KINETICS OF THE FORMATION OF PEPSIN FROM SWINE PEPSINOGEN AND IDENTIFICATION OF AN INTERMEDIATE COMPOUND

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Swine pepsinogen, the inactive precursor of swine pepsin, has recently been isolated from gastric mucosae and crystallized (1). This protein has no proteolytic or milk clotting action at pH 5.0–6.0 and is unchanged in solution at pH 8.5. When a solution of pepsinogen is made more acid than pH 5.0 it changes into the enzyme, pepsin. Pepsin, however, clots milk at pH 5.0–6.0 and is instantly inactivated at pH 8.5. Measurement of the milk clotting activity at pH 5.0–6.0, therefore, determines pepsin alone. Complete conversion of pepsinogen to pepsin at pH 2.0 followed by measurement at pH 5.0–6.0 determines pepsin plus pepsinogen; while pepsinogen alone may be estimated by inactivating the pepsin at pH 8.5 and then converting the pepsinogen at pH 2.0, followed by an estimation of the pepsin thus formed.

In the previous work (1) other methods of estimation were used to examine the formation of pepsin from crystalline pepsinogen at pH 4.6 and it was found that the reaction was autocatalytic; *i.e.*, a product of the reaction, pepsin, accelerated the formation of itself. It was found that this transformation at pH 4.6 could be quantitatively described by a simple autocatalytic equation which states that the rate of reaction at any time is proportional to both the pepsin and pepsinogen concentration. At pH 4.6 the decrease in pepsinogen concentration was accompanied by a concomitant and equal increase in the pepsin.

In the present kinetic study the previous results at pH 4.6 have been confirmed and it has been found that under all conditions of varied acidity and salt the formation of pepsin from pepsinogen is

essentially autocatalytic for addition of pepsin always accelerates the reaction. However, in solutions more acid than pH 4.0 the loss in pepsinogen was not immediately accompanied by an equivalent increase in pepsin. Since the amount of pepsin ultimately reached the value equivalent to the total original pepsinogen concentration and for other reasons it is concluded that this discrepancy is not due to a secondary reaction involving either the pepsin or pepsinogen. The experimental facts agree with the assumption that in the formation of pepsin from pepsinogen there is an intermediate compound. The presence of this intermediate compound is responsible for the deviations from the simple autocatalytic kinetics. The compound differs from pepsinogen in being alkali (pH 8.5) labile like pepsin but differs from pepsin in being enzymatically inactive. The compound (PI) is a reversibly dissociable compound of pepsin (P) and a low molecular weight inhibitor (I), the dissociation being nearly complete below pH 5.0 and very small at pH 6.0.

The inhibitor is a peptide which is slowly destroyed in the presence of pepsin. It is soluble and stable in boiling 2.5 per cent trichloracetic acid, insoluble in 0.7 saturated ammonium sulfate containing 2.5 per cent trichloracetic acid, and dialyzes only slowly through collodion.

The transformation reaction may be schematically represented as follows:

$$\text{Pepsinogen} \xrightarrow{\text{(Pepsin)}} \underset{\text{compound}}{\text{Pepsin inhibitor}} \underset{> \text{pH } 5.4}{\overset{< \text{pH } 5.4}{\rightleftharpoons}} \text{Pepsin} + \text{Inhibitor} \rightarrow \text{Pepsin} + X$$

The pepsin inhibitor and pepsin inhibitor compound have many properties in common with the trypsin inhibitor and trypsin inhibitor compound reported by Kunitz and Northrop (2). However, the trypsin inhibitor is not formed from trypsinogen upon conversion into trypsin but is a material occurring in the pancreas.

Experimental Results

The experiments reported here present a general survey of the kinetics of the transformation of pepsinogen into pepsin. The general forms of the curves under the various conditions are significant and have been obtained repeatedly with various preparations but quantitatively there is considerable variation, owing to the fact that the

reaction rate is very sensitive to slight changes in pH, salt concentration, temperature, and pepsin or pepsinogen concentration.

