Studies on α-Galactosidases (IV)

Multiplicity of the α-Galactosidases and Allied Glycosidases from Several Sources as shown by Paper Chromatography

By

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NISIZAWA has isolated by fractionation two kinds of β-galactosidases of different aglycon specificity from the hepatopancreas of a snail[1]. Later, NISIZAWA and WAKABAYASHI have observed that the aglycon specificity of the β-glucosidase of malt varied significantly with different fractions and they have postulated the presence of multiple β-glucosidases in this source[2][3]. On the basis of these results, NISIZAWA was of the opinion that several glycosidases of different specificity might exist in a given source.

JERMYN[4] has recently demonstrated that the β-glucosidase of *Aspergillus oryzae* was resolved into several components by paper chromatography. This was a very interesting finding since the β-glucosidase of Taka-diastase apparently behaved like a single enzyme on fractionation in an earlier experiment by NISIZAWA[5].

In a previous paper, WAKABAYASHI and NISIZAWA reported that α-galactosidases from several sources each showed a rather characteristic aglycon specificity[6]. However, it was not clear in any given case whether the specificity was that of a single enzyme or the resultant of those of several synergistically acting components.

The present paper deals with the resolution of α-galactosidases from different sources by paper chromatography and also with the aglycon specificity of each component separated. Similar investigations were carried out, for comparison, with the β-galactosidases and β-glucosidases from the same sources.

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Materials and Methods

1. *Preparation of initial enzyme solutions*

Each crude extract was prepared in the same way as described in a previous paper[6] from Taka-diastase, apricot kernel, malt and brewer's bottom yeast. Prior to use, 100 ml. of each was concentrated to about 4 ml. under reduced pressure at 30°C. The concentrates served as initial solutions.

2. *Substrates*

i) *α-Galactosides*:

Phenyl α-D-galactoside (m. p. 138~140°C), *m*-cresyl α-D-galactoside (m. p. 150~151°C), *p*-cresyl α-D-galactoside (m. p. 144~145°C), *p*-nitrophenyl α-D-galactoside (m. p. 167~168°C), melibiose (commercial, superior quality).

ii) *β-Galactosides*:

Phenyl β-D-galactoside (m. p. 143~144°C), *o*-cresyl β-D-galactoside (m. p. 193~194°C), *p*-nitrophenyl β-D-galactoside (m. p. 177~178°C).

iii) *β-Glucosides*:

Phenyl β-D-glucoside (m. p. 173~174°C), *o*-cresyl β-D-glucoside (m. p. 163~164°C), *p*-nitrophenyl β-D-glucoside (m. p. 164~165°C).

3. *Paper chromatography and the elution of enzymes*

Solvent systems:

Methanol : water (1 : 4), acetone : water (2 : 3), acetone : acetate buffer of 0.01 M at pH 4.8 (2 : 3), acetone : phosphate buffer of 0.01 N at pH 7 (2 : 3), acetone : NaCl of 0.7 M : phosphate buffer of 0.028 N at pH 7 (3 : 2 : 5), acetone : aqueous NaCl of 0.16%, w/v (2 : 3).

Temperature:

Room temperature, 0~5°C, 20~23°C.

Chromatography was carried out by the ascending technique with Toyo filter paper No. 50 (40 × 40 cm.) throughout the work.

Using a micropipette, 0.25 ml. of concentrated enzyme solution was horizontally applied to the paper at 6 cm. from the lower end. The enzyme band was confined to a width of 0.5 cm., and it was developed under the conditions indicated for each experiment. The filter paper was removed from the chamber after the solvent front had travelled 25~30 cm. beyond the starting line. Three guide strips each of 2 cm. in width were then quickly cut out from the paper at a distance of 4 cm. from the two sides and down the centre.

After the proteins on the guide strips had been located with the spray reagent, the portions of the residual filter paper corresponding to the spots were excised and each cut into small pieces. Equivalent areas from several sheets of filter paper were put together and the group extracted with 250 ml. of 0.05 N aqueous ammonia solution with continuous stirring for two hours at room temperature. After being filtered, the extracts were concentrated to about 20 ml. under reduced pressure at 30°C. They were used for enzyme assays. If necessary, these enzyme solutions were subjected to rechromatography by the same procedure. Since, however, the enzyme solutions thus obtained from Taka-diastase showed fairly low activities, the rechromatographed enzymes were determined directly without elution, the piece of filter paper containing them being cut to a uniform size (5×2 cm.).

