Studies on Protease

V. The cleavability of derived proteins by trypsin

(Directed by Prof. Toyoo Uchino in the Nagasaki Medical College)

By

Takashi UCHINO (内野 孝)

(From the Clinical Research Laboratory, Hokkaido Red Cross Hospital, Asahigawa)

and

Kikuo SUGAI (菅井規矩雄)

(From the Biochemical Institute, Nagasaki Medical College. Director: Prof. Dr. Toyoo Uchino)

(Received for publication, July 2, 1942)

Introduction

In an earlier paper one of the authors (Uchino, 1942) reported on the cleavability of various derived caseins by means of pepsin. In that investigation an attempt was made to examine the peptic hydrolysis of the derived caseins in relation to the initial reaction velocity as well as at the varying pH of the reaction mixture, since the split products, which appear in the course of the proteolysis, bring about the inhibition of the protease action, and moreover the optimum pH for an enzyme action is not necessarily the same for different substrates.

The present paper is a continuation of this work and deals with the action of trypsin on a series of derived proteins. The protein derivatives employed were alkali treated casein, deaminized casein, phosphorized casein, acetyl casein, iodized casein, phosphorized gelatin and phosphorized egg albumin.

Materials

1. Alkali treated caseins C_1, C_2, C_5, C_{24}, C_{36} and C_{48}, deaminized casein D_{90}, acetyl casein and iodized casein were the same products as mentioned in the preceding paper (Uchino, 1942).

2. The phosphorization of casein, egg albumin and gelatin was carried out according to the method described by Rimington (1926). The nitrogen and phosphorus contents of these phosphorized proteins are given in the following Table I, along with those of the original proteins.

Table I

Preparations	Total nitrogen (Microkjeldahl, % of dry weight)	Total phosphorus (Youngburg, % of dry weight)
Casein	14.15	0.808
Phosphorized casein	13.68	1.328
Gelatin	15.72	—
Phosphorized gelatin	15.63	1.324
Egg albumin	12.14	—
Phosphorized egg albumin	13.90	0.927

Experimental Procedure

The experimental details were mainly the same as those mentioned by Nakamura (1940). Trypsin and enterokinase were prepared according to the method of Waldschmidt-Leitz and his co-workers (Bertho-Grassmann's Biochemisches Praktikum, p. 104).

Into each of the six flasks were introduced 1.0 cc. of the trypsin and enterokinase solutions and the flasks were kept at 30° C for 30 minutes for activation. 2.0 cc. of 1 M glycine-NaOH buffer of varying pH and 4.0 cc. of the 2.0% substrate solution were then added to each of the flasks and the latters were kept in a thermostat at 30° C. The titrations were carried out by the formol titration method, using N/5 NaOH solution, immediately after the addition of the substrate as well as after the lapse of 10, 20, 40 and 80 minutes respectively. The solution in the remaining flask was employed for the determination of pH. The control experiments, which were performed with special precaution, gave always negligible values.

Experimental Results

1. Experiments with casein. Fig. 1 gives the initial hydrolysis rates of casein by trypsin at varying pH of the medium.

Fig. 1 shows us that the highest tryptic activity is attained at pH 9.02.

2. Experiments with alkali treated caseins. The initial tryptic digestion rates of the alkali treated caseins C_1, C_2, C_5, C_{24}, C_{36} and C_{48} are shown in Figs. 2–7 and summarized in Fig. 8.

Just as in the case of peptic digestion (Uchino, 1942), so also in this case the alkali treated caseins are attacked by trypsin with definitely less speed than the natural protein. The optimum pH for the highest initial tryptic activity undergoes no change and remains at about pH 9.0.

3. Experiments with deaminized casein. The action of trypsin on deaminized casein D_{90} at varying pH is demonstrated in Fig. 9.

