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The influence of salts on the conversion of pepsinogen to pepsin

Pepsinogen and pepsin are both considered to be single-chain polypeptide structures[1-3]. Further, the conversion of pepsinogen to pepsin in acid media has been shown to entail limited proteolysis which conforms to the kinetics of autocatalysis[4,5]. The process is reported to be influenced by pH and by the presence of salts. At a around pH 4, 1 M NaCl and 1 M $MgSO_4$ increase the conversion rates[5].

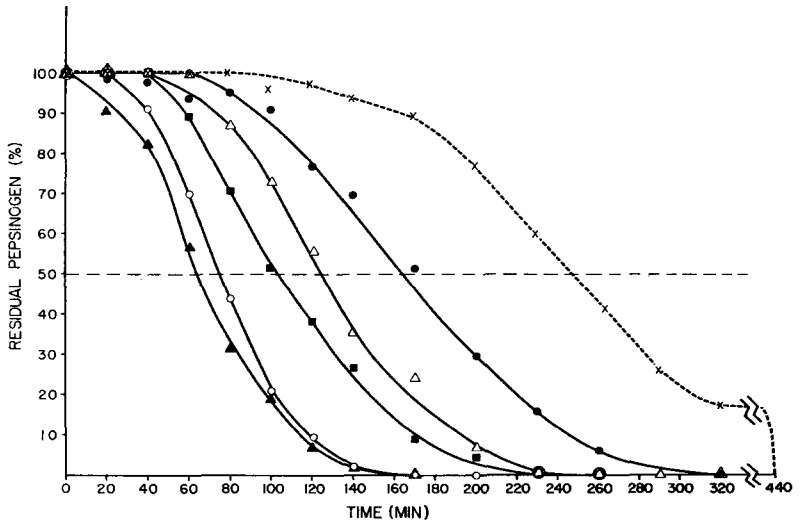
The present communication on the influence of NaCl shows that the conversion rate increases with increasing NaCl concentration and is a linear function of that concentration over the range 0.075–0.65 M NaCl. Further, it confirms the autocatalytic character of the conversion process under these conditions.

The pepsinogen (Worthington Biochemicals Corp., Lot 6007-10) used was shown to be about 97.5% pure physico-chemically by DEAE-cellulose chromatography, ultracentrifugation, free-boundary and cellulose acetate electrophoresis criteria[6]. Freshly prepared solutions of pepsinogen (12.8 mg/ml) in 0.002 M sodium phosphate buffer (pH 7.0) were used as the starting solutions for the experiments to be reported. Approx. 2% insoluble material was removed by centrifugation before use.

The conversions of pepsinogen to pepsin and determinations of residual pepsinogen were based on the work of HERRIOTT[4]. They involved the following steps in the order given: conversion of pepsinogen to pepsin, stopping of the reaction and destruction of newly formed pepsin, activation of the residual pepsinogen, and finally its determination (as pepsin) by assay of proteolytic activity using hemoglobin as substrate.

A typical procedure is described, as follows: The pepsinogen solution containing 12.8 mg/ml in 0.002 M phosphate buffer (pH 7.0) was mixed with an equal volume of 0.1 M acetate buffer (containing variable amounts of NaCl) to give a conversion mixture, pH 4.4 at 25°. A 0.05-ml aliquot was withdrawn at "zero-time." The remainder of the conversion mixture was then incubated at 37°, and similar aliquots were withdrawn periodically. These and the zero-time aliquot were mixed immediately on withdrawal with 0.45 ml of a satd. borax solution (approx. 0.15 M) containing HCl to give a final pH of 8.4, set at room temperature for 10 min, and then stored in ice until ready for activation. This treatment arrested the conversion of pepsinogen to pepsin and destroyed any pepsin which had been formed. Residual pepsinogen was activated at pH 1.7 for 15 min at room temperature by mixing 0.1 ml of the pH-8.4 solution with 0.4 ml of 0.075 M glycine containing enough 1 M HCl to achieve the proper final pH. Activated pepsinogen was then assayed for proteolytic activity using hemoglobin as substrate. The assay mixture was composed of 0.10 ml of activated pepsinogen solution, 0.50 ml of 0.1 M glycine–HCl buffer (pH 1.7) 2 ml of pH-1.7 hemoglobin–HCl solution and 0.40 ml H_2O, a final volume of 3.0 ml. The assay mixture was 1.67% with respect to the hemoglobin substrate. It was incubated for 10 min at 37° and then deproteinized by adding 5 ml of a 5% solution of trichloroacetic acid. Precipitated protein was filtered with Whatman No. 3 paper and the absorbancy of the filtrate was measured at 280 mμ in a Beckman model DU spectrophotometer.

In Fig. 1A the results are plotted as percentage residual pepsinogen *vs.* time, to show the time course of the conversions. The rate of conversion increases as the



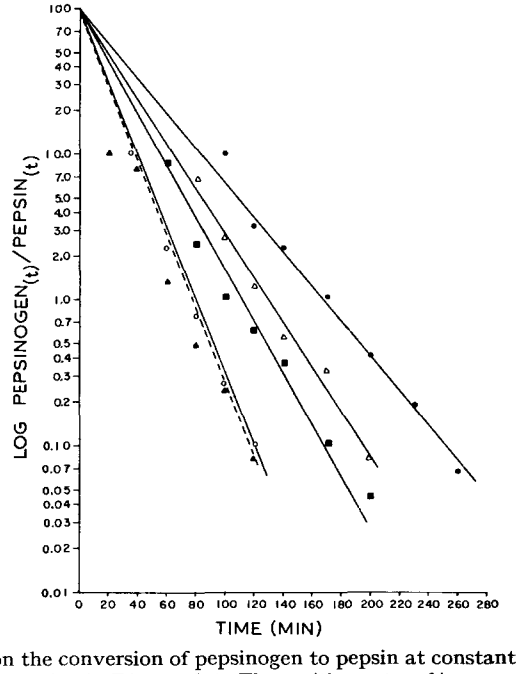


Fig. 1. Effect of NaCl on the conversion of pepsinogen to pepsin at constant acetate concentration (0.05 M). See text for details. A. Direct plot. The $t_{\frac{1}{2}}$ (time at 50% conversion) is given by the intersection of the dotted line with the curves. B. Semi log plot.

