Factors that Affect the Fragmentation of Growth Hormone and Prolactin by Hypophysial Proteinases

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Ellis et al. (1), Li and Papkoff (2) and Ellis (3) have reported changes in biological activity and electrophoretic behavior after treatment of growth hormone with acidic and alkaline buffers, and Lazo-Wasem and Graham (4) have studied the stability of growth hormone prepared by different procedures. Recently, it has been shown (5) that highly purified preparations of growth hormone are contaminated with proteinases. The study reported here concerns the various factors that influence the action of these enzymes and data are presented that show that many of the observations made on the stability of growth hormone can be explained by the presence of proteolytic enzymes in samples of the hormone.

EXPERIMENTAL PROCEDURE

Electrophoresis on Polyacrylamide—The method of disk electrophoresis1 as described by Ornstein and Davis (6) was used for analyzing mixtures of proteins. We have found that the addition of tetramethylethylenediamine to the large pore gel (spacer and sample sections of the columns) greatly increases the rate of polymerization of the gel. The gel polymerized slowly or not at all when the hormone samples used in these studies were mixed with the gel. The addition of 60 μl of the reagent to 100 ml of the large pore gel was sufficient. Tetramethylethylenediamine was used by Ornstein and Davis2 in their preliminary studies, but later they found it unnecessary for the type of samples they were analyzing. We have found no alteration in our electrophoretic patterns with the use of the reagent.

When applying 25 μl of a sample to a column, an equal volume of upper gel of twice the usual concentration was also added. Strongly acidic solutions, such as 1 M acetic acid, were neutralized with 1 M Tris before they were added to the column. Overheating during the electrophoresis produced an artifact in the pattern of the bovine growth hormone. A slowly migrating band behind the major component was observed when the current was greater than 5 ma per tube.

In the studies reported here, the standard gels were used unless stated otherwise: 7.5% acrylamide and a pH of 9 for the lower gel; the large pore gel was buffered at pH 10.5. The Tris-glycine buffer, pH 8.4, was used in the electrode compartments. These are the same conditions as used in our first report (5). Some samples were also analyzed on columns made with 4% acrylamide for the lower gel. The conditions of pH were the same as with the standard columns.

Disk electrophoretic analysis at an acidic pH was also carried out using the buffer system described by Reisfeld et al. (7). One modification in the composition of the buffer was introduced for these studies. As reported previously (5), the tetramethylethylenediamine interferes with the determination of protein in eluates of the polyacrylamide. By decreasing the amount of acetic acid used, the quantity of tetramethylethylenediamine could be decreased and polymerization still occurred in about 1 hour. Using the same notation as in the initial report (7), the composition of the A solution was 1 N KOH, 48 ml; glacial acetic acid, 10 ml; tetramethylethylenediamine, 1.0 ml; and water to make 100 ml. The pH of the lower gel was 4.6. The buffer for the electrode compartments was prepared by mixing 3.2 g of β-alanine with 13.9 ml of 1 M acetic acid and diluting to 1 liter with water.

Incubation Procedure—The degree of fragmentation of a hormone was observed by disk electrophoresis. Incubation mixtures were made with 0.2 ml of an 0.5% solution or suspension of hormone. Aliquots of stock solutions of the various reagents were then added. The mixtures were incubated at 37° or at room temperature without agitation. It was found that uniform heating at 37° such as in a water bath, or forced air incubator, was necessary if reproducible degradation was to be obtained. A drop of toluene was added to each tube before incubation.

The buffers used were 0.14 M acetic buffer, pH 4; 0.02 M citrate buffer, pH 5; 0.05 M phosphate buffer, pH 7.4; and 0.25 M Na2CO3 made to pH 9.6 with 3 N HCl.

Hormone Samples—Bovine, ovine, and human growth hormones and ovine prolactin were prepared in these laboratories by the methods described previously (5). A more detailed account of the procedure used for the isolation of human growth hormone has recently appeared (8). Samples of bovine growth hormone (NIH-B5) and ovine growth hormone (NIH-S3) obtained from the National Institutes of Health were used.

Inhibitors—Diisopropylphosphorofluoridate was employed as described previously (5). An 0.1 M solution of the disodium salt of ethylenediaminetetraacetic acid, adjusted to pH 7 with 3 N HCl, was used as a stock solution. The sodium salt of p-chloromercuribenzoate was suspended in 0.04 M phosphate buffer, pH 7.0, to make a 2 x 10−3 M solution. Approximately 10 μl of 1 N NaOH were added to dissolve the material. The pH was near

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† Career Development Awardee of the National Institutes of Health. Preparations from this source are designated by "NIH.”

