THE SPREADING OF PEPSIN AND OF TRYPSIN*

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(Accepted for publication, May 28, 1934)

In this article the behavior of pepsin and trypsin in a monolayer under various conditions will be described. Both substances[1] show all the characteristic properties of other proteins. Pepsin is admirably suited for the study of the influence of positive ions, as its isoelectric point lies far to the acid side. On trypsin the influence of negative ions can easily be shown.

Pepsin[2]

The pepsin was obtained in the form of a 5 per cent solution in glycerol (1 cc. containing 50 mg. of pepsin). It was diluted tenfold before use with 0.01 N hydrochloric acid (pH 2). 5 mm.[3] were blown out of a calibrated micropipette on the surface of the water in a Langmuir tray according to the method which we have used for several years.

Effect of pH.—The effect of pH on the amount of the spreading has been studied. For this purpose dilute hydrochloric acid solutions between pH 1 and 3, a 1/300 molar sodium acetate-acetic acid solution between pH 3.6 and 5.6 (or 1/350 molar veronal acetate buffer solutions according to Michaelis), and mixtures of HCl 1/300 N and Na_2CO_3 1/300 N were used.

It was possible to show that, as a rule, the influence of pH is the same as that observed with other proteins. The maximum was again a

* These experiments were made possible by a grant from the Pieter Langerhuizen Fund.

[1] These substances were kindly placed at my disposal by Dr. John H. Northrop.

[2] As the pepsin solutions contained 50 mg. pepsin per cc. glycerol, it was easy to calculate from these data the protein content of the solution used in the present experiments. The solutions were always made by adding dilute hydrochloric acid to a certain amount of the glycerol solution determined by weight. The specific gravity of the glycerol was taken at 1.25.

spreading of ±1 sq. m. per mg. On the acid side, however, there exists a small minimum only, whereas on the alkaline side of the isoelectric point a pronounced minimum is observed (Fig. 1).

It has been possible to demonstrate that the minimum (at pH 6.2) is strongly influenced by the addition of cations to the water in the tray. 1 milliequivalent of a bivalent cation has a distinct effect on the spreading: it tends to increase the size of the area at this point (Fig. 2). It was impossible to find any difference between Mg^{++}, Ca^{++}, Sr^{++}, and Ba^{++}, all having the same effect in very small amounts ($\frac{1}{2}$ milli-

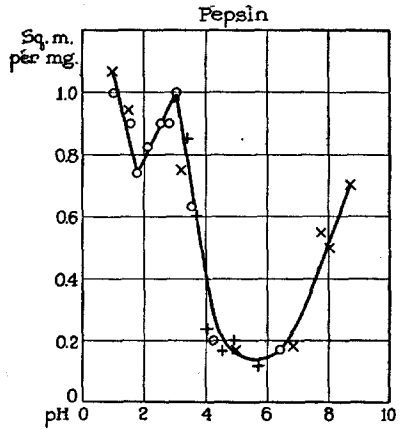

FIG. 1

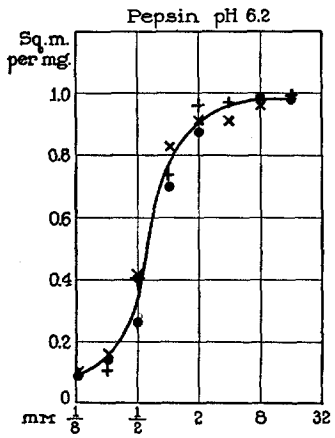

FIG. 2

FIG. 1. Influence of pH on the spreading of pepsin. The symbols °, ×, and + indicate different samples of pepsin.

FIG. 2. Influence of bivalent cations on the spreading of pepsin. Ba is omitted; its curve is the same as the others given. ×, Ca; +, Sr; •, Mg.

molar). This is in agreement with what we found on studying ovalbumin.

Univalent cations have the same effect as have bivalent cations, but much larger amounts are necessary to produce an increase in the size of the area (Fig. 3). Li^+, Na^+, and K^+ behave differently according to their atomic number, the larger atom having the stronger effect (Fig. 4).