Curve	*NaCl (M)*	*I*
×- - -×	—	0.015
●—●	0.075	0.092
△—△	0.200	0.219
■—■	0.350	0.370
○—○	0.450	0.470
▲—▲	0.650	0.672

ionic strength is increased. This is effected by reduction in the duration of the "lag" period as well as by an increased conversion rate in the exponential region of the process. Fig. 2, a plot of the reciprocal of the time for 50% conversion, $1/t_{1/2}$, *vs.* NaCl concentration establishes the direct linear relation which exists between NaCl concentration and the rate of conversion over the range tested. The straight lines obtained when the data is plotted as log (pepsinogen/pepsin) *vs.* time (Fig. 1B), confirms the autocatalytic nature of the conversion in each case*.

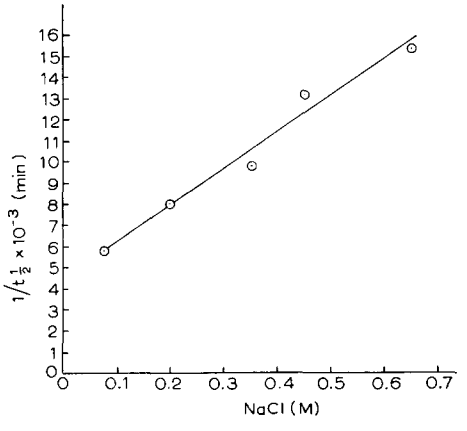


Fig. 2. Plot of $1/t_{\frac{1}{2}}$ *versus* NaCl concentration, in the conversion of pepsinogen to pepsin.

Graphic representation of HERRIOTT's data (Fig. 4 in ref. 5) in a plot of $1/t_{1/2}$ *vs.* ionic strength also shows the accelleration at pH around 4 to be a function of ionic strength (Fig. 3).

It is reasonable to suppose that the salts exert their effect by dissolution of electrostatic bonds that may normally stabilize pepsinogen and pepsin–pepsin inhibitor complex. The importance of electrostatic forces for the conformational

* In the kinetics of autocatalysis the rate of reaction is a function of the concentrations of reactant and product. In the conversion of pepsinogen (Pg) to pepsin (P) this is represented[4] as

$$-\frac{d\,Pg}{dt} = +\frac{dP}{dt} = k \cdot Pg \cdot P$$

The definite integral of this second-order reaction equation[4,7] is

$$k \cdot Pg_0 \cdot t = 2.3 \log \frac{P_t \cdot Pg_0}{Pg_t \cdot P_0}$$

assuming that P_0, the concentration of pepsin at time = 0, is very low.

On separation and rearrangement of terms this becomes

$$\log \frac{Pg_t}{P_t} = -\frac{k \cdot Pg_0 \cdot t}{2.3} + \log \frac{Pg_0}{P_0}$$

A plot of $\log \frac{Pg_t}{P_t}$ *versus* t should give a straight line with negative slope proportional to the overall reaction rate constant, k, and with an intercept equal to $\log \frac{Pg_0}{P_0}$ when $t = 0$.

The linear results obtained from such plots of the experimental data for the systems containing NaCl conform with these expectations for kinetics of simple autocatalysis.

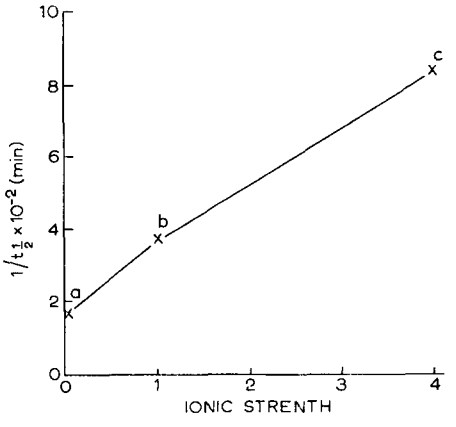


Fig. 3. Effect of salts on conversion of pepsinogen to pepsin. Plot of $1/t_{\frac{1}{2}}$ *versus* ionic strength. (Data calculated from results of HERRIOTT[5].)

System	*Acetate concentration (M)*	*I*	$t_{1/2}$ *(min)*	$1/t_{1/2}$ *(min)*
a. 0.1 M acetate (pH 4)	0.018	0.018	60	1.67×10^{-2}
b. 0.1 M acetate, 1 M NaCl (pH 4.25)	0.025	1.025	27	3.70×10^{-2}
c. 0.1 M acetate, 1 M $MgSO_4$ (pH 4.1)	0.020	4.020	12	8.33×10^{-2}

integrity of pepsinogen has also been suggested by PERLMANN[8], based on studies of the optical rotatory properties of pepsinogen, and may also be inferred from the observations by KATCHALSKI *et al.*[9] that the inhibition of pepsin (a polyanion) by polylysine (a polycation) can be reversed by heparin (a polyanion).

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