1 A commercial model may be obtained from the Canal Industrial Corporation, Bethesda, Maryland.
2 L. Ornstein and B. J. Davis, personal communication.
9. Crystalline trypsin inhibitor from egg white was purchased commercially. A $2 \times 10^{-3}$ M solution of iodoacetamide was prepared in the phosphate buffer, pH 7, described above.

For studies performed at pH 4, the inhibitors were dissolved in 0.14 M sodium acetate buffer, pH 4.

Activators—A stock solution of metal ions was prepared by dissolving MgCl$_2$ and ZnSO$_4$ in either 0.04 M phosphate buffer, pH 7, or 0.14 M sodium acetate buffer, pH 4, to give a solution that was $10^{-3}$ M in each ion. Adjustment of pH of the stock solution was made with 1 M NaOH.

A commercial sample of mercaptoethanol was assumed to be 12 M and dilutions were made in either the phosphate or acetate buffer.

Assay for Proteolytic Activity—Proteolysis of urea-denatured hemoglobin was used as a quantitative assay for proteolytic activity. The method has been described (5). Enzymatic activity at pH 4.0, 7.4, and 9.6 was measured. The buffers had the following composition: 0.14 M sodium acetate buffer, pH 4.0; 0.05 M potassium phosphate buffer, pH 7.4; and 0.025 M Na$_2$CO$_3$ made to pH 9.6 with 3 N HCl. The buffers were made 6 M in urea. The hemoglobin and test samples were dissolved in these buffers.

The spectrophotometric assay for trypsin and chymotrypsin as described by Hummel (9) was also used to measure proteolytic activity of the hormone samples.

$N_H$-terminal Amino Acids—Fluorodinitrobenzene was used as described previously (5). The diiodophenylated amino acids were cut from the paper chromatogram and eluted with 1.2 ml of 1% NaHCO$_3$ instead of 4 ml.

RESULTS

Influence of Temperature on Fragmentation—The degree of fragmentation was observed by means of disk electrophoresis. At pH 7 and above, hydrolysis was noted to proceed in two different manners depending on the temperature. In general, when a preparation was allowed to stand at room temperature at pH 7, the fragmentation took place in a stepwise fashion, whereas at 37° an over-all decrease in concentration of all components occurred. This is illustrated in Fig. 1. In the determination of the optimal pH for breakdown at pH 5 and above, room temperature was employed. When the action of inhibitors was investigated at pH 7, the incubations were done at 37° since hydrolysis at room temperature proceeded too slowly in the presence of inhibitors. At pH 4 the hydrolysis occurred so rapidly at room temperature that the reactions were carried out at 5°.

Electrophoresis at Acidic pH—During the degradation of bovine growth hormone at an alkaline pH, a slowly migrating component forms as the original bands undergo fragmentation (5). Since proteins with an alkaline isoelectric point migrate upward and cannot be detected when the standard disk electrophoretic method is used, we analyzed a highly degraded sample of hormone at an acidic pH in order to determine whether any basic components were formed during the fragmentation. The undergraded hormone showed one major band and three minor ones with very little material at the buffer front. After fragmentation at pH 7.5 the sample had two of the same minor bands but the major component was missing and no new component was seen except for a heavily stained buffer front. The material at the buffer front was probably low molecular weight, basic fragments. After fragmentation at pH 4, bovine growth hormone showed no new components when examined under acidic conditions. As with the degradation under alkaline conditions, a disappearance of the major component was the principal change.

Electrophoresis on Large Pore Gel—By decreasing the concentration of acrylamide, the pore size of the polyacrylamide is increased (6), permitting molecules of very large molecular weight to enter the gel. To determine whether the disappearance of the components of bovine growth hormone after incubation at pH 4 was a result of aggregation of the hormone to a molecular size that could not penetrate the 7.5% polyacrylamide, electrophoresis was also performed with gels containing 4% acrylamide. Bovine growth hormone was analyzed before and after incubation at pH 4. No components were seen with the 4% acrylamide that were not observed with the standard gel.

