PEPSINOGEN:

ACTIVATION BY A UNIMOLECULAR MECHANISM

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SUMMARY. Pepsinogen rapidly develops proteolytic activity in the presence of high concentrations of denatured hemoglobin. At the same time, it is transformed into a species which is denatured at pH 8.5, showing that the activity is due to the formation of pepsin by a unimolecular mechanism, not to an active form of the zymogen.

Recent experiments have clearly shown that the related zymogens prochymosin (prorennin) and pepsinogen are capable of self-activation, without the intervention of the derived proteases (1-4). However, the kinetic order of this activation is still open to some doubt. On the basis of kinetic studies of activation, Foltmann suggested a proteolytically active form of prochymosin, at low pH, which could digest a second molecule to form chymosin (1). On the other hand, Bustin and Conway-Jacobs showed that pepsinogen developed proteolytic activity in the presence of high concentrations of a pepsin substrate and concluded that this was due to an intramolecular activation of the zymogen, since the activation seemed to be independent of pepsinogen concentration and was not enhanced by added pepsin (2).

Studies of mine on the kinetics of activation of pepsinogen above pH 4, using a characteristic difference spectrum, which appears irreversibly as pepsin is formed, showed deviations from auto-catalytic behavior compatible with either of the above hypotheses (3). Below pH 4, most of the absorbance change occurred rapidly and reversibly, with no detectable formation of pepsin, leading me to suggest a rapid conformational change to a form of pepsinogen which was capable of self-activation.

Kassell and Kay (5) have suggested that all the above observations could be reconciled if this postulated intermediate showed proteolytic activity towards

other zymogen molecules and to pepsin substrates, but could not convert itself to pepsin, in the presence of a large excess of hemoglobin. This intriguing idea made it reasonable to extend the experiments of Bustin and Conway-Jacobs to identify the species giving rise to the proteolytic activity which they observed.

Fig. 1 shows two experiments which essentially confirm the results of

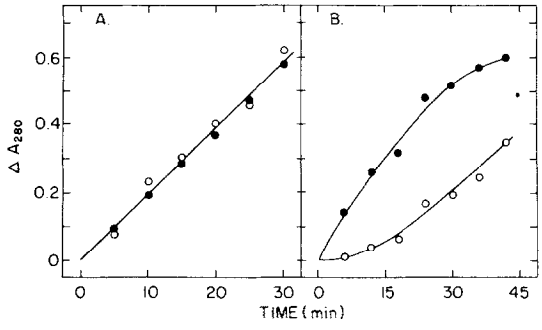

FIG. 1. Digestion of hemoglobin at (A) pH 1.8 and (B) pH 3.0, 25°, by solutions of pepsin (•) and pepsinogen (o). The peptides liberated during the times shown were measured after CCl_3COOH precipitation.

Bustin and Conway-Jacobs (2). Aliquots of 25 μl of 0.7 mg/ml solutions of pepsin (Worthington Lot #PM1BA) or pepsinogen (Worthington Lot #PG1GB) were added to 5 ml samples of a 25 mg/ml solution of hemoglobin (Worthington Lot #HB3CA) at pH 1.8 or 3.0 and 25°. At 5 or 6 min intervals, 5 ml of 10% CCl_3COOH were added, the solution clarified by centrifugation and the absorbance at 280 nm measured. At the lower pH, both pepsin and pepsinogen solutions show equal activity, whereas at pH 3.0, pepsinogen shows less activity and a lag time in its occurrence compared with pepsin.

The identity of the active species in the solutions to which pepsinogen was added was determined by the following experiments. Pepsinogen was added to acid solutions of hemoglobin, as above. This time, at 5 or 6 min intervals, the solutions were adjusted to pH 9.5 by the addition of 0.2 N sodium hydroxide and kept at that pH for 1 hr. The solutions were then brought below pH 2 with 1 ml

of 1 N hydrochloric acid. After a further 30 min, an equal volume of 10% CCl_3COOH was added, the solution clarified and the absorbance at 280 nm measured. In a second series of experiments, this procedure was repeated, except that the solutions were adjusted to pH 8.5 during the middle time period. The results of these experiments are shown in Fig. 2.

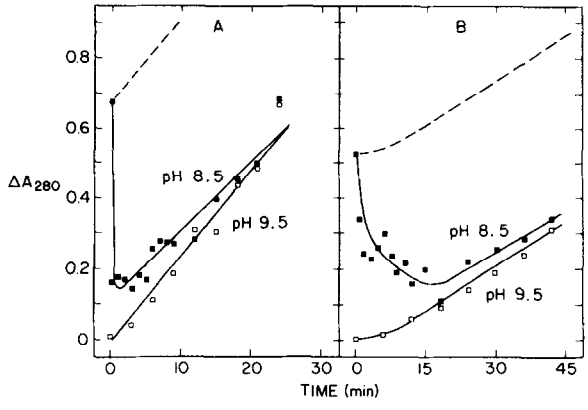

FIG. 2. Digestion of hemoglobin at (A) pH 1.8 and (B) pH 3.0, 25°, by solutions of pepsinogen. After the times shown the solutions were taken to pH 8.5 (■) or pH 9.5 (□) for 1 hr and then back below pH 2 for 30 min. The peptides liberated during the combined incubations were measured after CCl_3COOH precipitation. The dashed lines show the expected results, if the activity formed during the first incubation survived exposure to pH 8.5 and could digest the substrate during the further 30 min at low pH.

The experiments reported here confirm the observation that pepsinogen shows marked proteolytic activity at low pH, even in the presence of a large excess of denatured hemoglobin, which would inhibit any auto-catalytic activation by contaminating pepsin (2). The new experiments, shown in Fig. 2, take advantage of the fact that both pepsinogen and pepsin are irreversibly denatured at pH 9.5, whereas only pepsin is destroyed at pH 8.5 (6).

The zero time points of the upper curves of Figs. 2A and 2B show the products formed by the active species generated from pepsinogen which had received no initial incubation at low pH, but had been held at pH 8.5 for 1 hr and then taken to low pH for 30 min. If the activity formed during the first incubation at low pH were due to a form of pepsinogen, stable at pH 8.5, all the following

points would be higher (dashed lines) since this activated form would be able to attack substrate during the first and third incubations. In fact all later points are lower than the first, showing that the species formed was, like pepsin, unstable at pH 8.5. The lower curves of Figs. 2A and 2B represent the activity generated in the first incubation only, since both proteins are destroyed in the middle incubation at pH 9.5 (compare Fig. 1). After the first 30 sec the two curves of Fig. 2A approximately coincide, indicating that the pepsinogen has been converted completely to a form labile at pH 8.5. At the higher pH of Fig. 2B, this conversion is slower and is complete in about 15 min.

Similar results to those shown in Fig. 2 were obtained if the exposure to high pH took place in two stages; 30 min at pH 5.5, followed by 30 min at pH 8.5 or 9.5. This ruled out the possibility that an active form of pepsinogen would be more sensitive to high pH than the form found at neutral pH. Therefore, the activity observed is due to the formation of pepsin, which is denatured at this pH. These experiments do not rule out the possibility that pepsinogen could show proteolytic activity at low pH, similar to that detected with other zymogens (5). However, they do show that the activity detected by Bustin and Conway-Jacobs arose from the irreversible activation of pepsinogen to pepsin. Since this occurred in the presence of an enormous excess of a possible substrate, it seems certain that the activation is an intramolecular reaction (7).

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