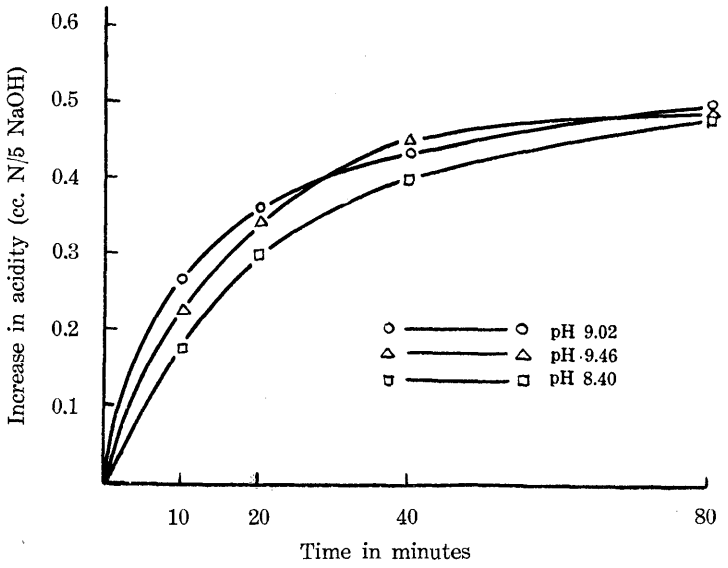

Fig. 1. Action of trypsin on casein

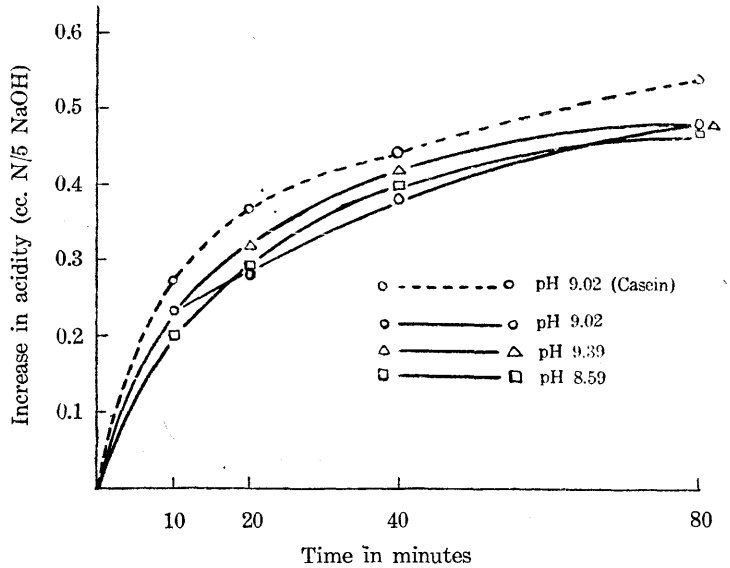

Fig. 2. Action of trypsin on alkali treated casein C_1

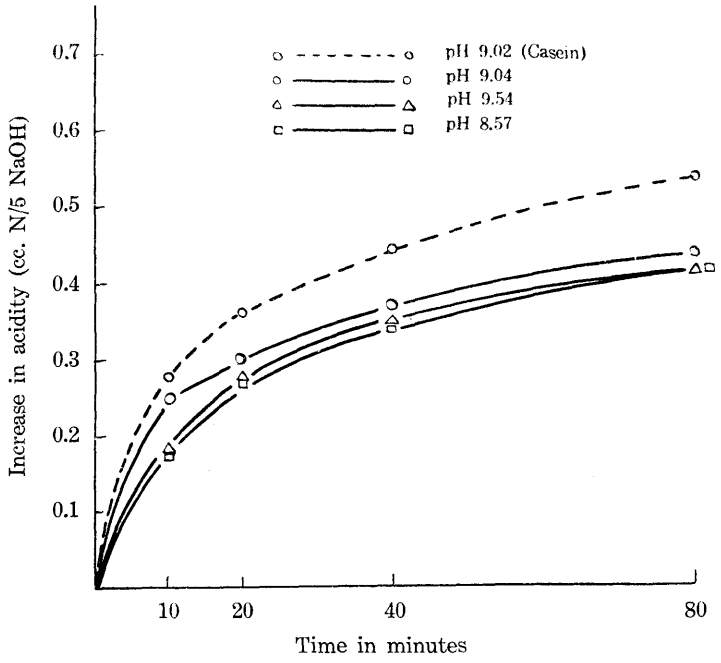

Fig. 3. Action of trypsin on alkali treated casein C_2