Optimal pH for Fragmentation—In previous studies (5) it was found that the hydrolysis of our bovine growth hormone by contaminating proteinases occurred at both pH 7 and 10, but was most rapid at the higher pH. Hydrolysis under acidic conditions was not reported. We have subsequently determined the optimal conditions of pH for fragmentation of several pituitary hormone preparations. The results are summarized in Table I. In incubation at room temperature was used for these studies. When bovine growth hormone was permitted to degrade under alkaline conditions until none of the original bands remained, the degraded product was always more insoluble in alkaline buffers than was the original material.

At pH 7.4 there was a considerable variation in the time required to produce hydrolysis in the various preparations. For example, our bovine growth hormone preparation fragmented readily at room temperature within 3 hours. For the same degree of breakdown in our human growth hormone, 2 days at room temperature or overnight at 37° were required. Various batches of bovine growth hormone prepared by the same procedure varied considerably with respect to rate of fragmentation.

The hormones that degraded most rapidly at pH 9.6 were the bovine growth hormone and the two preparations of human growth hormone, all of which were made in these laboratories.

**Fig. 1. Two ways in which bovine growth hormone is observed to degrade at pH 7.** a, Unincubated hormone; b, incubated at 37° for 3 hours; c, after 18 hours at room temperature. Note the overall decrease in intensity of the bands in b, and the stepwise fragmentation in c.
TABLE I

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Inhibitor*</th>
<th>pH†</th>
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<tr>
<td></td>
<td>DFP</td>
<td>CMB</td>
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<tr>
<td>Bovine GH...</td>
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<td>++</td>
</tr>
<tr>
<td>(NIH)</td>
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<td></td>
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<tr>
<td>Ovine GH...</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Ovine GH</td>
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<td></td>
</tr>
<tr>
<td>(NIH)</td>
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<td></td>
</tr>
<tr>
<td>Ovine prolactin...</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Human GH...</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Human GH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Raben-type)</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

* Symbols used in inhibitor column: ++++, strongly inhibitory; ++, moderately inhibitory; +, weakly inhibitory; -, no inhibition.
† Symbols used in pH column: ++++, rapid degradation of hormone; ++, moderate rate of degradation; +, slow degradation; -, no degradation observed.
† The abbreviations used are: DFP, diisopropylphosphorofluoridate; CMB, p-chloromercuribenzoate; GH, growth hormone.

The ovine prolactin and growth hormone prepared by us, and the samples of bovine and ovine growth hormone from the National Institutes of Health underwent fragmentation more rapidly at pH 7 than at 9.6.

No breakdown was noted at pH 5 in any of the samples after 18 hours at room temperature, whereas at this temperature transformations had occurred at pH 7 in all but the human growth hormone samples. A disappearance of the bands in our ovine and bovine growth hormones (whole gland preparations) could be demonstrated at pH 5 if the samples were incubated overnight at 37°C.

When incubated at pH 4 at room temperature for 16 hours in acetate and citrate buffers, the characteristic bands seen in all the ovine and bovine growth hormone preparations disappeared with no indication of a stepwise conversion. Our human growth hormone required 24 hours at room temperature before a change was noted. The human growth hormone prepared by the method of Raben (10) had to be incubated at 37°C for 16 hours in order to show a decrease in intensity of the bands. Ovine prolactin showed only minor loss in intensity of its bands after 24 hours at 37°C. A bovine growth hormone preparation (5) that failed to fragment at an alkaline pH readily underwent degradation at pH 4. Data presented in a following section indicate that the disappearance of the bands at pH 4 is a result of a proteolytic degradation mediated by contaminating enzymes.

Evidence for formation of a new component at an acidic pH could be demonstrated by allowing bovine growth hormone to stand at 5°C for 18 hours (Fig. 2, a, b, and c). A new band appeared which migrated behind the major component. After disappearance of the original bands, the new compound still remained. Formation of the new band was more effectively shown in glycerine-HCl buffer, pH 3.6, than in acetate buffer of the same pH.