4. *Detection of enzymes and nonenzymic protein*

Both enzymic and nonenzymic protein on the chromatograms was detected as a rule by spraying with 0.1 per cent butanol solution of ninhydrin and heating at 110°C for 5 minutes.

For the detection of α-galactosidase activity, guide strips were sprayed with 0.0048 M *p*-nitrophenyl α-galactoside in 0.05 M acetate buffer, pH 4.8. They were then quickly placed horizontally on a sheet of paraffin paper and incubated in a glass cylinder at 30°C. After 30 minutes, they were hung over a concentrated ammonium hydroxide solution and exposed to the ammonia vapor for a while. If a yellow color appeared, it indicates the presence of α-galactosidase activity.

In the amylase test a 0.5 per cent starch solution was sprayed over the guide strips and the latter were then incubated at 50°C for 10 minutes and dried at 105°C. The presence of amylase could be detected by the appearance of a colorless to red-violet spot against a blue-black background when the strips were dipped into a bath containing 0.005 N iodine[7].

5. *Enzyme assays*

Enzyme solutions (1 part) obtained from paper chromatograms were incubated with 0.012 M substrate solutions (1 part) and 0.05 M acetate buffer of pH 4.8 (2 parts) at 30°C. 1.0 ml. aliquots of the reaction mixture were removed at intervals and reducing power measured by SHAFFER-HARTMANN-SOMOGYI'S[8] method. Alternatively, liberated aglycon was estimated colorimetrically. The average reaction constant, K, was evaluated from determinations

by either method, assuming that the reaction is first order.

The ratio of the values of K for substrates of different aglycon was used as a measure to compare the aglycon specificity of glycosidases.

6. *Preparation of crystalline Taka-amylase A from the commercial Sankyo Taka-diastase*

Taka-amylase A used as a control in the present work was purified and recrystallized thrice according to the method of AKABORI[9]. This product was electrophoretically and ultracentrifugally homogeneous.

7. *Egg albumin*

Partially purified commercial egg albumin (Wakô Co.) was also used as a control for the paper chromatography of enzymes.

Results

1. *Activities of enzyme fractions from Taka-diastase*

i) *Fractionation by the acetone-water solvent system*

The paper chromatography of a Taka-diastase solution at room temperature with the acetone-water solvent system gave three spots as shown in Fig. 1・(1), which were referred to as upper, middle and lower fractions. After being eluted, these fractions were tested for their glycosidase activities. The results are shown in Table 1-(1).

The activity ratios of the upper and middle fractions towards α- and β-galactosides were very similar, but the ratios of the lower fraction towards the same galactosides were considerably different from those of the other two, especially in the ratios for *p*-nitrophenyl and *p*-cresyl α-galactosides and for *o*-cresyl β-galactoside. On the other hand, the β-glucosidase activities of the upper and lower fractions were similar and they were considerably different from that of the middle one. Furthermore, it may be noticed that the ratios of the α-galactosidase activities towards phenyl α-galactoside for upper, middle and lower fractions were in the order 25 : 5 : 1, while those of the β-galactosidase and β-glucosidase activities towards the corresponding phenyl glycosides for these fractions were in the order 25 : 5 : 1 and 35 : 2.5 : 1, respectively.

Table 1-(1). Enzyme activities of upper, middle and lower spots from Taka-diastase towards various glycosides.

