Inhibition of Glycosidases by Aldonolactones of Corresponding Configuration

THE C-4- AND C-6-SPECIFICITY OF β -GLUCOSIDASE AND β -GALACTOSIDASE

By J. CONCHIE, A. L. GELMAN AND G. A. LEVY

Rowett Research Institute, Bucksburn, Aberdeen, and North of Scotland College of Agriculture, Aberdeen

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1. In barley, β -glucosidase and β -galactosidase are separate enzymes. The former also displays β -D-fucosidase activity. 2. In the limpet, *Patella vulgata*, β -glucosidase activity is associated with the β -D-fucosidase, previously shown to be a separate entity from the β -galactosidase also present. 3. Almond emulsin presents all three activities as a single enzyme. Each is equally inhibited by glucono-, galactono- and D-fucono-lactone. 4. In rat epididymis, there is no significant β -glucosidase activity, nor is there appreciable inhibition of the β -galactosidase and β -D-fucosidase activities of the preparation by gluconolactone.

Heyworth & Walker (1962) have produced convincing evidence that, in almond emulsin, the β -glucosidase and β -galactosidase activities reside in a single enzyme. However, from the earlier literature reviewed by these authors it would appear that in preparations from certain other sources the two enzymes are separate.

Levy & McAllan (1963a) found that β -galactosidase could be separated from β -D-fucosidase in extracts from the limpet, *Patella vulgata*. Although both enzymes were inhibited by galactonolactone and fuconolactone, inhibition was in each case much more pronounced when the inhibitor was derived from the same sugar as the substrate. In mammalian tissues, however, it was concluded that β -galactosidase and β -D-fucosidase activities were due to a single enzyme, in spite of some conflicting evidence (Levy & McAllan, 1963b). The relative inhibitory powers of fuconolactone and galactonolactone were determined not so much by the type of substrate as by the origin of the enzyme preparation. Thus β -galactosidase and β -D-fucosidase activities in rat epididymis were both powerfully inhibited by galactonolactone and only feebly by fuconolactone (see also Conchie, Hay, Strachan & Levy, 1967), whereas in ox liver fuconolactone was much more effective than galactonolactone against both types of enzyme activity (Levy & McAllan, 1963b).

The relationship between the C-4- and C-6-specificity of these glycosidases presents a problem that has now been studied with enzyme preparations from four sources.

EXPERIMENTAL

Enzyme preparations

Barley. Barley (71g.) of the Ymer variety, moisture content 10%, was ground slowly in a hand-mill, care being taken to avoid heat generation. The ground barley was suspended in 0.2N-acetic acid-NaOH buffer, pH5.0, containing 0.5% (w/v) NaCl, and homogenized for 1 min. in an Ultra-Turrax TP18/2N disintegrator (Janke und Kunkel Kg., Staufen i. Br., Germany), the final volume being 500 ml. The suspension was autolysed for 1 hr. at 37°, centrifuged at 10000g for 15 min., and the supernatant solution fractionated with $(\text{NH}_4)_2\text{SO}_4$ at 0°, the fraction precipitated between 20 and 80% saturation limits being retained. After centrifuging at 10000g for 10 min., this precipitate was dissolved in 0.01N-acetic acid-NaOH buffer, pH5.0 (110 ml.). The β -glucosidase and β -galactosidase activities of the preparation were 36200 and 11000 o-nitrophenol units/g. of dry barley respectively, corresponding to 86 and 89 % of the glycosidase activities in the original homogenate, and the β -D-fucosidase activity was 12400 p-nitrophenol units/g. of dry barley. The solution could be stored at -20° without loss in activity and was diluted 1:40 for β -glucosidase and β -D-fucosidase assays, and 1:20 for β -galactosidase assays. The pH values for optimum activity in 0.2M- Na_2HPO_4 -0.1M-citric acid buffer (McIlvaine, 1921) were 3.75 and 4.9 for β -galactosidase and β -glucosidase respectively, but assays were done throughout at a common pH value of 4.5 to make kinetic values comparable.