The strong influence of polyvalent positive ions on the spreading at the alkaline side of the isoelectric point of a protein, like pepsin, can also be observed when making use of organic bases like spermine or

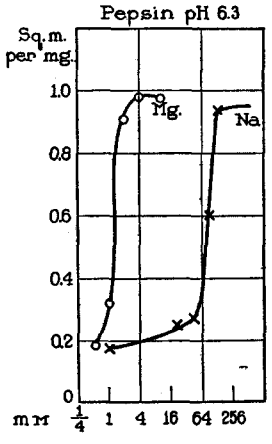

FIG. 3

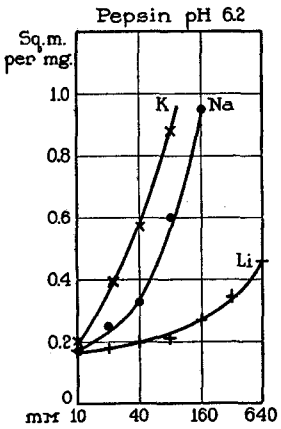

FIG. 4

FIG. 3. Influence of uni- and bivalent ions on the spreading of pepsin. ×, Na; ○, Mg.

FIG. 4. Influence of lyotropic series on the spreading of pepsin. ×, K; •, Na; +, Li.

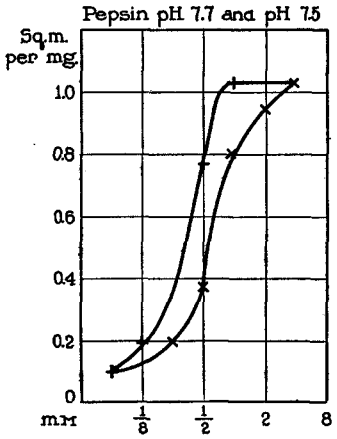

FIG. 5

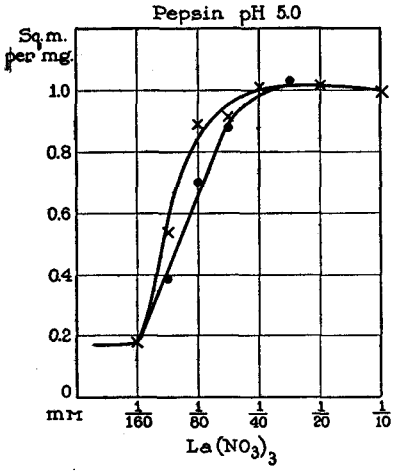

FIG. 6

FIG. 5. Influence of spermine and agmatine on the spreading of pepsin. +, spermine; ×, agmatine.

FIG. 6. Influence of time on the spreading of pepsin after addition of lanthanum nitrate. •, 6 minutes; ×, 12 minutes.

agmatine (Fig. 5). Exactly the same amount of these substances was sufficient to produce an increase in the spreading, when added to the water in the tray, at a pH of ±7.5 as was the case with Ca. A trivalent positive ion has a strong effect on the spreading at pH 5.0. $La(NO_3)_3$ has a distinct influence in a 1/8 millimolar solution. As in most of the experiments time has the same influence on the end result, chiefly when the spreading has an intermediate value (Fig. 6).

It is obvious that pepsin is a protein that shows this influence extremely well, because its isoelectric point lies far on the acid side (pH 2.7).

With substances like guanidin, methylguanidin, creatin, and creatinine, no effect on the spreading of pepsin was obtained at pH 6.2, when the same amounts were used as in the case of monovalent ions like potassium and sodium.

Trypsin

The trypsin was obtained in the form of a cake containing some ammonium sulfate and having a water content of ±70 per cent. It was dissolved in 0.001 molar hydrochloric acid. Solutions containing respectively 26.3, 27, and 28 mg. cake per cc. were used.[3]

Quite a different type of curve was found, when plotting pH against size of spreading area (Fig. 7). A definite maximum of spreading at the isoelectric point could not be observed when using the original preparation containing ammonium sulfate.