Assay with Hemoglobin as Substrate—A comparison was made between the rate of hydrolysis of urea-denatured hemoglobin by the contaminating proteinases and the ease of fragmentation of the hormone as judged by disc electrophoresis. The bovine growth hormone and ovine prolactin prepared in these laboratories were used since they underwent fragmentation very rapidly at pH 7.4 and contained sufficient enzymes to be detected by the proteinase assay. No hydrolysis of hemoglobin could be detected with hormone preparations that fragmented slowly, such as our ovine growth hormone or the bovine growth hormone obtained from the National Institutes of Health. Fig. 3 illustrates that the enzymes in prolactin degraded the hemoglobin quite effectively at pH 7.4 but only slightly at pH 9.6. The proteinases of the growth hormone preparation were about...
equally active at these two pH values. At pH 7.4 diisopropylphosphorofluoridate (10⁻³ M) inhibited the hydrolysis of the hemoglobin by the bovine growth hormone preparation. These results are consistent with the fragmentation data (Table I).

No hydrolysis of the urea-denatured hemoglobin could be detected at pH 4 when 5 mg of our bovine growth hormone preparation were used.

**Reaction toward Synthetic Substrates**—The bovine growth hormone and ovine prolactin samples that hydrolyzed hemoglobin were assayed for chymotrypsin-like and trypsin-like activities using N-benzylo-l-tyrosine ethyl ester and p-toluene-sulphonyl-l-arginine methyl ester as substrates. No hydrolysis was noted at pH 7 when as much as 100 µg of hormone was used. The methods could detect 2 µg of chymotrypsin and 0.5 µg of trypsin.

**Action of Activators**—Fragmentation of our bovine growth hormone at both pH 4 and pH 7 was accelerated slightly by 5 X 10⁻² M mercaptoethanol. A mixture of Mn²⁺, Mg²⁺, and Zn²⁺, each 5 X 10⁻⁴ M, had no acceleratory effect.

**Action of Inhibitors**—Table I summarizes the results obtained in a study of the action of inhibitors on the proteinases contaminating the hormone preparations. All inhibition studies were carried out at pH 7, in 0.05 M phosphate buffer. Inhibition of either the stepwise fragmentation or the over-all decrease in the intensity of the bands could be demonstrated depending on the temperature used (see Fig. 1).

Diisopropylphosphorofluoridate (10⁻³ M) was the most effective inhibitor in every instance. The inhibitor could not be removed by dialysis at pH 7. When used at 10⁻⁴ M, it was definitely less inhibitory. Demonstration of inhibition by the reagent was most difficult with human growth hormone prepared by the method of Raben (10). Fragmentation of this material proceeded only when the pH was above 8, at which diisopropylphosphorofluoridate has been shown to be less effective (5).

Degradation of human growth hormone prepared by a different procedure (8) was readily inhibited by the substance.

p-Chloromercuribenzoate (2 X 10⁻³ M) was a less efficient inhibitor and failed to prevent the hydrolysis of ovine prolactin. When diluted to 10⁻⁴ M, the reagent caused no inhibition in any of the preparations. Mercure ion (10⁻³ M) produced about the same degree of inhibition as did the organic mercurial when tested with our bovine growth hormone, and cysteine (2 X 10⁻⁴ M) prevented this inhibition. Ethylenediaminetetraacetic acid (10⁻³ M) acted as an inhibitor in a few cases but very ineffectively. In a number of experiments, treatment of human growth hormone and prolactin with p-chloromercuribenzoate or ethylenediaminetetraacetic acid actually produced an increase in the number of components seen. The fragmentation was also atypical in that the components were closer together than usual. Iodoacetamide (2 X 10⁻³ M) failed to inhibit the fragmentation in all cases. The trypsin inhibitor of egg white was inhibitory only when used in an amount equal to that of the hormone. It was tested only with our bovine growth hormone preparation.

Adams and Smith (11) have reported that phosphate inhibits the alkaline proteinases of the hypophysis during the hydrolysis of urea-denatured hemoglobin. A comparison of the rate of fragmentation of bovine growth hormone, as judged by disk electrophoresis, was made using 0.05 M and 0.2 M phosphate buffers, pH 7.5. Fragmentation of bovine growth hormone prepared in our laboratories was only slightly inhibited by 0.2 M phosphate whereas breakdown of the hormone obtained from the National Institutes of Health (NIH-B5) was greatly slowed by the high concentration of phosphate.

No inhibitor was found for the enzymes that functioned at pH 4. Treatment of our bovine growth hormone first at pH 7 with diisopropylphosphorofluoridate and then lowering the pH to 4 failed to inhibit the hydrolysis.