Enzyme fraction	α-D-Galactoside								β-D-Galactoside				β-D-Glucoside			
	Phenyl		*p*-Nitro-phenyl		*m*-Cresyl		*p*-Cresyl		Phenyl		*o*-Cresyl		Phenyl		*o*-Cresyl	
	K	r	K	r	K	r	K	r	K	r	K	r	K	r	K	r
Upper	0.19	1	0.180	0.95	0.184	0.97	0.196	1.03	1.1	5.8 (1)	1.165	6.13 (1.06)	1.48	7.8 (1)	0.178	0.94 (0.12)
Middle	0.036	1	0.040	1.11	0.040	1.11	0.039	1.08	0.21	5.8 (1)	0.277	7.7 (1.3)	0.11	3.1 (1)	0.028	0.78 (0.25)
Lower	0.007	1	0.012	1.70	0.008	1.14	0.011	1.57	0.044	6.3 (1)	0.076	10.8 (1.7)	0.043	6.1 (1)	0.007	1.0 (0.16)
(Initial)*		1		0.66		1.17		0.97								

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1. For the relative activities in the parentheses, activities for phenyl β-galactoside and phenyl β-glucoside are each taken as 1.
Developed with the acetone-water solvent system at room temperature.
* Figures are cited from a previous paper[6].

ii) *Fractionation by the acetone-acetate buffer solvent system*

Another Taka-diastase solution was similarly chromatographed at 0~5°C with the acetone-acetate buffer solvent system. Three fractions (upper, middle and lower) were also developed on the paper, as shown in Fig. 1・(2). The enzyme activities of the eluates of the three components are shown in Table 1-(2).

As may be clearly seen from the ratios, the aglycon specificity of the α-galactosidases of the upper and lower fractions was almost the same, while that of the middle fraction was significantly different from them. The aglycon specificity of the β-galactosidase of the upper fraction was considerably different from those of the middle and lower ones, while for the β-glucosidase, the lower fraction was the one which showed distinctive features. Further, the activities of the three fractions towards phenyl α- and β-galactosides and phenyl β-glucoside were conspicuously different. The ratios of the activities of the upper, middle and lower fractions towards phenyl α-galactoside were in the order 2.5 : 1 : 3, while those towards phenyl β-galactoside and β-glucoside were respectively 1 : 85 : 60 and 1 : 8 : 25.

Table 1 – (2). Enzyme activities of upper, middle and lower fractions from Taka-diastase towards various glycosides.

Enzyme fraction	α-D-Galactoside				β-D-Galactoside				β-D-Glucoside			
	Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl	
	K	r	K	r	K	r	K	r	K	r	K	r
Upper	0.068	1	0.15	2.21	0.054	0.8 (1)	0.28	4.12 (5.2)	0.21	3.1 (1)	0.063	0.92 (0.3)
Middle	0.027	1	0.12	4.44	4.68	173 (1)	9.34	346 (2.0)	1.64	60.7 (1)	0.19	7.04 (0.12)
Lower	0.083	1	0.17	2.05	3.19	38.4 (1)	5.97	71.9 (1.9)	5.25	63.2 (1)	0.35	4.21 (0.07)
(Initial)*		1		0.66								

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1. Activities for phenyl β-galactoside and phenyl β-glucoside are each taken as 1 for the values of r in the parentheses.
Developed with the acetone-acetate buffer solvent system at 0° to 5°C.
* Figures are cited from a previous paper[6].

iii) *Rechromatography of the eluates from the upper and lower fractions of Taka-diastase used in the experiments i) and ii)*

The eluates from both the upper and the lower fractions of Taka-diastase used in experiment i) and ii) were again chromatographed to examine whether or not the enzyme activity in these extracts could be further resolved into components. The rechromatography was carried out under the same conditions as the first chromatography. Results are shown in Fig. 1 • ((3)~(6)). As seen in the figures, one spot (in Fig. 1 • (4) and (6)) could be detected on rechromatography of the lower fraction and two spots (in Fig. 1 • (3) and (5)) on rechromatography of the upper. The activities of the enzymes on the paper strips corresponding to the spots were determined without elution. The results are summerized in Table 2 – (1) and (2) and in Table 3 – (1) and (2).

The lower fractions obtained by rechromatography showed an activity towards α-galactosides so low that it was practically zero in certain cases, while that towards other glycosides still remained measurable (Table 2– (1) and (2)). It is interesting that the β-glucosidase activity of the lower fraction in both Tables 1 – (1) and 2 – (1), where the acetone-water solvent system

Table 2-(1). Enzyme activities of upper and lower fractions obtained by rechromatography from the upper fraction in Table 1-(1).