Effect of Variation in Pepsin and Pepsinogen Concentration on the Reaction

It was shown in the writer's previous work (1) that transformation of pepsinogen to pepsin at pH 4.6 followed the simple autocatalytic equation:

$$-\frac{d\,(\text{pepsinogen})}{dt} = +\frac{d\,(\text{pespin})}{dt} = K\,(\text{pepsinogen})\,(\text{pepsin})$$

or on integration:

$$K = \frac{2.3}{(\text{pepsinogen}_{(0)})\,t}\log_{10}\left[\frac{(\text{pepsinogen}_{(t)})}{(\text{pepsinogen}_{(0)}) - (\text{pepsinogen}_{(t)})}\right] + C$$

where pepsinogen $_{(0)}$ is the concentration of pepsinogen at time = 0, pepsinogen $_{(t)}$ is the concentration of pepsinogen at time = t, and C the integration constant is equal to the logarithm of the ratio of pepsinogen concentration at time = 0 to the pepsin concentration at time = 0. This equation predicts that increasing the concentration of either pepsin or pepsinogen will result in an increase in rate of change in pepsinogen concentration per unit of time.

EXPERIMENTAL PROCEDURE

A. To 3.4 ml. water was added 0.4 ml. M/1 pH 4.5 acetate buffer and 0.2 ml. of a pepsinogen solution containing 2 mg. protein nitrogen per ml. The solution was kept at 35.5°C. At intervals of time 0.5 ml. samples were removed and the pepsinogen content determined as described under Experimental methods.

In a second tube 0.5 ml. of a solution of crystalline pepsin prepared from Parke Davis pepsin at pH 4.5 containing 1 mg. protein nitrogen per ml. was added to 2.9 ml. water followed by the acetate buffer and pepsinogen and treated in a similar manner.

B. The same procedures and solutions were used in this experiment as in *A* with the exception that the temperature was 0°C. and the buffer instead of pH 4.5 acetate was M/1 acetic acid. Pipettes and solutions were all cooled to 5°C. before using.

C. To 4 ml. of 5 N hydrochloric acid was added 15.6 ml. water at 0°C. and 0.4 ml. of pepsinogen containing 5 mg. protein nitrogen per ml. The tube containing the solution was kept in cracked ice and at intervals of time 1 ml. samples were removed with previously chilled pipettes and the pepsinogen estimated in the

usual way. In the alkaline borate solution used in the estimation of pepsinogen an equivalent of alkali was present in the borate solution and this solution was chilled to 0°C. before introduction of the acid in order to minimize the effect of the strong alkali on the protein. In the second tube was 13.6 ml. water, 2 ml. of 1 mg. protein nitrogen per ml. crystalline pepsin solution, 4 ml. 5 N hydrochloric acid and 0.4 ml. pepsinogen. This was treated in a manner analogous to that used on the solution in the first tube.

In Fig. 1 are the results of experiments in which the effect of adding pepsin to pepsinogen solutions was examined at pH 4.6, pH 3.0,

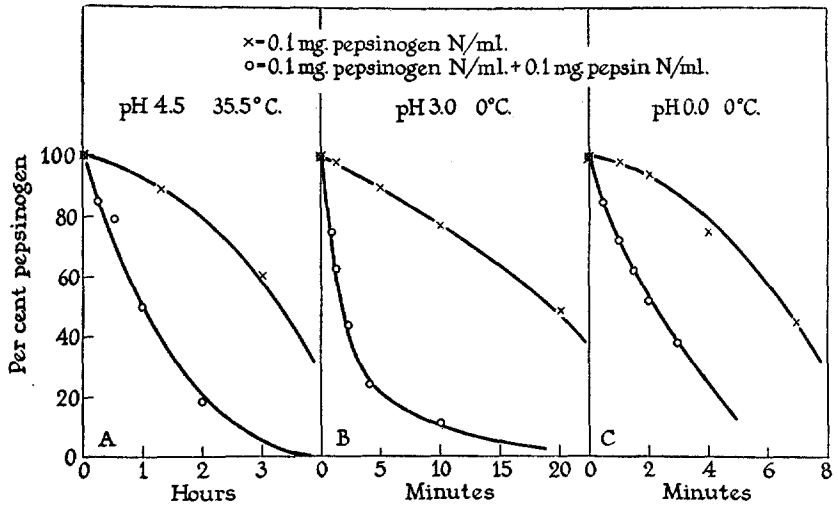

FIG. 1. Effect of pepsin on the transformation of pepsinogen at different pH

and pH 0. In each instance the addition of pepsin caused an increase in the rate of reaction as measured by the loss in the pepsinogen. This result is in agreement with the hypothesis that the system is essentially autocatalytic in nature at all pH.