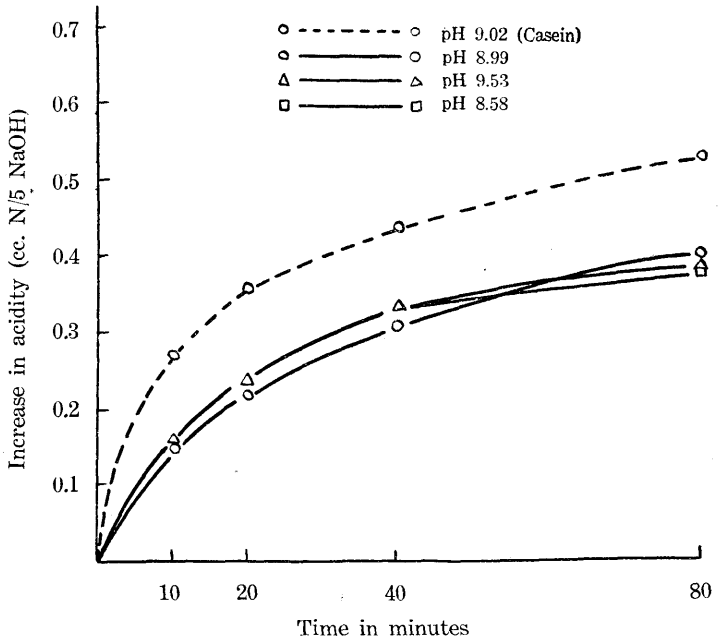

Fig. 4. Action of trypsin on alkali treated casein C_5

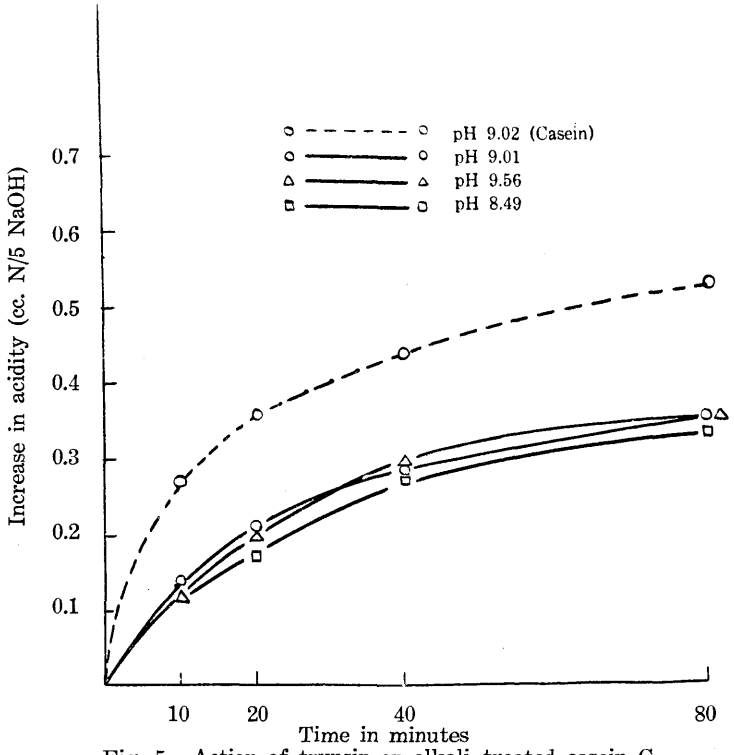

Fig. 5. Action of trypsin on alkali treated casein C_{24}

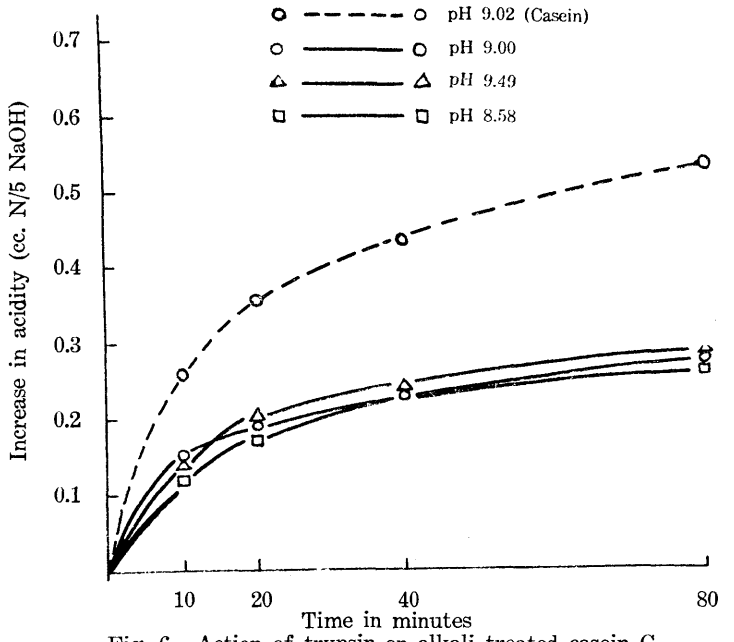

