THE UTZINO LABORATORY

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The Utzino Laboratory was put into operation in 1942 under Prof. Utzino who was transferred to the Faculty of Medicine, Kyoto University from Tohoku University in the preceeding year.

On the Amidase and Protease Action of Bacillus Natto

Both autolysate and acetone powders splitted asparagine (optimum pH 7), glutamine, acetamide (pH 7.5-8) and, though slightly, benzamide. Salicylamide and creatine, however, were never splitted. Aspartic acid was desaminated by both preparations, though slightly.

Both autolysate and acetone powders attacked proteins (optimum pH 7.5) and the degree of hydrolysis at pH 7.5 diminished in the following order: Casein, gelatin, fibrin, egg albumin, edestin. Protamine was also attacked. The gelatin hydrolysis at pH 7.5 was not affected by cysteine, but was inhibited by the addition of metal salts (0.01 mol) in the following order: $AgNO_3$, $CuSO_4$, Hg_2Cl_2, $NiSO_4$ and $FeSO_4$. $Co(NO_3)_2$ and $MnSO_4$ seemed to give no effect upon it. Gelatin hydrolysis seemed to be inhibited by addition of $FeSO_4$, $CoCl_2$ and $MnCl_2$ between 0.02 and 0.004 mol, and the hydrolysis of casein appeared to be depressed by a higher concentration of $FeSO_4$ or $CoCl_2$, whereas $MnCl_2$ showed accelerating effect upon it. Dipeptides and tripeptides could also be split and the diglycine-splitting was also accelerated by $FeSO_4$, $MnSO_4$ or $CoCl_2$, and was inhibited by cysteine. Although all of benzoylpeptides examined were remarkably hydrolysed, acetylpeptides were unattacked except acetyl-L-phenylalanine which could slightly be attacked at pH 6.0. On the contrary, Cl-acetylleucine and Cl-acetylphenylalanine were broken down at pH 6 and 7.5.

On the Protease Action and the Chemical Composition of Penicillium Notatum

Both maceration and acetone powder of Pen. notatum hydrolysed various proteins at a pH range of 5 to 9, and the optimum pH was found to be at pH 6.8 for gelatin, at pH 5.8 for casein and from 6.4 to 6.8 for peptone. Protamine was also attacked. The acetone powder underwent partial inactivation. The degree of hydrolysis at pH 7 diminished in the following order: Casein, fibrin, gelatin, albumin and edestin. Proteinase could be easily extracted by glycerine and was very resisitant to keeping at 37°C. The proteolytic action was neither accelereted nor inhibited by addition of H_2S, cysteine or KCN both in acid and alkaline reaction. On the contrary, the proteinase was remarkably inhibited by egg albumin in a cencentration of more than 0.02%. Penicillium contained also dipeptidase (diglycine), tripeptidase (triglycine, leucyldiglycine), hippurase, carboxydipeptidase,

acylase (acetylglycine, acetylglutamic acid) and halogenacylase (Cl-acetylleucine, Cl-acetylphenylalanine). Optimum reaction for the hydrolysis of diglycine was found to be at pH 7.5, that for benzoylglycine at pH 7 and that for benzoyldiglycine near at pH 6. Mold enzymes were unstable against acid, leaving only peptonase and hippurase by keeping at pH 4 and 37°C for 5 min., but these actions diminished after 90 min. treatment. They were rather resistant to alkali, and showed no inactivation, when keeping at pH 9 and 37°C 17 hrs. But at pH 11.5 and 37°C, they lost activity of gelatin- and benzoyldiglycine-splitting after 120 min. remaining only peptone- and slight benzoylglycine-splitting activity. Peptonase and hippurase were more resistant against both treatments than others. These observations led to the conclusion that the protease action of Penicillium is quite different from that of animals as well as higher plants.