EXPERIMENTAL PROCEDURE

A. To 7.65 ml. water, 1 ml. M/1 pH 4.65 acetate buffer, and 0.35 ml. N/10 hydrochloric acid was added 1 ml. of 10 mg. protein nitrogen per ml. pepsinogen solution. The mixture was kept at 35.5°C. and at intervals samples were removed and analyzed for the amount of pepsinogen as described in Experimental methods.

Immediately after mixing the above reaction mixture 1 ml. was removed and added to 7.65 ml. water, 0.35 ml. N/10 sodium chloride, and 1 ml. M/1 pH 4.65

acetate all at 35.5°C. This solution now contains 0.1 ml. protein nitrogen per ml. Aliquots were removed from time to time and analyzed for pepsinogen.

As soon as the above solution containing 0.1 mg. protein nitrogen per ml. was mixed 1 ml. was removed and added to 7.65 ml. water, 0.35 ml. N/10 sodium chloride, and 1 ml. M/1 pH 4.65 acetate and handled just as in the above instance. This solution contains 0.01 mg. protein nitrogen per ml.

B. The three solutions in *B* containing respectively 1.0, 0.1, and 0.01 mg. protein nitrogen per ml. were made up as described below and kept at 0°C. Samples were analyzed for pepsinogen.

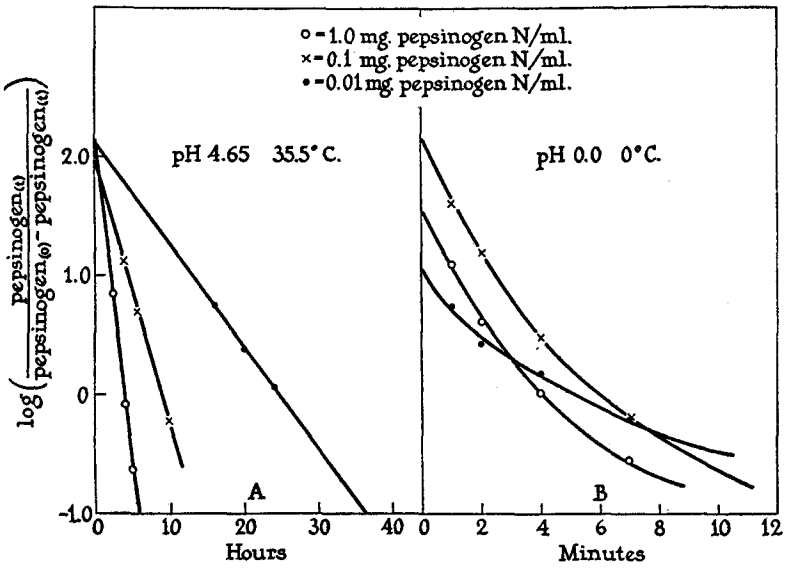

FIG. 2. Effect of pepsinogen concentration on the rate of transformation of pepsinogen at different pH.

"1.0"—4 ml. 5 mg. protein nitrogen per ml. pepsinogen was added to 4 ml. 5 N hydrochloric acid + 0.3 ml. N/1 hydrochloric acid + 11.7 ml. water all at 0°C.

"0.1"—4 ml. of 0.5 mg. protein nitrogen per ml. pepsinogen + 4 ml. 5 N hydrochloric acid + 12 ml. water.

"0.01"—0.4 ml. of 0.5 mg. protein nitrogen per ml. pepsinogen + 4 ml. 5 N hydrochloric acid + 15.6 ml. water.

In Fig. 2 are the results of experiments in which the pepsinogen concentration was varied at pH 4.6 and pH 0. At pH 4.6 an increase in pepsinogen concentration always resulted in an increase in the rate of reaction as measured by the loss in pepsinogen. Further

proof of the autocatalytic nature of the reaction is shown by the fact that the determined values fall on a straight line when the $\log_{10}\left(\frac{\text{pepsinogen}_{(t)}}{\text{pepsinogen}_{(0)} - \text{pepsinogen}_{(t)}}\right)$ is plotted against the time, where pepsinogen $_{(t)}$ is the concentration of pepsinogen at time t and pepsinogen $_{(0)}$ is the original pepsinogen concentration.