Fig. 6. Action of trypsin on alkali treated casein C_{36}

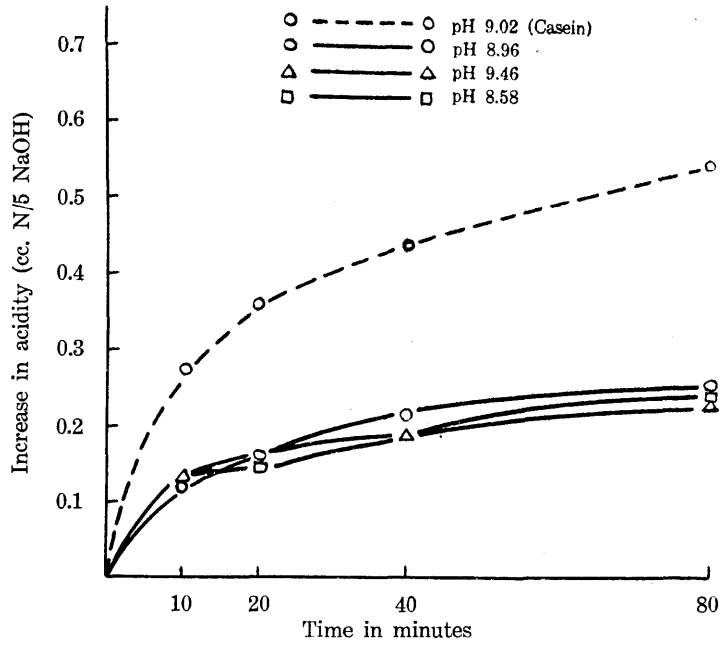

Fig. 7. Action of trypsin on alkali treated casein C_{48}

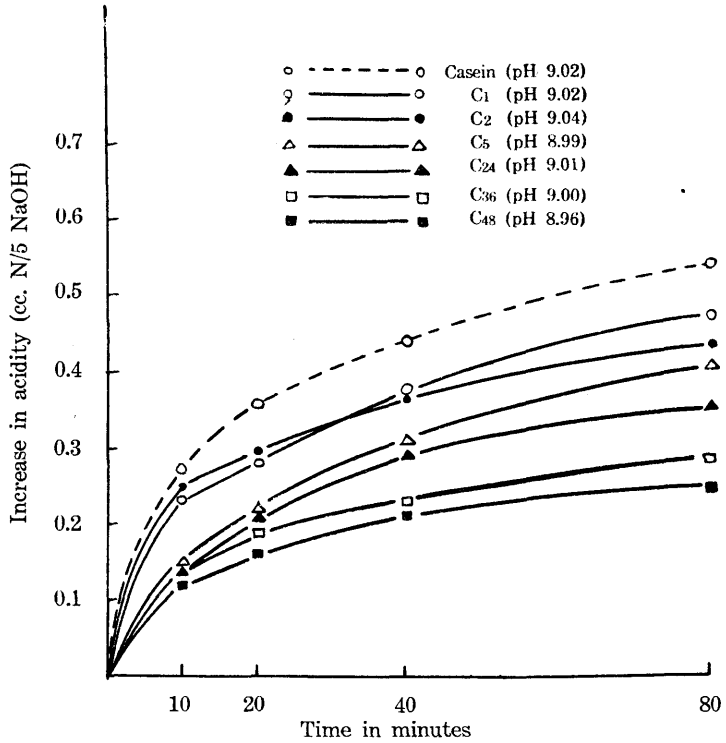

Fig. 8. Initial hydrolysis rates of ordinary and alkali treated caseins by trypsin