Enzyme fraction	α-D-Galactoside				β-D-Galactoside		β-D-Glucoside	
	Phenyl		p-Nitrophenyl		Phenyl		Phenyl	
	K	r	K	r	K	r	K	r
Upper	0.012	1	0.0077	0.64	0.105	8.75	0.082	6.83
Lower	0.000	—	0.000	—	0.0068	—	0.01	—

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1.
Developed with the acetone-water solvent system at room temperature.

was used, was far lower than that of the upper fraction in the same tables while, on the contrary, the activity of this enzyme in the lower fractions in Tables 1-(2) and 2-(2), where the acetone-buffer solvent system was used, was far higher than that in the upper fractions.

Table 2-(2). Enzyme activities of upper and lower fractions obtained by rechromatography from the upper fraction in Table 1-(2).

Enzyme fraction	α-D-Galactoside				β-D-Galactoside				β-D-Glucoside			
	Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl	
	K	r	K	r	K	r	K	r	K	r	K	r
Upper	0.006	1	0.016	2.66	0.000	—	0.000	—	0.004	0.66	0.000	—
Lower	0.000	—	0.003	—	0.006	(1)	0.0043	(0.7)	0.0076	(1)	0.0066	(0.9)

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1. For relative activities in the parentheses, activities for phenyl β-galactoside and phenyl β-glucoside are each taken as 1.
Developed with the acetone-acetate buffer solvent system at 0~5°C.

Although two fractions, namely, the upper and the lower, were again obtained by rechromatography from the upper fractions in Table 1-(1) and (2), the lower fractions in the same tables each appeared as a single component on rechromatography. Furthermore, the relative activities of these components against β-glucosides were nearly equal (Table 3-(1) and (2)),

while they were distinct from those of the lower fraction in Table 2-(2), which had originated from the upper fraction of Table 1-(2). However, the β-glucosidase activity of the single component in Table 3-(2) was very different from that of the lower fraction in Table 1-(2), even though it originated directly from the latter and no other component with measurable activity had appeared when this fraction was rechromatographed.

It is noteworthy that practically no α-galactosidase activity was found in these single components, while some measurable β-galactosidase and β-glucosidase activities remained. There was a significant difference between the ratio of the activities of these two latter glycosidases in the component of Table 3-(1) and that of Table 3-(2).

Table 3-(1). Enzyme activities of the rechromatographed lower fraction in Table 1-(1).

Enzyme fraction	α-D-Galactoside				β-D-Galactoside		β-D-Glucoside			
	Phenyl		p-Nitrophenyl		Phenyl		Phenyl		p-Nitrophenyl	
	K	r	K	r	K	r	K	r	K	r
A single component	0.000	—	0.0078	0.06	0.103	0.75	0.136	1	0.022	0.16

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl β-glucoside is taken as 1.
Developed with the acetone-water solvent system at room temperature.

Table 3-(2). Enzyme activities of the rechromatographed lower fraction in Table 1-(2).

Enzyme fraction	α-D-Galactoside				β-D-Galactoside				β-D-Glucoside			
	Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl	
	K	r	K	r	K	r	K	r	K	r	K	r
A single component	0.000	—	0.000	—	0.117	0.26 (1)	0.24	0.5 (1.9)	0.449	1	0.081	0.18

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl β-glucoside is taken as 1. For activity in the parentheses, activity for phenyl β-galactoside is taken as 1.
Developed with the acetone-acetate buffer solvent system at 0~5°C.

2. *Activities of enzyme fractions from apricot emulsin*

A crude and a tannin-purified[10] apricot emulsins were chromatographed respectively with the methanol-water and the acetone-water solvent systems at room temperature. The chromatograms obtained are shown in Fig. 1・(7) and (8), respectively. Two components were evident, an upper and a lower.

Enzyme activities of the eluates from the upper and the lower fractions towards β-galactosides and β-glucosides were determined. With the crude enzyme, only one glycoside of each type was used, but two of each were used with the purified one. The results are shown in Table 4-(1) and (2).

Table 4-(1). Glycosidase activities of the upper and lower fractions from a crude apricot emulsin.

Enzyme fraction	β-D-Galactoside		β-D-Glucoside	
	o-Cresyl		*o*-Cresyl	
	K	r	K	r
Upper	0.027	1	0.43	15.9
Lower	0.019	1	0.25	13.2

Note:
K, the value is multiplied by 10^3.
r, activity for *o*-cresyl β-galactoside is taken as 1.
Developed with the methanol-water solvent system at room temperature.