**Evidence for Enzymatic Degradation at pH 4**—Since at pH 4 no inhibitor of the enzymes that altered bovine growth hormone could be found and because no hydrolysis of urea-denatured hemoglobin could be detected, evidence for proteolytic contamination had to be obtained indirectly.

The most convincing data indicating proteolysis during incubation at pH 4 were the changes observed in the NH₂-terminal amino acids of ovine growth hormone (Table II). The ovine

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**Table II**

<table>
<thead>
<tr>
<th>Dinitrophenylamino acid</th>
<th>O.D.₅₆₀ of eluted spot</th>
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<tbody>
<tr>
<td>Aspartic acid</td>
<td>0.035</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.066</td>
</tr>
<tr>
<td>Serine</td>
<td>0.14</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.08</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.02</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.19</td>
</tr>
<tr>
<td>Phenytoalanine</td>
<td>0.79</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Hydrolysis of urea-denatured hemoglobin by bovine growth hormone (GH) and ovine prolactin. The optical density values represent the absorption of trichloroacetic acid filtrates. Incubation was at 37° for 16 hours with 20 mg of hemoglobin as substrate. Note the similar rate of hydrolysis with the growth hormone at the two pH values while the enzymes in the prolactin were most active at pH 7.4.
hormone was allowed to stand at 37° overnight in 0.14 M acetate buffer, pH 4. This caused a complete disappearance of components as judged by disk electrophoresis. The NH₂-terminal amino acids were then determined. Alanine decreased whereas glutamic acid and serine increased. The data also indicate that the peptide chain with phenylalanine as NH₂-terminal is not attacked. During hydrolysis at an alkaline pH both peptide chains were altered.

As mentioned above, ovine prolactin underwent alteration at pH 4 very slowly. It was reasoned that if the disappearance of the bovine growth hormone bands were mediated by a proteinase, then addition of some of the growth hormone to prolactin should accelerate the disappearance of the prolactin bands. Equal quantities of the two hormones were mixed and incubated for 16 hours at 37° and pH 4. For controls, solutions of the individual hormones were incubated separately and analyzed both individually and after mixing just before electrophoresis. Incubation of growth hormone and prolactin together accelerated the disappearance of the latter. The growth hormone bands completely disappeared. Addition of growth hormone to prolactin just before electrophoresis did not change the pattern of the prolactin. The accelerated decrease in concentration of the bands seen in prolactin when incubated with growth hormone is seen in Fig. 2, d and e.

With hope of inactivating any proteinases present in bovine growth hormone preparations, the hormone was incubated at pH 4 in 50% ethanol for 1 hour at 37° and then lyophilized. A control sample was treated in the same manner only without ethanol. The samples were then incubated overnight in 0.14 M acetate buffer, pH 4. The bands of the control completely disappeared whereas the ethanol-treated material still showed the characteristic pattern when analyzed by disk electrophoresis although the intensity of the bands was definitely weaker than with unincubated material.

**Discussion**

There have been reports (1, 2) regarding the changes in electrophoretic behavior of bovine growth hormone after treatment with acidic and alkaline buffers but inconsistencies are apparent, as authors of the reports have pointed out. For example, growth hormone undergoes different alterations when prepared by different procedures (1, 2) or even when isolated by the same method (1). Such behavior could be explained by varying amounts and kinds of proteolytic contaminants. Not only the method of isolation but also the completeness of the separation of anterior and posterior lobes of the hypophysis would be of importance for it has been shown (5) that bovine growth hormone is less contaminated with proteinases when anterior lobes are used instead of whole glands.

Bovine growth hormone isolated by alkaline extraction loses biological activity when treated with acid, whereas preparations prepared using glacial acetic acid for extraction show no loss of activity when stored in acidic buffers (4). Differing degrees of proteolytic contamination of the two preparations would explain this observation. The hot glacial acetic acid could be expected to inactivate the hypophysial proteinases at least partially, a view consistent with the disk electrophoretic data. Electrophoresis indicated a much slower rate of fragmentation of human growth hormone prepared by the method of Raben (10) than for material isolated under milder conditions. The inactivation of growth hormone by proteinases, such as the acid proteinase of the hypophysis described by Ellis (12) would then be more likely to occur in the growth hormone preparation with the higher amount of contaminating enzymes.

The failure of prolactin to degrade at pH 4 can also be explained by a low concentration of acid proteinase in the material. The initial step in the isolation of prolactin is an acid-aceton extraction at room temperature, a process likely to inactivate enzymes. Ellis (12) has shown that the hypophysial acid proteinase is very sensitive to organic solvents.