Limpet. A limpet (*Patella vulgata*) preparation (2.5 ml./g. of tissue), made by $(\text{NH}_4)_2\text{SO}_4$ fractionation of an aqueous extract as described by Conchie & Levy (1957), had β -glucosidase and β -galactosidase activities of 63500 and

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63700 *o*-nitrophenol units/g. of tissue respectively and a β -D-fucosidase activity of 45800 *p*-nitrophenol units/g. of tissue. The solution was diluted 1:100 for all assays. Further fractionation followed the method of Levvy & McAllan (1963a).

A 'fucosidase-enriched' preparation was prepared by diluting 5 ml. of the above solution to 70 ml., adjusting to pH 8.5 and incubating for 2 hr. at 27°. After readjusting to the original pH (5.3), the solution was made up to 100 ml. This preparation had β -glucosidase and β -galactosidase activities of 36400 and 5670 *o*-nitrophenol units/g. of tissue respectively and β -D-fucosidase activity of 30000 *p*-nitrophenol units/g. of tissue. The solution was diluted 1:2.5 for β -glucosidase and β -D-fucosidase assays and used undiluted for β -galactosidase assays.

A 'galactosidase-enriched' preparation was made by diluting 5 ml. of the original preparation to 70 ml., adjusting to pH 3 and heating for 10 min. at 50°. After cooling, the solution was adjusted to the original pH (5.3) and made up to 100 ml. This preparation had β -glucosidase and β -galactosidase activities of 475 and 20400 *o*-nitrophenol units/g. of tissue respectively. The solution was used undiluted for β -glucosidase assays and diluted 1:2.5 for β -galactosidase assays.

Emulsin. Partially-defatted sweet-almond meal (0.5 g.), kindly supplied by Dr P. G. Walker, Royal National Orthopaedic Hospital, Stanmore, Middlesex, was suspended in 0.01 N-acetic acid-NaOH buffer, pH 5.0 (10 ml.), shaken gently for 15 min., and the suspension centrifuged at 1500 g for 10 min. The supernatant solution had β -glucosidase and β -galactosidase activities of 2370000 and 424000 *o*-nitrophenol units/g. of emulsin respectively and a β -D-fucosidase activity of 1760000 *p*-nitrophenol units/g. of emulsin. The preparation was diluted 1:400 for β -glucosidase and β -D-fucosidase assays and 1:100 for β -galactosidase assays.

Rat epididymis. A rat epididymis preparation (2 ml./g. of tissue) was fractionated with $(\text{NH}_4)_2\text{SO}_4$ as described by Levvy, Hay & Conchie (1964). The β -D-fucosidase activity of the preparation was 3700 *p*-nitrophenol units/g. of tissue and the β -galactosidase activity 28600 *o*-nitrophenol units/g. of tissue. The solution could be stored without loss in activity at -20°, and was diluted 1:8 for β -D-fucosidase assays and 1:50 for β -galactosidase assays.

Enzyme assays

β -Glucosidase. The incubation mixture contained 2 ml. of 0.2 M- Na_2HPO_4 -0.1 M-citric acid buffer (McIlvaine, 1921) (pH 4.5 for the enzymes from barley and emulsin, and pH 5.0 for the limpet enzyme), 0.5 ml. of 40 mM-*o*-nitrophenyl β -glucoside and 0.5 ml. of enzyme preparation, the volume being made up to 4 ml. with water or inhibitor. After 1 hr. at 37°, 4 ml. of 0.4 M-glycine-NaOH buffer, pH 10.4, was added and the liberated nitrophenol measured on a Spekker photoelectric absorptiometer with Ilford no. 601 violet filters (peak transmission 430 m μ).

β -Galactosidase. The hydrolysis of 5 mM-*o*-nitrophenyl β -galactoside in 0.2 M- Na_2HPO_4 -0.1 M-citric acid buffer, pH 4.5, by barley and emulsin enzymes was measured as described for β -glucosidase assays. With the limpet and rat enzymes the pH of the assay mixture was 3.1 and the final concentration of substrate 2.5 mM.