Table 4-(2). Glycosidase activities of the upper and lower fractions from a tannin-purified apricot emulsin.

Enzyme fraction	α-D-Galactoside				β-D-Galactoside				β-D-Glucoside			
	Phenyl		*p*-Nitrophenyl		Phenyl		*o*-Cresyl		Phenyl		*o*-Cresyl	
	K	r	K	r	K	r	K	r	K	r	K	r
Upper	0.034	1	0.052	1.53	0.032	0.94 (1)	0.26	7.65 (8.1)	0.11	3.24 (1)	3.25	95.6 (30)
Lower	0.0026	1	0.0027	1.04	0.019	7.31 (1)	0.08	30.8 (4.2)	0.096	36.9 (1)	0.79	303.8 (8.2)

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1. For the values of r in the parentheses, activities for phenyl β-galactoside and phenyl β-glucoside are each taken as 1.
Developed with the acetone-water solvent system at room temperature.

As these tables show, the ratios between the β-galactosidase and β-glucosidase activities in both fractions from crude emulsin were roughly of the same order as those in both fractions from the purified one, ranging from 1 : 16 to 1 : 10. Further, the activity ratio of the α-galactosidase in the two fractions from the latter was similar, although the activity in the upper fraction was about thirteen times higher than that in the lower. In contrast, the aglycon specificities of both β-galactosidase and β-glucosidase were different in the two fractions. Activities of these enzymes in the upper fraction were somewhat higher than those in the lower, with some difference for individual substrates, the ratios ranging from 1 : 1 to 4 : 1.

3. *Activities of enzyme fractions from malt*

An extract from malt was subjected to similar examination. Guide strips revealed the presence of two spots (upper and lower) in this case also (Fig. 1・(9)). Two components were eluted in the usual fashion from both upper and lower fractions and their enzyme activities were investigated. Table 5 shows the results.

Table 5. Glycosidase activities of the upper and lower fractions from a malt extract.

Enzyme fraction	α-D-Galactoside				β-D-Galactoside				β-D-Glucoside			
	Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl		Phenyl		p-Nitrophenyl	
	K	r	K	r	K	r	K	r	K	r	K	r
Upper	0.013	1	0.031	2.39	0.133	10.2 (1)	0.196	15.1 (1.5)	0.018	1.38 (1)	0.096	7.5 (5.4)
Lower	0.03	1	0.073	2.43	0.016	0.53 (1)	0.023	0.78 (1.5)	0.029	0.97 (1)	0.097	3.2 (3.3)

Note:
K, the value is multiplied by 10^3.
r, activity for pheny α-galactoside is taken as 1. For the values of r in the parentheses, activities against phenyl β-galactoside and β-glucoside are each taken as 1.
Developed with the acetone-NaCl-phosphate buffer solvent system at 5°C.

The ratios of the enzyme activities in these malt fractions altered only within a narrow range, in contrast to Taka-diastase and emulsin, and no special differences in the aglycon specificities of the three kinds of glycosidases seem to be present between the upper and lower components. This was unexpected, considering the results of previous work by Nisizawa and

WAKABAYASHI[2][3]. Probably the similarity of the aglycon specificity of these fractions is only apparent and they could be further resolved by rechromatography into components which had a different specificity. However, each glycosidase in the upper fraction was somewhat different in activity from that in the lower one, the activity ratios being roughly 1 : 2, 8 : 1 and 1 : 1 respectively for α- and β-galactosides and β-glucoside.

4. *Activities of the α-galactosidases of enzyme fractions from bottom yeast*

An extract which had been prepared in the same way as in a previous paper[6] gave two components on paper chromatography, that is, upper and lower frations (Fig. 1・(10)). The enzyme activities of eluates from these fractions are shown in Table 6. The activities of both fractions were similar except for some difference in their activities against phenyl α-galactoside.

Table 6. Enzyme activities of upper and lower fractions from a crude extract of bottom yeast.