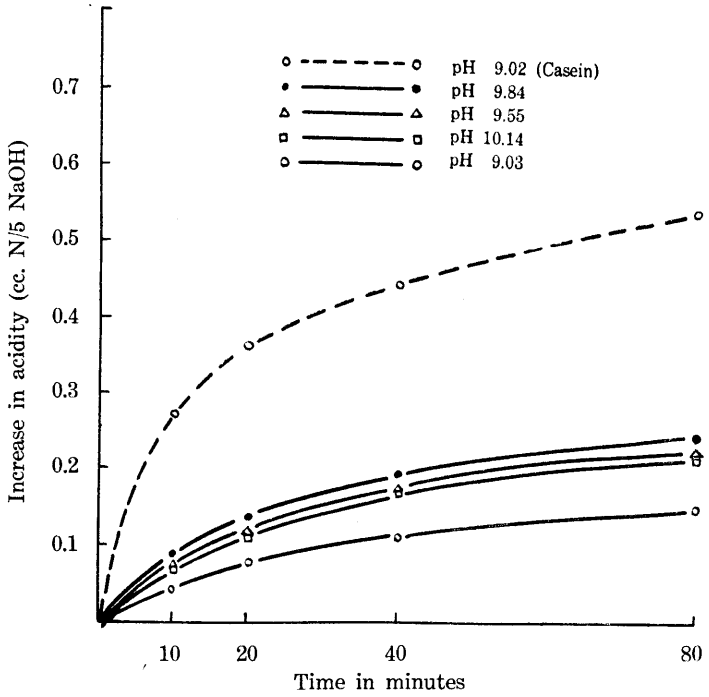

Fig. 9. Action of trypsin on deaminized casein D_{90}

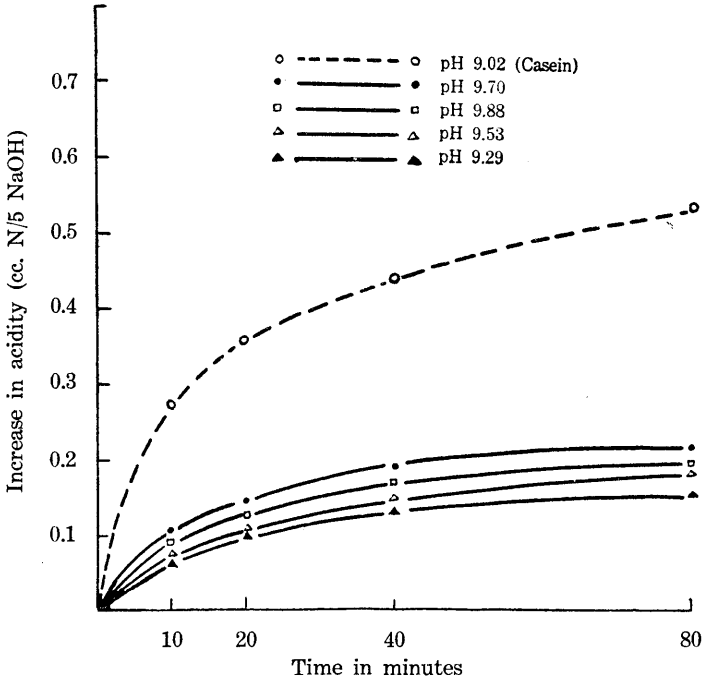

Fig. 10. Action of trypsin on acetyl casein

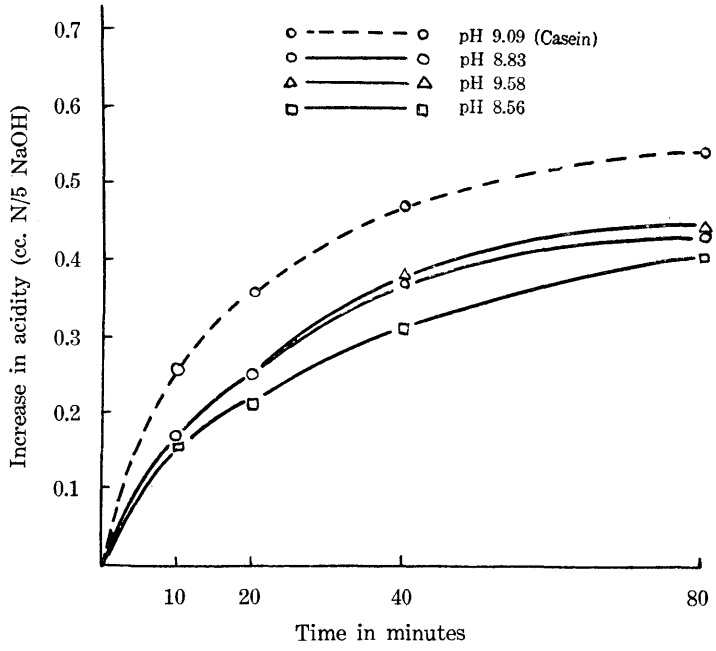

Fig. 11. Action of trypsin on ordinary and iodized casein

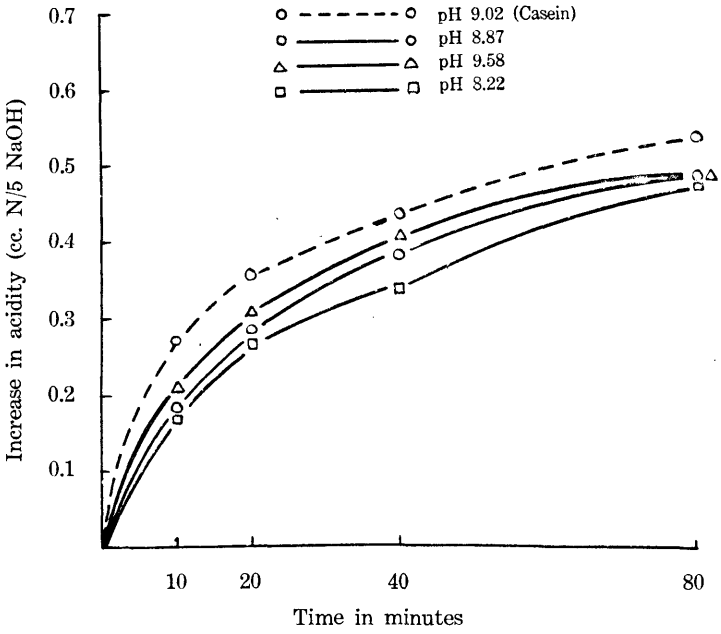

Fig. 12. Action of trypsin on ordinary and phosphorized casein

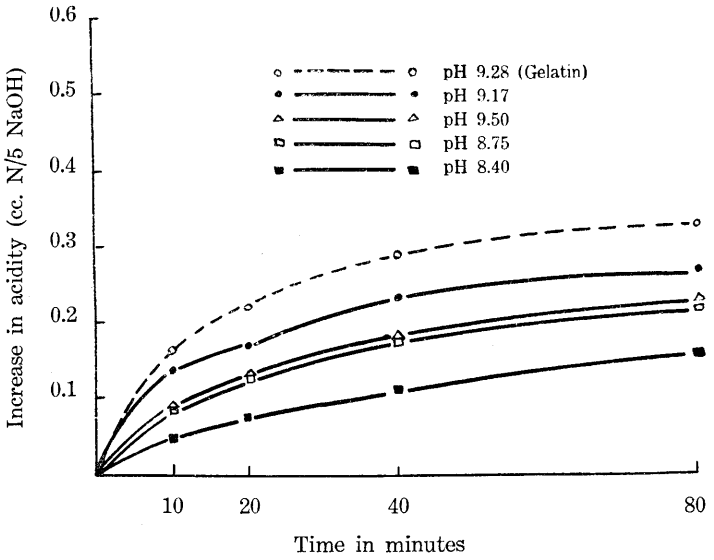

Fig. 13. Action of trypsin on ordinary and phosphorized gelatin

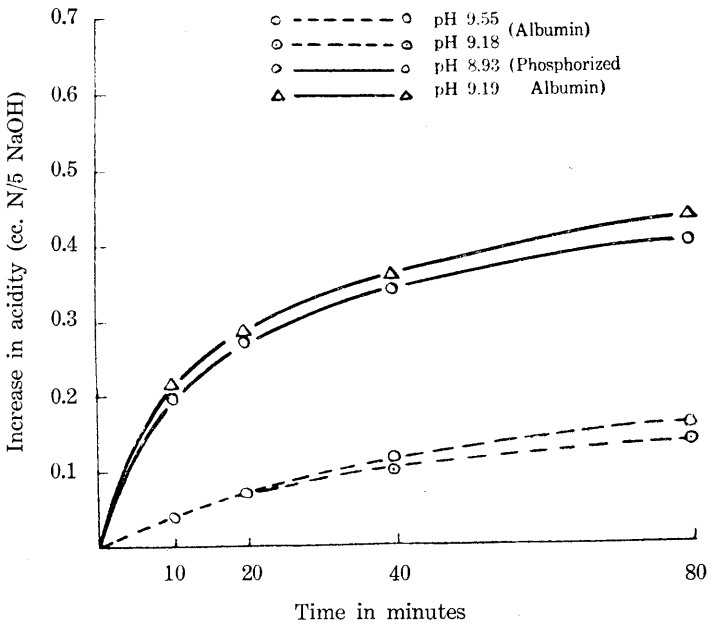

Fig. 14. Action of trypsin on ordinary and phosphorized albumin

It can be seen from Fig. 9 that deaminized casein is split by trypsin with marked slowness as compared with the untreated casein. It must be further noted that in this case the optimum pH is shifted to the alkaline side and lies at pH 9.84.

4. Experiments with acetyl casein. Fig. 10 shows the results of the experiments with acetyl casein.

