STUDIES OF MACERATING ENZYME ACTING ON MIDDLE LAMELLA PECTIN

II Adsorption of Macerating Enzyme and Polygalacturonase on Ion Exchange Resin Duolite CS-101.

Akira KAJI and Yoshio ANABUKI (Laboratory of Technical Microbiology)

(Received December 5, 1955)

It was already reported by the authors that polygolacturonase (PG) produced by *Cl. felsineum* var. *sikokianum* was adsorbed on ion exchange resin Amberlite IRC-50 and the efficiency of the elution of this enzyme was found to be 97%[1]. One of the authors announced that macerating enzyme (ME) was also adsorbed on IRC-50 and some different characters of the two enzymes were pointed out in their manners of adsorption and elution, so that ME was isolated from partially purified enzyme solution[2].

This report describes the experiments which were carried out with another resin Duolite CS-101 instead of Amberlite IRC-50. The noticeable difference was pointed out in the adsorption of PG and ME on Duolite CS-101, and ME could be removed successfully from the mixed enzyme solution.

Experiments and Results

The microorganism used in this experiment was the same as in the previous reports; *Cl. felsineum* var. *sikokianum*[3].

I. Assay methods. Activity of PG was expressed by the decrease of viscosity caused by the action of enzyme on 0.5% pectic acid solution for 1 hour, as was already mentioned in the previous papers[1,4].

Activity of ME was expressed by the degree of separation of fibers by the action of enzyme on a piece of Ganpi bark of the size of 1×1 cm, and the volumes of the enzyme solution here employed were in each case noted, as were mentioned in the previous paper[2].

II. Adsorption of polygalacturonase and macerating enzyme on Duolite CS-101. Twenty cc of the resin swelled by water was employed in each experiment. The size of the column of the resin was 2.3×5.0 cm, and its flow rate (SV) was 5 to 6. The enzyme solution used in adsorption was prepared as follows: culture medium of *Cl. felsineum* var. *sikokianum* was centrifuged at 3,000 r.p.m. for 15 min. and then the medium passed through a column of Duolite A-7 which would adsorb colouring matter and some parts of impurities in the centrifuged solution, while PG and ME were not caught on A-7. The activities of two enzymes are shown in Table 1. Solution (a) was employed in H-form of resin and in buffered resins of pH 5.0, 5.8 and 6.0; solution (b) was charged on buffered resin at pH 6.2. Numbers in brackets represent volume(cc) of the enzyme solution employed in the reaction media.

Table 1. Activities of PG and ME in the centrifuged solution.

	pH	PG unit/cc	ME (Degree of separation of fibres)		
(a)	4.6	96	⫼(0.5)	⫲(0.25)	⫩(0.1)
(b)	4.6	80	⫼(0.5)	⫲(0.25)	⫩(0.1)

The adsorption was carried out with H-form of resin, and with buffered resins of pH 5.0, 5.8, 6.0 and 6.2 by the method of HIRS, STEIN and MOORE.[5] As shown in Table 2, no difference between PG and ME was recognized with H-form or buffered resin of pH 5.0, but 13.8% of PG was found to pass through the

resin of pH 5.8, while the adsorption of ME on the same resin was observed to be almost complete. At pH 6.0, the amount of PG which was passed through the resin was increased to 39.6%, but no activity of ME was detected by the action of 2cc of passed solution on Ganpi fibre. However, a small amount of ME was found to pass through the resin of pH 5.8 or 6.0, since low activity of enzyme was noted when large amount (20cc) of the enzyme solution was employed. When buffered pH was adjusted to 6.2, the amount of PG adsorbed on the resin was more decreasing, but the activity of ME in passed solution was increased. That is to say, adsorption of PG was remarkably dropped to a low level and a noticeable reduction of the efficiency of adsorption of ME according to the increase of pH values. Therefore, ME could be removed so easily from mixed enzyme solution and PG rich solution would be obtained so easily by the adsorption of Duolite CS-101, as was compared with Amberlite IRC-50.

