase that can catalyze the hydrolysis of N-acetyl-L-tyrosine ethyl ester and can be inhibited by the blood inhibitor preparation of Beloff (6). Preliminary work dealing with this enzyme and the evidence for its non-identity with Proteinase C will be given in another paper in this series. The term B activity was used previously (7) to identify the water-soluble proteinase activity obtained by treatment of the Beloff and Peters' type extract with acetone and which comprised several different types of proteolytic enzymes.
Although extensive use of the term "ionic strength" has been employed in this paper, with the attendant connotation that any salt would replace KCl, the authors are aware that this is not the case. Preliminary work has shown that some salts are completely incapable of dissociating Proteinase C from its inhibitor even when the concentration of the various salts employed was such that their mean ionic activity was equivalent to that of reference KCl solutions. It may be that specific ion effects are operative or that the mean ionic activities of different salts are not equivalent in the presence of skin-extracted proteins and the casein substrate.

The importance of the magnitude of the ionic environment was also evident in studies of the pH and thermal stability of the proteinase activity in E-1.6. In general, it can be said that the activity is more stable to a particular hydrogen ion concentration and to a specific temperature when the Proteinase C activity is in a state of combination with CIn. The Proteinase C-CIn interaction, then, not only masks Proteinase C activity but also protects the enzyme from destruction under conditions in which, if Proteinase C was dissociated from CIn, extensive loss of Proteinase C activity would occur.

The results presented in this paper demonstrate that the extraction procedure of Beloff and Peters can be markedly improved. Thus, in our experiments, an approximate 10-fold increase in yield was obtained when extraction was performed at 1° with an extractant of 1.6 ionic strength. However, it will be recalled that the enzyme extracts prepared and assayed by the Beloff and Peters procedure were not exposed at any time to ionic strength 1.6 and thus a major portion of the activity was in an inactive state. When extracts prepared by their procedure were exposed to ionic strength 1.6 before assay, an approximate 5-fold increase in activity resulted. Actually, therefore, our extraction conditions effected a 2-fold increase in the yield of total activity.

The enhancement of activity in the Beloff and Peters type extract by freezing and thawing, by exposure to ionic strength 1.6, and after acetone precipitation (fraction EP-1) are all procedures leading to activation of Proteinase C. Whereas, the first two procedures activate Proteinase C reversibly, treatment with acetone appears to destroy some of the postulated inhibitor. This tentative conclusion would account for the lessened activation of the EP-1 fraction when exposed to ionic strength 1.6 and would also explain the increased yield of activity in EP-1 relative to the Beloff and Peters type extract when assayed without activation.

SUMMARY

The conditions for the extraction and activation of proteinase activity from skin acetone powder have been described. Both extraction and activation have been found to be markedly dependent upon ionic strength.
An enzyme labeled Proteinase C has been postulated to exist in skin extracts in reversible combination with an inhibitor (CIn) and the degree of association and dissociation is primarily a function of ionic strength. The ionic strength (1.6) for maximal dissociation of the Proteinase C-CIn interaction is markedly different from the ionic strength requirement of Proteinase C for the maximal rate of digestion of casein (0.6). The extent and rate of the Proteinase C and CIn interaction are also greatest about ionic strength 0.6. This interaction has been found to be pH-independent over the range amenable to study.

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