At pH 0 the results are not so clear. The reaction is more complicated since increasing the pepsinogen concentration does not cause a marked increase in rate of transformation and the reaction is not exactly linear when plotted as $\log_{10}\left(\frac{\text{pepsinogen}_{(t)}}{\text{pepsinogen}_{(0)} - \text{pepsinogen}_{(t)}}\right)$ but proceeds more slowly as the reaction continues. There are a

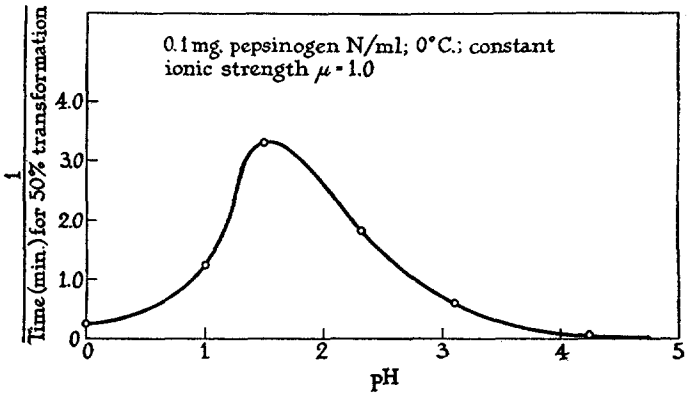

FIG. 3. pH-pepsinogen transformation curve

number of explanations for these deviations but unfortunately experimental difficulties prevent a decisive answer on this point. However, since pepsin increases the rate of conversion at this pH it is highly probable that the reaction is still autocatalytic in nature but complicated for reasons to be described later in this paper.

Effect of pH on the Rate of Transformation

If the transformation is autocatalytic one would expect a maximum rate in the region of acidity where pepsin is most active or efficient; *i.e.*, about pH 2.0. Since salts were known to affect the reaction it was necessary to have a constant ionic strength. The results of the experiments carried out in the presence of molar chloride ion at 0°C. are shown in Fig. 3 in which the reciprocal of the time necessary

for 50 per cent conversion is plotted against the pH. The curve is qualitatively similar to the pH digestion curve of pepsin (3) and is therefore a further indication that the reaction is catalyzed by pepsin.

EXPERIMENTAL PROCEDURE

All the solutions used in this experiment were M/10 with respect to acetate ion and M/1 with respect to chloride ion. The pH was determined electrometrically by a glass electrode standardized against M/10 pH 4.0 acetate buffer. The solutions were kept in the ice bath during the entire experiment and chilled pipettes were used throughout. The volume, 5 ml., and pepsinogen concentration of 0.1 mg. protein nitrogen per ml. was the same in all tubes. The tubes differed only in the amount of hydrogen ion which was regulated by acetate buffer and hydrochloric acid. The pepsinogen was a heat denatured and reversed preparation which had subsequently been fractionated and dialyzed.

Changing the pepsinogen concentration changes the rate of reaction more on the alkaline side of the maximum than on the acid side. The shape of the pH-rate of reaction curve will, therefore, vary somewhat with the pepsinogen concentration.

Effect of Salt on Reaction

If the change in pepsinogen catalyzed by pepsin is similar to the ordinary digestion of proteins by pepsin it would be expected that the rate of transformation would be depressed by the addition of neutral salts. It was found, however, as may be seen in Fig. 4 *A* that the rate of reaction as measured by the loss in pepsinogen is increased by the addition of salts at pH 4.0. The pH of the solution was shifted as much as a half a pH unit but after correcting for this effect there is still a very definite accelerating effect. The change in pepsinogen still follows the autocatalytic equation as may be seen from the type of plotting in Fig. 4 *A*. The addition of salt on the acid side of the pH transformation maximum has the opposite effect, as seen in Fig. 4 *B*. Here the pH is also affected by the addition of salts but even correcting for this the salt depresses the rate of reaction. It might be pointed out that these experiments in Fig. 4 *B* follow the autocatalytic plot more nearly than those at pH 0 in Fig. 2 *B*.

EXPERIMENTAL PROCEDURE

The pepsinogen content of the solutions in both *A* and *B* was 0.1 mg. protein nitrogen per ml. and the temperature was 25°C. and 0°C. respectively. Aliquots

were removed from time to time and pepsinogen content determined as described under Experimental methods. The pH was determined electrometrically with a hydrogen electrode standardized against M/10 pH 4.0 acetate buffer and M/10 hydrochloric acid.

A. In the presence of no salt M/10 pH 4.0 acetate was used. With sodium chloride and magnesium sulfate pH 4.45 acetate was used and the resulting pH is recorded on Fig. 4 *A*.