Enzyme fraction	α-D-Galactoside					
	Phenyl		*p*-Nitrophenyl		Glucosyl	
	K	r	K	r	K	r
Upper	0.067	1	0.26	3.88	0.13	1.94
Lower	0.051	1	0.28	5.49	0.096	1.90
(Initial)*		1		2.95		1.29

Note:
K, the value is multiplied by 10^3.
r, activity for phenyl α-galactoside is taken as 1.
Developed with the acetone-phosphate buffer solvent system at 5°C.
* Figures are cited from a previous paper[6].

5. *Paper chromatography of Taka-amylase A and egg albumin*

Commercial egg albumin tailed on chromatography, so that individual spots could not be discriminated, while crystalline Taka-amylase A was chromatographed as a single spot even in the presence of egg albumin. These chromatograms are shown in Fig. 1・(11) to (13).

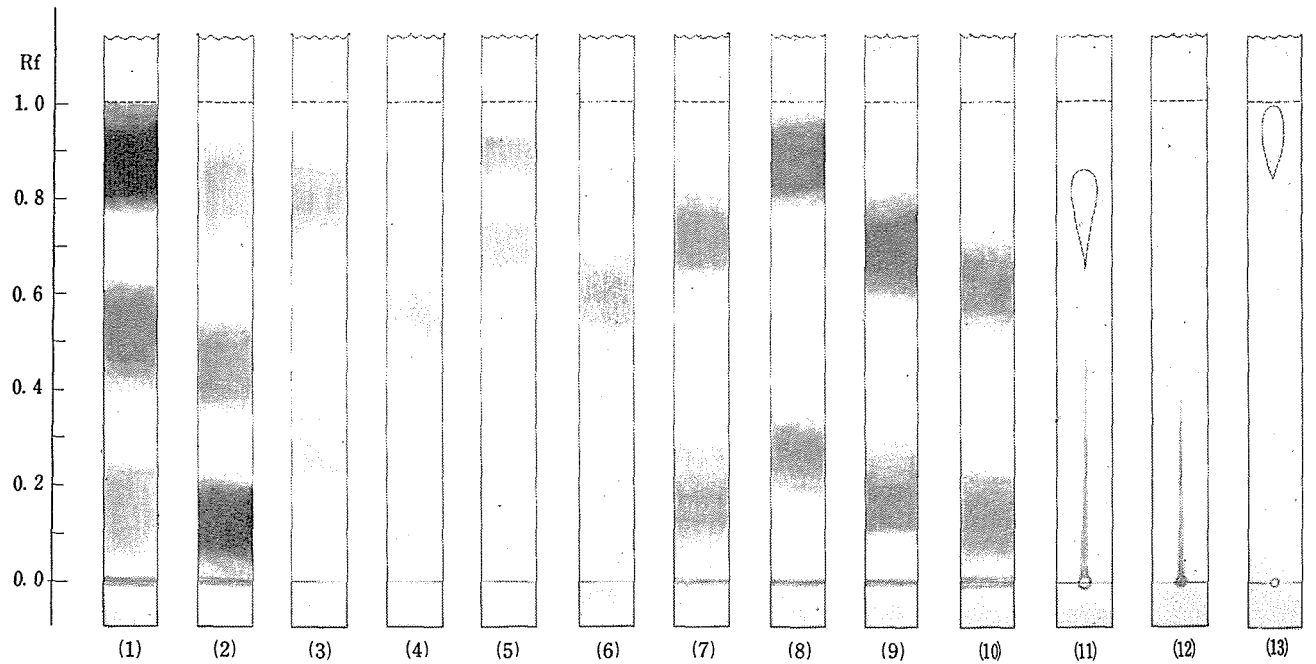

Fig. 1. Diagrams showing the paper chromatograms of glycosidase fractions from several different sources. Taka-amylase A and egg albumin as control.