The dried powder of mycelium of Penicillium consisted of 13.0% water, 4.3% lipid, 35.1% protein, 14.1% carbohydrate, 18.9% fibre and 8.2% ash. Ergosterol (0.075%) and mixed fatty acids were isolated from the ether extract, which shows 129 of iodine value, by saponification. And mannitol (0.6% of the ether-extracted mycelium) and betaine were isolated from the alcohol extract of ether extracted residue. Lysine (0.07‰), arginine (crude, 0.02‰) and betaine (0.03%) could be separated from hot water extract, and the presence of histidine was indicated by color reaction. Lysine (0.07%), histidine (0.02%) and arginine (0.45%) were successfuly isolated from the HCl hydrolysate of ether and alcohol extracted mycelium.

1) On the Amidase Action of Bacillus Natto.
Acta Scholae Medicinalis Universitatis in Kioto, Japonia, **27**, 224 (1950).
2) On the Protease Action of Bacillus Natto.
Acta Scholae Medicinalis Universitatis in Kioto, Japonia, **27**, 247 (1950).
3) On the Protease Action of Penicillum notatum. I.
Journal of Biochemistry, **37**, 51 (1950).
4) On the Protease Action of Penicillium notatum. II.
Journal of Biochemistry, **37**, 237 (1950).
5) On the Chemical Composition of Penicillium notatum.
Acta Scholae Medicinalis Universitatis in Kioto, Japonia, **28**, 1 (1950).

On the Specificity of Ficin and its Vermicidal Effect

The hydrolysis of gelatin or casein by ficin was executed in the active optimum range of pH 4 to 5. The rate of the hydrolysis of different proteins decreased in the following order: edestin, gelatin, casein, egg albumin, and ricin. Peptones had the same optimum pH range as genuine proteins. This suggests the presence of a cathopeptidase in ficin. Like the other intracellular enzymes, ficin could split dipeptides such as diglycine etc. neither at acid nor alkaline reaction. The tripeptides i.e. DL-leucyldiglycine or DL-leucylglycyl-DL-phenylalanine (β) were markedly splitted at pH 4.5, and from the enzymatic hydrolyzate of the former leucine was isolated. It is assumed that a catheptic aminopeptidase may be responsible for the hydrolysis of DL-leucyldiglycine at pH 4.5. Hippuricase and acylase action were not present in ficin. On the other hand, benzoyl-DL-leucylglycine and benzoyldiglycine were remarkably splitted at pH 4.5, and from the

enzymatic split-product of the latter hippuric acid were directly isolated.

0.5 per cent solution of fig sap filtrate in physiological saline solution caused at pH 8 and 38° the death of human *Ascaris* in 5 hours, and dissolved them completely in 24 hours.

The Asymmetric Anilide Synthesis of Amino Acids by the Action of Ficin

When in the presence of ficin acylated amino acid was incubated with aniline or phenylhydrazine at pH 4.5 and 37°, anilide or phenylhydrazide was synthesized. Optimum condition of enzymatic formation of hippurylanilide was at pH 4.5, at which the ficin was favorably able to perform hydrolysis of proteins. When benzoyl derivative of DL-phenylalanine was incubated with aniline and ficin, there resulted the quantitative precipitation of benzoyl-L-phenylalanine anilide only, and benzoyl-D-phenylalanine remained in solution. After acid hydrolysis, the former compound gave L-phenylalanine, and the latter D-phenylalanine. The antipodal specificity of ficin may be utilized as a method to resolve racemic amino acid into their optical components. In this manner DL-methionine and DL-lysine were resolved with ease by means of asymmetric anilide synthesis with ficin.

1) Yoneya, T.: "On the Specificity of Ficin", J. Biochem., **37**, 105 (1950).
2) Utzino, S. and Yoneya, T.: "The Asymmetric Synthesis of Peptide Bonds by the Action of Ficin", Jap. Med. J., **2**, 303 (1949).
3) Yoneya, T.: "Studies in Lysine and its Derivatives. I. The Asymmetric Anilide Synthesis of Lysine Derivatives by the Action of Ficin," J. Biochem., in press.