β -D-Fucosidase. The hydrolysis of 2.5 mM-*p*-nitrophenyl β -D-fucoside in 0.2 M- Na_2HPO_4 -0.1 M-citric acid buffer,

pH 4.5, was measured as described for β -glucosidase assays. With the rat enzyme the pH of assay was 3.2.

Inhibitors

Galactono-(1 $\rightarrow$ 4)-lactone was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.), and 'most inhibitory solutions' from it were prepared as described by Levvy, McAllan & Hay (1962). Such solutions are called galactono-(1 $\rightarrow$ 5)-lactone throughout this paper.

Glucono-(1 $\rightarrow$ 5)- and glucono-(1 $\rightarrow$ 4)-lactone were purified as described by Levvy *et al.* (1964).

Solutions of D-fucono-(1 $\rightarrow$ 5)-lactone were obtained by platinum-catalysed oxidation of D-fucose as described by Conchie *et al.* (1967).

All inhibitor solutions were freshly prepared and used immediately, and concentrations are expressed in terms of starting material.

RESULTS

Inhibition by lactones

Table 1 shows the concentrations of D-fucono-(1 $\rightarrow$ 5)-, glucono-(1 $\rightarrow$ 5)- and galactono-(1 $\rightarrow$ 5)-lactone (prepared as described in the Experimental section) required for 50% inhibition of the β -glucosidase, β -D-fucosidase and β -galactosidase activities in preparations from limpet, barley and emulsin. (Wherever the concentration of inhibitor permitted, inhibition could be made to approach 100%.) With all three enzyme preparations, fucono-(1 $\rightarrow$ 5)-lactone was an equally powerful inhibitor of both β -glucosidase and β -fucosidase activities. It was a relatively poor inhibitor of limpet and barley β -galactosidase activities, but inhibited emulsin β -galactosidase as powerfully as it did β -glucosidase and β -fucosidase from this source. Glucono-(1 $\rightarrow$ 5)-lactone displayed an identical pattern of behaviour, as did glucono-(1 $\rightarrow$ 4)-lactone, which was generally a slightly less efficient inhibitor than the (1 $\rightarrow$ 5)-lactone. Galactono-(1 $\rightarrow$ 5)-lactone, in the form of a 'most inhibitory solution' was a very poor inhibitor of both limpet and barley β -glucosidase activities and a powerful inhibitor of β -galactosidase from these sources. It was a moderately powerful inhibitor of all three emulsin glycosidases.

In view of the known specificity of inhibition of these lactones (Conchie & Levvy, 1957; Levvy & McAllan, 1963a; Levvy *et al.* 1964) the results strongly suggested that β -glucosidase and β -fucosidase activities in all three preparations were due to a single enzyme and that this enzyme was distinct from β -galactosidase, except in emulsin where all three glycosidase activities seemed to be due to one enzyme. It has already been shown that in the limpet β -D-fucosidase and β -galactosidase are distinct enzymes (Levy & McAllan, 1963a) and that in emulsin β -glucosidase and β -galactosidase

Table 1. Concentrations of D-fucono-, glucono- and galactono-(1 $\rightarrow$ 5)-lactone causing 50% inhibition of the hydrolysis of substrates by enzymes from various sources

For description of lactones, enzyme preparations and methods of assay see the text. Curves were drawn for percentage inhibitions at various inhibitor concentrations and the 50% values found by interpolation.