	Enzyme preparation	Solvent system	Temperature
(1)	Taka-diastase	Acetone-water (2:3, v/v)	Room temperature
(2)	Taka-diastase	Acetone-acetate buffer of 0.01 M at pH 4.8 (2:3, v/v)	0～5°C
(3)	Upper fraction of (1)	Acetone-water (2:3, v/v)	Room temperature
(4)	Lower fraction of (1)	Acetone-water (2:3, v/v)	Room temperature
(5)	Upper fraction of (2)	Acetone-acetate buffer of 0.01 M at pH 4.8 (2:3, v/v)	0～5°C
(6)	Lower fraction of (2)	Acetone-acetate buffer of 0.01 M at pH 4.8 (2:3, v/v)	0～5°C
(7)	Crude apricot emulsin	Methanol-water (1:4, v/v)	Room temperature
(8)	Partially purified apricot emulsin	Acetone-water (2:3, v/v)	Room temperature
(9)	Malt	Acetone-NaCl of 0.7 M-phosphate buffer of 0.028 N at pH 7 (3:2:5, v/v)	5°C
(10)	Brewer's bottom yeast	Acetone-phosphate buffer of 0.01 N at pH 7 (2:3, v/v)	5°C
(11)	Mixture of Taka-amylase A and egg albumin	Acetone-aqueous NaCl of 0.16%, w/v (2:3, v/v)	20～23°C
(12)	Egg albumin	As in (11)	As in (11)
(13)	Taka-amylase A	As in (11)	As in (11)

(11) 1.0 ml. of crystalline Taka-amylase A (0.1%, w/v) was mixed with 2.0 ml. of egg albumin (2%, w/v) in an aqueous NaCl solution (0.2%, w/v). 0.01 ml. of the mixture was spotted.

(12) 0.01 ml. of the above egg albumin solution was spotted.

(13) 0.01 ml. of the above Taka-amylase A solution was spotted.

Discussion

Several enzyme solutions from different sources were subjected to paper chromatography to investigate the possible multiple nature of some glycosidases.

The chromatographic resolution of the glycosidases of Taka-diastase was better than any other, and several fractions of similar or different activity ratios could be demonstrated (Tables 1, 2 and 3). Of these components, two upper fractions obtained by using different solvent systems were both resolved into a few further components by rechromatography (Tables 2-(1) and 2-(2)), but two kinds of lower fractions each behaved as a single component upon rechromatography with a different solvent system (Tables 3-(1) and 3-(2)). Their aglycon specificities for β-glucosides were alike, but distinct from that of the lower component derived from the upper fraction (Table 2-(2)). This suggests that the components derived from the lower fractions represent a single β-glucosidase. However, the aglycon specificity of the single component in Table 3-(2) was different from that of the lower fraction in Table 1-(2), although it was directly derived from the latter. This fact possibly indicates that the lower fraction in Table 1-(2) did not represent a single component with regard to the β-glucosidase activity, even though the lower fraction appeared like a single component on rechromatography as shown in Table 3-(2). Probably, any other β-glucosidase component contained in the lower fraction might have been lost during the treatment.

The ratios between the activities of three glycosidases of Taka-diastase varied from fraction to fraction and also with the solvent system used (Tables 1-(1) and 1-(2)). In some fractions, no α-galactosidase activity was found while measurable activities of β-galactosidase and β-glucosidase remained, as seen in Tables 3-(1) and 3-(2). All these facts suggest that these glycosidase activities are the properties of enzymes that are not identical.

Apricot emulsin and the glycosidases of malt and bottom yeast were resolved in the same fashion into at least two or three components. Components from the same origin could show either similar or different aglycon specificities.

Thus, a number of glycosidases from four sources have been resolved into components by paper chromatography and some of these components seem to have their own characteristic patterns of aglycon specificity. In this respect, resolution by paper chromatography appears not to be an "artifact" of chromato-

graphy, as has been postulated by JERMYN[4] for the β-glucosidase of *Aspergillus oryzae*. This conclusion is supported by the results with Taka-diastase which show that the β-glucosidase activities of the lower fractions were always lower than those of the upper ones on rechromatography as well as on initial chromatography when the acetone-water solvent system was used, while the reverse relationship was observed when the acetone-buffer solvent system was used (Tables 1-(1) and (2), 2-(1) and (2)).

Summary

Several glycosidases including the α-galactosidases of Taka-diastase, apricot kernel, malt and bottom yeast were resolved into fractions by paper chromatography. In general, the ratios of the activities towards a number of substrate varied for any glycosidase with the fraction in which it occurred. Some of these fractions were further resolved into a few components when they were chromatographed again, but some behaved upon rechromatography as a single component, the aglycon specificity of which seemed to be characteristic.

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