Table 2. Adsorption of polygalacturonase and macerating enzyme on Duolite CS-101 of various pH values.

pH of buffered resin	Volume of soln. passed through resin (cc)	pH of passed soln.	PG unit/cc in passed soln.	Ratio of adsorption of PG(%)	Activity of macerating enzyme in passed soln. (Degree of separation of fibres)		
H-form	0-200	4.0	1	99.3	−(2.0)	−(1.0)	−(0.5)
	200-300	4.2	0		−(2.0)		
	300-400	4.2	1		−(2.0)		
	400-500	4.2	1		−(2.0)		
	500-600	4.2	0		−(2.0)		
5.0	0-100	5.0	1	99.5	−(2.0)		
	100-200	5.0	1		−(2.0)		
	200-300	5.0	0		−(2.0)		
	300-400	5.0	0		−(2.0)		
	400-500	5.0	0		−(2.0)		
5.8	0-100	5.8	11	86.2	−(2.0)		
	100-200	5.8	15		−(2.0)		
	200-300	5.8	13		−(2.0)		
	300-400	5.8	14		−(2.0)		
	400-500	5.8	13		−(2.0)		
6.0	0-100	6.0	40	60.4	−(2.0)		
	100-200	6.0	39		−(2.0)		
	200-300	6.0	40		−(2.0)		
	300-400	6.0	40		−(2.0)		
	400-500	6.0	40		−(2.0)		
6.2	0-100	6.2	32	53.8	+(2.0)		
	100-200	6.2	39		+(2.0)		
	200-300	6.2	40		+(2.0)		
	300-400	6.2	40		±(2.0)		
	400-500	6.2	34		±(2.0)		

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to prof. H. KATAGIRI of Kyoto Univ. for his guidance and encouragement, and also to thank prof. K. OKUNUKI and Dr. B. HAGIHARA of Osaka Univ. for their kind suggestions.

REFERENCES

(1) KAJI, A, ANABUKI, Y.: *J. Agr. Chem. Soc. Japan*, **29**, 755 (1955).

(2) KAJI, A.: *Bull. Agr. Chem. Soc. Japan*, **20**, 8 (1956).

(3) KAJI, A., SAITO, H.: *J. Ferm. Tech. Japan*, **30**, 242 (1952).

(4) KAJI, A.: *J. Agr. Chem. Soc. Japan*, **27**, 699 (1953).

(5) HIRS, C. H. W., STEIN, W. H., MOORE S.: *J. Biol. Chem.*, **200**, 493 (1953).

中葉ペクチン溶解酵素に関する研究

Ⅱ イオン交換樹脂 Duolite CS-101 による中葉ペクチン溶解酵素とポリガラクチュロナーゼの吸着

梶 明，穴吹吉夫

Cl. felsineum var. *sikokianum* の醱酵液中には中葉ペクチン溶解酵素(ME)と液化型ポリガラクチュロナーゼ(PG)とが主として生産される．醱酵液を遠沈した後 Duolite A-7 を通過させると，ME 及び PG は吸着されないが液中の色素及び不純物の一部が除去された．この A-7 通過液の pH を 5.0，5.8，6.0 並びに 6.2 に調節して，同じ pH 価に緩衝化した Duolite CS-101 を通過させる．完全酸性化及び pH が低いときは ME，PG 共によく吸着されたが，pH 価が 5.8，6.0，6.2 と高くなるに従って先づ PG が通過し始め，次に ME が通過液中に増加してきた．その結果は第2表に示す通りであった．この吸着差は既報の如く Amberlite IRC-50 においても観察されたところであるが，CS-101 の場合には両酵素の挙動に鋭い差が確認された．特に pH6.0 に緩衝化した樹脂を通過した液中には PG が主として存在し，ME は殆ど除去されていた．

終始御懇篤なる御指導を賜わった京大片桐英郎教授に深甚の謝意を表する．