As can be seen from Fig. 10 the acetyl casein is hydrolyzed by trypsin much slower than the original protein. Moreover the optimum pH is shifted to the alkaline side as in the case of deaminized casein.

5. Experiments with iodized casein. The results of the experiments with iodized casein are indicated in Fig. 11.

From Fig. 11 it follows that the iodized casein is hydrolyzed by trypsin a little slower than the natural protein.

6. Experiments with phosphorized proteins. The tryptic digestion of the phosphorized casein, gelatin and egg albumin was then compared with that of the natural proteins. Figs. 12–14 give the results obtained.

From Figs. 12–14 it is clear that the phosphorized casein and gelatin are acted upon by trypsin with slightly less speed than the untreated proteins, whereas the phosphorized egg albumin is split by trypsin with considerable rapidness as compared with the natural one.

Summary

The action of trypsin on a series of derived proteins was examined in relation to the initial rates of hydrolysis as well as to the optimum pH of the enzyme action.

1. Alkali treated casein is split by trypsin much slower than the natural protein.
2. Deaminized casein and acetyl casein are attacked by trypsin with marked slowness in comparison with the untreated casein, in both cases the optimum pH for the tryptic activity being shifted to the alkaline side.
3. Iodized casein is hydrolyzed by trypsin to a slightly less extent than the original casein.
4. Phosphorized casein and gelatin are split by trypsin with slightly less speed than the natural proteins, while on the contrary phosphorized egg albumin is split markedly more rapidly than the natural one.

In conclusion we wish to express our sincere thanks to Prof. Dr. T. Uchino for his kind guidance throughout this research.

References

Nakamura (1940): J. of Riochem., **31**, 311.
Rimington (1926): Biochem. J., **21**, 272.
Uchino (1942): Act. Med. Nagasakiensia, **3**, 137.