B. The concentration of hydrochloric acid and sodium chloride and the E.M.F. of the activation mixture are indicated on Fig. 4 *B*.

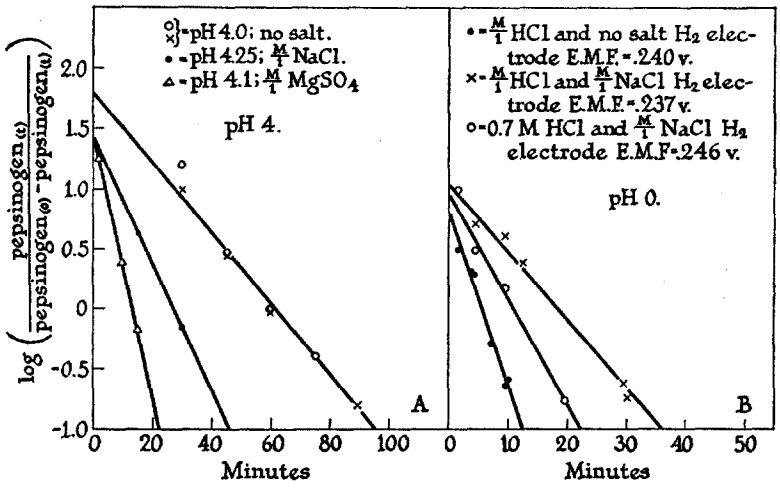

FIG. 4. Effect of salts on the rate of transformation of pepsinogen at different pH.

Evidence for an Intermediate Compound, (PI)

As pointed out earlier in this paper, the decrease in pepsinogen should be equal to the increase in pepsin if the reaction consists simply of the conversion of pepsinogen to pepsin. This was the case in the experiments first reported (1) which were carried out at pH 4.6. However, when similar experiments were carried out in solutions more acid than pH 4.0 it was found that the decrease in pepsinogen was faster than the increase in pepsin. This is shown clearly in Fig. 5 where the changes in pepsinogen and pepsin were examined at pH 4.6, pH 4.0, pH 0, and at pH 4.0 in the presence of molar magnesium sulfate. It may be seen that at pH 4.6 the amount of pepsin found is equal at any time to the amount of pepsinogen lost, thus confirming the previous work. Under the other conditions, however, when

the pepsinogen was completely altered there was only a fraction of the pepsin present. On longer standing the pepsin concentration always reached a value equivalent to the total original pepsinogen concentration. The discrepancy between the pepsinogen lost and the pepsin found cannot be due to the irreversible destruction of pepsin nor to any secondary reaction involving either pepsin or pepsinogen

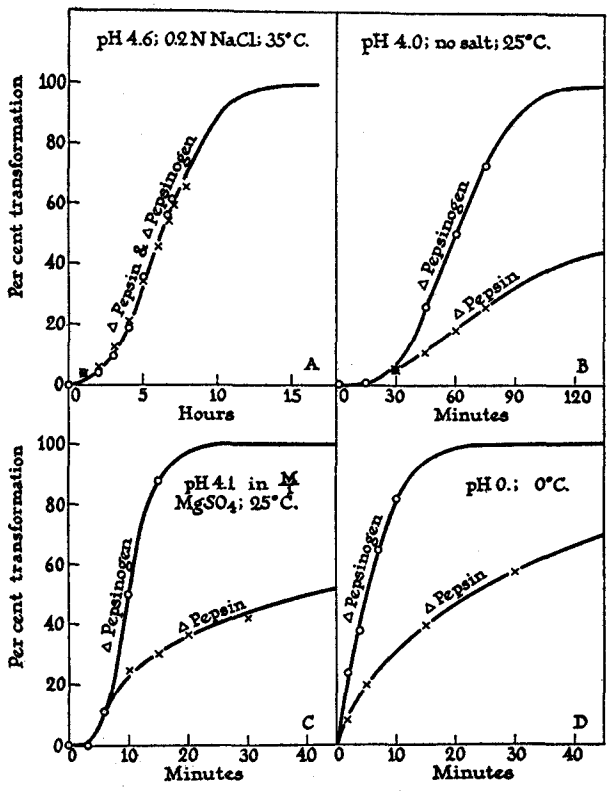

FIG. 5. Comparison of increase in pepsin with decrease in pepsinogen during transformation under different conditions.

but must be due to an intermediate inactive substance which later is converted into active pepsin.