Unequivocal proof for contaminating enzymes active at an acidic pH was more difficult to obtain than for the enzymes which function under alkaline conditions (5). Certain facts that indicate proteolysis at pH 4 and which would be difficult to explain otherwise are the altered NH₂-terminal amino acid pattern, acceleration of the disappearance of the characteristic bands of prolactin when mixed with growth hormone, the slow degradation of human growth hormone prepared by the method of Raben, the slowed alteration of growth hormone after treatment with ethanol, and the acceleration of disappearance of the components of bovine growth hormone when treated with mercaptoethanol. Failure to detect hydrolysis of urea-denatured hemoglobin may be a result of a low concentration of enzymes in the hormone preparations.

Detection of proteolysis by measurement of the disappearance of electrophoretic components is complicated by the fact that aggregation of growth hormone occurs at pH 4 (13, 14). Since no additional component was seen when the pore size of the polyacrylamide was increased, an aggregate too large to enter the gel was not indicated. The new slowly migrating band seen after incubating growth hormone at pH 4 may be the aggregated hormone but even this disappeared upon prolonged standing.

When it was observed (5) that the bovine growth hormone prepared in these laboratories under went most rapid degradation at pH 10, there was concern as to whether alkaline conditions were not causing the breakdown. The observation that certain preparations, such as the growth hormone from the National Institutes of Health, fragmented more slowly at pH 9.6 than at 7.4 indicates that this was not the case. Also, bovine growth hormone prepared from anterior lobes is highly resistant to fragmentation at both pH 7.4 and 9.6 (5). Inhibition of the fragmentation of growth hormone and prolactin by a heat-labile, nondialyzable substance in serum (13) also supports the view that the degradation of the hormones is a result of proteolysis. Inability to detect proteolysis of urea-denatured hemoglobin by certain preparations is interpreted to mean that the degree of contamination was low in these samples. Without exception we have observed a direct correlation between rate of fragmentation as detected by disk electrophoresis and the amount of proteolysis in the hemoglobin assay.

The different rates of fragmentation and different values of pH optima of the various preparations indicate varying degrees and kinds of contamination. For example, the fact that our bovine growth hormone degrades most rapidly at pH 10 whereas the NIH material fragmented best at pH 7.4 suggests two enzymes that function under alkaline conditions. Additional support for this view is that phosphate ion inhibits fragmentation of the NIH bovine hormone but has less pronounced effect on the material prepared in these laboratories.

The relatively ineffective inhibition produced by diisopropylphosphorofluoridate when added to human growth hormone prepared by the method of Raben can be explained as a result of
a spontaneous reactivation of the enzymes during incubation. The preparation has a low concentration of contaminating proteinases which necessitates a long incubation period to demonstrate a fragmentation, and the enzymes in the sample are most active at an alkaline pH. These conditions would favor a spontaneous reactivation of the enzymes as has been shown with diethylphosphoryl trypsin (16). The rate of reactivation of the trypsin inhibitor compound increased with increasing pH. The fact that inhibition by diisopropylphosphorofluoridate can be demonstrated readily with growth hormone that fragments easily would be consistent with the view of spontaneous reactivation in the case of the Raben-type material. An alternate explanation would be that the hypophysial enzymes which are inhibited by diisopropylphosphorofluoridate are essentially inactivated by glacial acetic acid whereas peptidases unaffected by the inhibitor are more stable to the acetic acid. These peptidases would then be responsible for the major portion of the observed fragmentation although small amounts of diisopropylphosphorofluoridate-inhibited enzymes would also contribute to the degradation.

SUMMARY

The effect of diisopropylphosphorofluoridate, p-chloromercuribenzoate, ethylenediaminetetraacetic acid, mercaptoethanol, divalent metals, and phosphate ion on the proteolytic fragmentation of a number of different samples of growth hormone and prolactin was studied. The pH optimum for the fragmentation was also examined. The degree of inhibition and pH optima were found to be different in the various preparations indicating that the method of isolation of the hormone determined the amount and kind of contaminating proteinase. These data were used to explain the differences in physicochemical and biological stability of various hormone preparations.

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Factors that Affect the Fragmentation of Growth Hormone and Prolactin by Hypophysial Proteinases

U. J. Lewis


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