

On the Hypoglycemic Activity of Protease Preparation of *Streptomyces griseus*, Pronase P

Commercial protease preparation, "Pronase P," (Kaken Chemical Mfg. Co., 45,000 PUK/g.) which was isolated from the culture broth of *Streptomyces griseus* by Nomoto, *et al.*¹⁾ is most active at neutral PH range and its optimum temperature is 40~60°. On the contrary with trypsin, chymotrypsin, or other proteases, Pronase P has a broad substrate specificity though only peptide-bonds of -gly-gly-*¹ and -gly-L-pro are not hydrolyzed. Recently, proteases are clinically used as antiphlogistic agents but it remains to be elucidated what are the *in vivo* effects of proteases on various enzymes, hormones, and endocrine systems. During examination on the degradation of insulin by proteases, the present authors found that Pronase P showed hypoglycemic activity on rabbits.

Methods—One mg. of pronase P was dissolved in 1 ml. of 0.05M phosphate buffer (PH 6.0) and this solution was injected subcutaneously to 3~5 male rabbits weighing 2.5~3 kg. after fasting for 24 hr.

Trypsin (Nutritional Biochemicals Mfg. Co., salt-free crystalline preparation) and insulin (Shimizu Seiyaku Mfg. Co., 20 u/ml.) were used as control. The blood sugar level was measured by Hagedorn-Jensen's method.²⁾ The incubations of insulin with pronase P or trypsin were carried out at 35°, in consideration of the prevention of heat denaturation of insulin, for 1 hr. in 0.05M phosphate buffer of PH 6.0. All the results are given as percentage of initial blood sugar level.

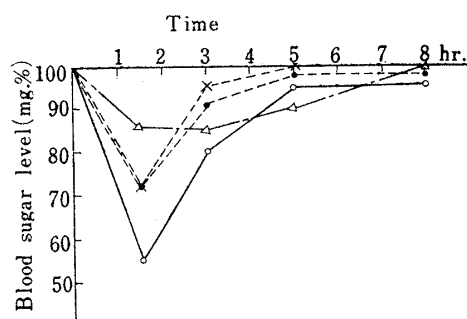


Fig. 1. Effect of Pronase P on Blood Sugar Level of Rabbits

- Pronase P 0.15 mg./kg.
- ×---× Pronase P 0.03 mg./kg.
- △---△ Pronase P 0.015 mg./kg.
- insulin 0.3 u/kg.

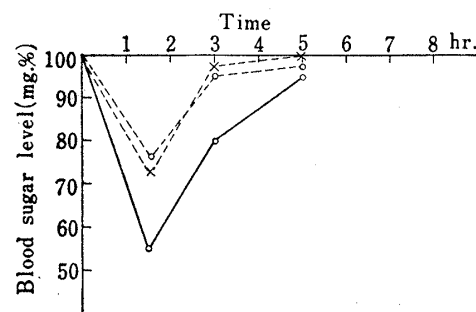


Fig. 2. Effect of Pronase P on Hypoglycemic Activity of Insulin

- insulin 0.3 u/kg.
- ×---× Pronase P 0.03 mg./kg.
- mixture of insulin (0.3 u/kg.) and Pronase P (0.03 mg./kg.), after incubation for 1 hr.

Results—Fig. 1 shows the results of hypoglycemic activity of Pronase P and insulin. In doses of 0.15 mg./kg. or 0.03 mg./kg. of Pronase P, blood sugar level was decreased by 28% 1.5 hr. later and restored to initial level 5 hr. after the injection. In a dose of 0.015 mg./kg., the decrease was 15% 3 hr. later and the time of the peak decrease shifted from 1.5 hr. to 3 hr. In the administration of 0.3 u/kg. of insulin, the decrease of 45% was observed at 1.5 hr., 20% at 3 hr., and no decrease at 5 hr.