EXPERIMENTAL PROCEDURE

The solutions in all four parts of Fig. 5 contained 0.1 mg. protein nitrogen per ml. and the changes in pepsinogen and pepsin were determined by the procedures outlined in the Experimental methods.

The solutions were made up as follows:

A. 1 ml. heat denatured and reversed pepsinogen containing 1 mg. protein nitrogen per ml. was added to a solution made up of 1 ml. 2 N hydrochloric acid + 4 ml. N/1 sodium acetate + 4 ml. water. The reaction was carried out at 35.5°C.

B. 0.1 ml. of 10 mg. protein nitrogen per ml. pepsinogen was added to 10 ml. of M/10 pH 4.0 acetate at 25°C.

C. 0.1 ml. of 10 mg. protein nitrogen per ml. pepsinogen was added to a solution made up of 5 ml. 2 M magnesium sulfate + 1 ml. M/1 pH 4.45 acetate and 4 ml. water, all at 25°C.

D. 0.1 ml. of 10 mg. protein nitrogen per ml. pepsinogen was added to 10 ml. of N/1 hydrochloric acid at 0°C.

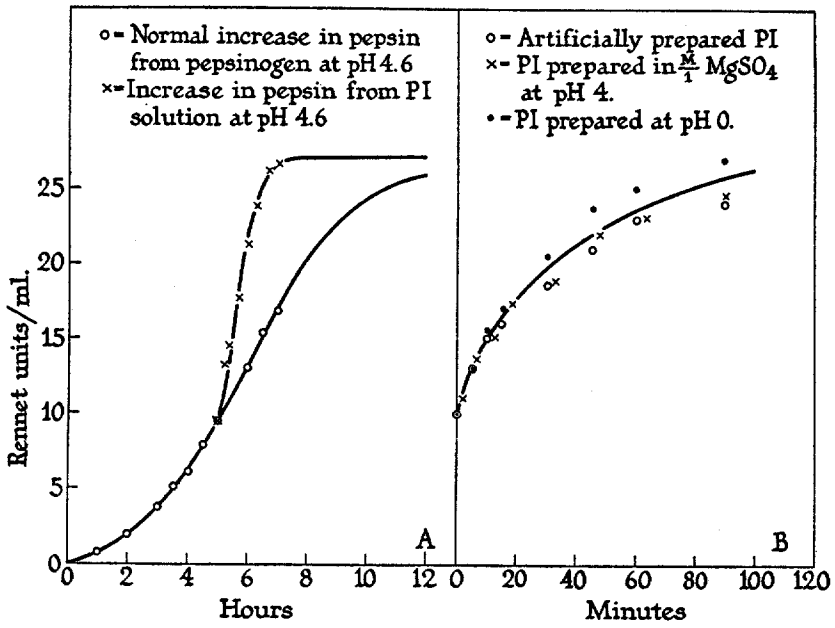

FIG. 6. *A.* Comparison of rate of formation of pepsin from pepsinogen and the intermediate compound, *PI*, at pH 4.6. *B.* Comparison of rate of formation of pepsin from *PI* prepared by different procedures.

Further evidence of an intermediate compound is shown in Fig. 6 *A*. A pH 0 transformation mixture in which all the pepsinogen had disappeared but which contained only a small fraction of its full pepsin activity was shifted to pH 4.6 and the rate of pepsin formation examined. This was compared with the rate of formation of pepsin in a similar pepsinogen solution which had been at pH 4.6 throughout the entire reaction. It may be seen in Fig. 6 *A* that pepsin is formed

in the two solutions at entirely different rates and therefore the two solutions must contain different substances. At pH 0, therefore, an intermediate compound, *PI*, accumulates in the solution and at pH 4.6 this dissociates into pepsin and inhibitor.

In Fig. 6 *B* are the curves showing the rate of pepsin formation from the intermediate compound called *PI* prepared in three quite different ways. Since the materials change into pepsin at about the same rate it is probable that they are the same substance.

EXPERIMENTAL PROCEDURE

A. Normal Transformation at pH 4.6.—1 ml. of dialyzed purified heat denatured and reversed pepsinogen solutions containing 1 mg. protein nitrogen per ml. was added to a solution made up of 1 ml. 2 N hydrochloric acid, 4 ml. N/1 sodium acetate, and 4 ml. water. The solution was kept at 35.5°C. and analyzed at varying intervals of time for active pepsin by the rennet method after a dilution of 0.1/10 in water.