With trypsin having a minimum at pH 3 it was easy to study the effect of anions. It was possible to demonstrate:

1. The valency effect in the series Cl^-, SO_4^{--}, and MTS^{---}* (Fig. 8).
2. The lyotropic series Cl^-, Br^-, I^-, and CNS^- (Fig. 9).

* MTS means methanetrisulfonic acid $CH(SO_3H)_3$.

[3] The trypsin content of the cake was determined by means of the activity coefficient according to Northrop, modified somewhat by Dr. Meyer. It was found that the cake contained 24 per cent active trypsin This was assumed as the total protein content. Direct nitrogen determinations were not possible as the cake contained ammonium sulfate. This determination of the activity had the great advantage that it showed that the preparation had not been transformed into inactive material.

Dr. Northrop also sent trypsin in a dry state. It contained 92 per cent trypsin according to a N determination made by my collaborator Dr. Meyer. This served for the last series of experiments.

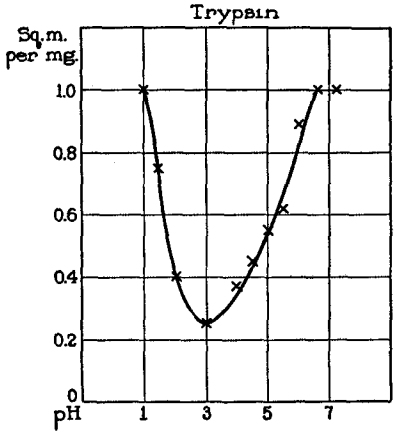

FIG. 7

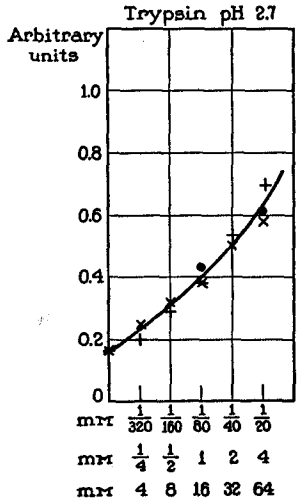

FIG. 8

FIG. 7. Influence of pH on the spreading of trypsin.
FIG. 8. Valency effect on trypsin. +, MTS; ×, SO_4; •, Cl.

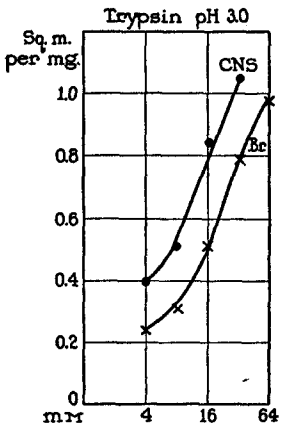

FIG. 9

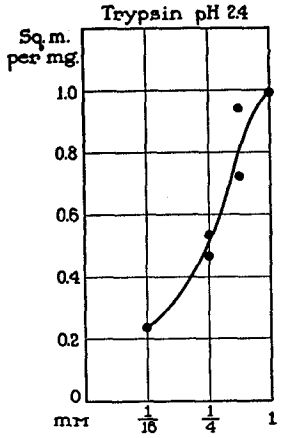

FIG. 10

FIG. 9. Lyotropic series. Influence on spreading of trypsin. Cl and I are not given. Their curves are lying at the right place. •, CNS; ×, Br.
FIG. 10. Influence of glutathione on the spreading of trypsin.

The same observations could be made on the acid side of the isoelectric point when studying trypsin and when using substances like glutathione. This substance had a pronounced effect on the spreading when it was added to the water in the tray to make a $\frac{1}{4}$ millimolar solution, whereas the maximum was obtained with a $\frac{1}{2}$ millimolar solution (Fig. 10).

On the other hand glutamic acid had no such influence and did not enhance the spreading, even when 528 mg. per liter was used; that is, a 4 millimolar solution.

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