As shown in Fig. 2, when insulin was given to rabbits after the incubation with Pronase P, the level of blood sugar at the peak decrease took significantly high value as

*1 Abbreviation used are as follows: gly, glycine; pro, proline.

1) M. Nomoto, Y. Narahashi: J. Biochem., **46**, 653 (1959).

2) H. C. Hagedorn, B. N. Jensen: Biochem. Z., **135**, 46 (1923).

compared with that when insulin alone was given.

This result shows that insulin was degraded by Pronase P *in vitro*. Nevertheless, it is interesting that Pronase P showed hypoglycemic activity.

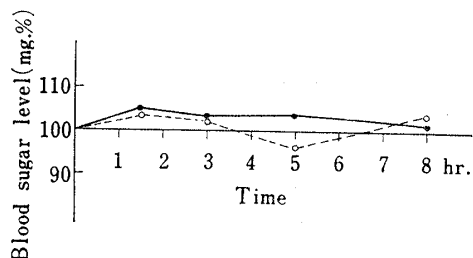


Fig. 3. Effect of Trypsin on Hypoglycemic Activity of Insulin

●—● trypsin 0.15 mg./kg.
○---○ mixture of insulin (0.3 u/kg.)
and trypsin (0.15 mg./kg.),
after incubation for 1 hr.

As shown in Fig. 3, when insulin was incubated with trypsin, trypsin entirely counteracted hypoglycemic activity of insulin and trypsin only was ineffective on blood sugar level in a dose of 0.15 mg./kg. It will be worth notice that a difference of physiological activity exists between the two proteases.

Recently, Dixit, *et al.*³⁾ reported that acid-alcohol extracts of larval foods of the honeybee showed insulin-like activity. This report shows the possibility that such active substances can be taken out of invertebrates.

The pattern of curve of hypoglycemic activity of Pronase P was analogous to that of insulin.

It is not yet known, however, whether or not Pronase P itself shows insulin-like activity or activates the enzyme participating in insulin-activity.

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3) P. K. Dixit, N. G. Patel : *Nature*, **202**, 189 (1964).

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Dehydro-deoxynupharidine : A New Alkaloid of *Nuphar japonicum* DC.

A new unstable base, $C_{15}H_{21}ON$ (I) was isolated from the root of *Nuphar japonicum* DC., and designated as dehydro-deoxynupharidine. It formed a crystalline perchlorate, m.p. 159~161°, $[\alpha]_D +130.1^\circ$ ($CHCl_3$) (*Anal.* Calcd. for $C_{15}H_{22}O_5NCl$: C, 54.28; H, 6.69; N, 4.22. Found: C, 54.19; H, 6.77; N, 4.41). The infrared spectrum of the perchlorate in chloroform showed bands at 877, 1510 and 3128 cm^{-1} attributable to the furan ring, and at 1640 cm^{-1} due to the iminium salt which conjugates with the furan ring, while its nuclear magnetic resonance spectrum contained three signals (δ 8.09, 7.56, 6.82), each corresponding to one proton, which were obviously attributable to the furan ring.

Sodium borohydride reduction of the perchlorate of I afforded an oily amine, II, b.p.₃ 125° (oil bath temp.), $[\alpha]_D -114.1^\circ$ ($CHCl_3$), IR $\nu_{\text{max}}^{\text{liquid}}$ 2764, 2792 (*trans*-quinolizidine) 874, 1032, 1505, 3140 cm^{-1} (furan). II formed a perchlorate, m.p. 203~204.5° (*Anal.* Calcd. for $C_{15}H_{24}O_5NCl$: C, 53.49; H, 7.18; N, 4.16. Found: C, 53.58; H, 7.33; N, 4.11), and a picrate, m.p. 154.5~155.5° (*Anal.* Calcd. for $C_{15}H_{23}ON \cdot C_6H_3O_7N_3$: C, 54.54; H, 5.67; N, 12.12. Found: C, 54.68; H, 5.67; N, 12.27). II was identical with an authentic specimen