Transformation of (PI) at pH 4.6.—1 ml. of the same pepsinogen as used in the above experiment but cooled to 0°C. was added to 1 ml. 2 N hydrochloric acid also at 0°C. This was kept at this temperature for 10 minutes after which 8 ml. of cold N/2 sodium acetate was mixed with it. The tube containing the solution was placed at 35.5°C. and analyzed immediately for pepsinogen and at varying intervals of time for pepsin activity as in the above experiment. The pepsinogen content was only 10 per cent of the original amount; *i.e.*, there had been a 90 per cent conversion.

B. Artificially Prepared PI.

Inhibitor.—145 ml. 1 mg. protein nitrogen per ml. pepsinogen at room temperature pH 6.5 + 3 ml. 0.2 N hydrochloric acid for 30 minutes at room temperature; 1.5 ml. N/1 sodium hydroxide added and solution left 10 minutes after which 4.0 ml. N/1 hydrochloric acid added and solution allowed to stand 30 minutes; suspension filtered. Filtrate titrated to pH 3.5. Analysis showed the presence of no pepsin activity in this filtrate.

PI.—0.5 ml. of dialyzed purified pepsin from pepsinogen solution was added to 1 ml. N/1 sodium acetate and 2 ml. of the above described inhibitor solution pH 6.0 (yellow to methyl red and brom thymol blue). After 90 minutes at 25°C. this solution was cooled to 0°C. and mixed with a solution of the following composition at 35°C.: 1 ml. 4 N hydrochloric acid, 11.5 ml. water, 2 ml. 2 M magnesium sulfate, and 1.9 ml. 4 M sodium acetate. This solution after thorough mixing was brought to 35.5°C. and samples removed and diluted 0.1/10 in water and analyzed immediately after dilution for pepsin by the rennet method.

PI Prepared from Pepsinogen at pH 4.0 in the Presence of M/1 Magnesium Sulfate.—1 ml. dialyzed fractionated reversed heat denatured pepsinogen containing 2 mg. protein nitrogen per ml. was mixed with 1 ml. 4 M pH 4.6 acetate and 2 ml. 2 M magnesium sulfate and allowed to stand at 0°C. for 155 minutes.

This solution was then mixed with a solution at 35.5°C. containing 2 ml. 2 M sodium chloride, 2 ml. 2 M pH 4.6 acetate, and 12 ml. water. After thorough mixing 0.1 ml. samples were removed, diluted to 10 ml. in water, and activity determinations made as in above solution.

PI Prepared from Pepsinogen at pH 0.—1 ml. of the pepsinogen used in the above experiment containing 2 mg. protein nitrogen per ml. was added to 1 ml. 4 N hydrochloric acid and 2 ml. water at 0°C. where it was kept for 10 minutes. A solution made up of 2 ml. 2 M magnesium sulfate, 2 ml. 4 M sodium acetate, and 12 ml. of water at 35°C. was added. There was less than 7 per cent of the original pepsinogen left; *i.e.*, there was 90 per cent conversion. Samples were analyzed as in the above experiments after dilution of 0.1/10 in water.

General Nature of the Intermediate Compound, PI

The amount of demonstrable pepsin activity in activation mixtures below pH 4 varies with the dilution, time, and pH of the solution used for measurement. This suggested the existence of a dissociable pepsin inhibitor compound as the intermediate compound. It was found that if solutions which had developed full pepsin activity were titrated to pH 6.0 the activity decreased slowly with time, suggesting that the dissociation of the pepsin inhibitor compound was reversible and a function of pH. This is actually the case and the midpoint in the dissociation equilibrium is the vicinity of pH 5.4 with complete dissociation in solutions more acid than pH 4.0 and nearly complete association at pH 6.0.

Mechanism of the Transformation

The entire reaction may be represented schematically as follows:

$$\text{Pepsinogen} \xrightarrow{\text{(Pepsin)}} \underset{\text{compound}}{\text{Pepsin inhibitor}} \underset{> \text{pH } 5.4}{\overset{< \text{pH } 5.4}{\rightleftharpoons}} \text{Pepsin} + \text{Inhibitor} \rightarrow \text{Pepsin} + X$$

The first reaction from pepsinogen to pepsin inhibitor compound is catalyzed by pepsin while the second reaction from the compound to free pepsin and the inhibitor is a reversible dissociation. The third reaction is the destruction of the inhibitor in the presence of pepsin.

Since the pepsin inhibitor compound can be demonstrated in transformation mixtures at pH 0–pH 4, it cannot be supposed that the pepsin and inhibitor are first formed separately in solution and then combine to form *PI* compound, for the equilibrium at these pH is in

favor of the dissociated free pepsin and the enzyme, P, should therefore not combine with the inhibitor, I.

At pH 4.6 where the changes in pepsin and pepsinogen are equivalent the first reaction is the slowest and determines the rate with which pepsin is produced; *i.e.*, the PI dissociates liberating active pepsin as fast as it is formed. In solutions more acid than pH 4.0 the first reaction is faster than the second or dissociation reaction. Consequently the dissociation reaction determines the rate of formation of free pepsin and accounts for the accumulation of the PI compound in the solution.

General Nature of the Inhibitor, I

The inhibitor may be prepared free of pepsin by alkali denaturation and acid precipitation of the pepsin in a freshly activated solution. When the filtrate containing the inhibitor is mixed with solutions of crystalline pepsin at pH 6.0 the pepsin activity decreases slowly. This change may be reversed by mere acidification of the solution. If, however, the activation solution is allowed to stand at pH 2.0–5.0 for a number of hours the inhibitor slowly disappears. In the absence of pepsin the inhibitor is quite stable over this range. It is stable and soluble in boiling 2.5 per cent trichloracetic acid, insoluble in 0.7 saturated ammonium sulfate containing 2.5 per cent trichloracetic acid, and dialyzes only slowly through collodion.

EXPERIMENTAL METHODS

Rennet Activity Measurements.—The rennet method was carried out as described previously (1) using "20 per cent Klim" in M/10 pH 5.0 acetate buffer. The Klim solution was prepared at least 12 hours before used and discarded 2 days after preparation. In a given experiment all samples were diluted to the same volume and unless the concentration of acid or buffer was too high the solvent was distilled water. The estimation was made immediately after diluting unless otherwise stated in the procedure of the particular experiment.

Determination of Pepsinogen.—This measurement involves the alkali denaturation of pepsin and pepsin inhibitor compound at pH 8.65 and room temperature for 15–30 minutes followed by full activation of the pepsinogen at pH 1.0–2.0 and activity estimations of the resulting pepsin. The solutions used and technique varied somewhat depending on the concentration of buffer and acid in the sample containing pepsinogen which was made alkaline. When the sample contained as much as 1 N hydrochloric acid, as in mixtures at pH 0, so that an equivalent of alkali was required, the alkaline solution was kept at 0°C. until mixing and neu-

tralization were complete. This diminished secondary effects due to localization of the strong alkali.

The details may best be given by the following example. 0.5 ml. sample of activation mixture was mixed with 1.5 ml. of N/3 pH 8.65 borate buffer containing alkali equivalent to acid in the sample. This solution remained at 25°C. for 15–30 minutes after which 1 ml. of N/2 sulfuric acid was added or sufficient acid to make the solution just pink to thymol blue. After standing 30 minutes at 25°C. this solution was diluted in water and analyzed by the rennet method or hemoglobin method. The analyses by both methods are quantitatively comparable. Since the estimation of pepsin in the original activation mixture was and must be carried out by the rennet method all of the measurements in the determination of pepsinogen in the present work were made by the rennet method.

Determination of Pepsin in the Transformation Mixture.—Estimation of pepsin in a transformation mixture was made by merely diluting a sample in water or dilute acetate buffer of such concentration and pH that the pH of the dilution did not get above pH 4.65. The estimations were made immediately after diluting and mixing.

Pepsinogen Preparations.—Nearly all pepsinogen used in this work was prepared as previously described (1) but without crystallization. The preparation was dialyzed against dilute pH 6.8 phosphate buffer and then frozen and dried as described previously (1). In this way salt and buffer free stable materials of reproducible nature were used.

pH.—pH estimations unless otherwise noted were colorimetric using indicators of Clark and Lubs.

SUMMARY

A study of the kinetics of the transformation of swine pepsinogen into pepsin under a variety of conditions has been made. The results show that the transformation as a whole is essentially autocatalytic in nature under all conditions.

Evidence is presented to show the existence of a compound intermediate between pepsinogen and pepsin. This compound was found to be a reversibly dissociable complex of pepsin and a low molecular weight inhibitor.

Some of the general properties of the intermediate compound and of the inhibitor have been examined.

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