Source of enzyme	Substrate	Concn. for 50% inhibition (mM)		
		Fuconolactone	Gluconolactone	Galactonolactone
Limpet	5 mM-o-Nitrophenyl β -glucoside	0.081	0.16	31% inhibition with 20 mM
	2.5 mM-p-Nitrophenyl β -D-fucoside	0.014	0.058	—
	2.5 mM-o-Nitrophenyl β -galactoside	0.92	No inhibition with 2 mM	0.15
Limpet: 'fucosidase-rich' prep.	5 mM-o-Nitrophenyl β -glucoside	0.058	0.15	41% inhibition with 20 mM
Limpet: 'galactosidase-rich' prep. Barley	2.5 mM-p-Nitrophenyl β -D-fucoside	0.013	0.057	—
	2.5 mM-o-Nitrophenyl β -galactoside	0.75	No inhibition with 2.5 mM	0.075
	5 mM-o-Nitrophenyl β -glucoside	0.12	0.023	31% inhibition with 37 mM
	2.5 mM-p-Nitrophenyl β -D-fucoside	0.071	0.021	23% inhibition with 5 mM
	5 mM-o-Nitrophenyl β -galactoside	1.5	32% inhibition with 5 mM	0.20
Barley: 'glucosidase-rich' prep.	5 mM-o-Nitrophenyl β -glucoside	—	0.016	36% inhibition with 40 mM
	2.5 mM-p-Nitrophenyl β -D-fucoside	—	0.027	10% inhibition with 5 mM
	5 mM-o-Nitrophenyl β -galactoside	—	39.8	0.69
Barley: 'galactosidase-rich' prep. Emulsin	5 mM-o-Nitrophenyl β -D-fucoside	—	35% inhibition with 40 mM	0.15
	5 mM-o-Nitrophenyl β -galactoside	0.022	0.087	17.4
	2.5 mM-p-Nitrophenyl β -glucoside	0.029	0.085	37% inhibition with 5 mM
Rat	5 mM-o-Nitrophenyl β -D-fucoside	0.018	0.071	11.6
	2.5 mM-p-Nitrophenyl β -galactoside	4.7*	10% inhibition with 5 mM	0.012†
	2.5 mM-o-Nitrophenyl β -galactoside	9.6*	No inhibition with 5 mM	0.058*†

* Quoted from Conchie *et al.* (1967).

† Lactone solution prepared by catalytic oxidation of galactose.

activities are due to one enzyme (Heyworth & Walker, 1962).

Both β -D-fucosidase and β -galactosidase activities from a rat-epididymal preparation are powerfully inhibited by galactono-(1 $\rightarrow$ 5)-lactone and feebly inhibited by fucono-(1 $\rightarrow$ 5)-lactone (Conchie *et al.* 1967). Tests with glucono-(1 $\rightarrow$ 5)-lactone showed negligible inhibition of both these enzymes (Table 1). The β -glucosidase activity of the preparation was very low (198 *o*-nitrophenol units/g. of tissue). It appears probable that in this case the β -D-fucosidase and β -galactosidase activities are due to one enzyme (Levy & McAllan, 1963b) and that the very low β -glucosidase activity observed is a side action of this enzyme.

Fractionation of limpet and barley glycosidases and effect of inhibitors

Limpet. The β -D-fucosidase and β -galactosidase activities of a limpet preparation can be separated from each other by heat inactivation at suitable pH values (Levy & McAllan, 1963a). In a 'fucosidase-enriched' preparation, made as described in the Experimental section, the ratio of β -fucosidase to β -galactosidase rose from 0.72 in the original preparation to 5.3. There was a similar rise in the ratio of β -glucosidase to β -galactosidase from 1 to 6.4. Tests with glucono-(1 $\rightarrow$ 5)- and fucono-(1 $\rightarrow$ 5)-lactone gave 50%-inhibition values (Table 1) little different from the corresponding values for the original preparation.

A 'galactosidase-enriched' preparation (see the Experimental section) had a β -galactosidase to β -glucosidase ratio 43, compared with a ratio 1 for the original preparation. Inhibition values for glucono-(1 $\rightarrow$ 5)- and fucono-(1 $\rightarrow$ 5)-lactone tested against β -galactosidase (Table 1) were unchanged from the original ones, whereas galactono-(1 $\rightarrow$ 5)-lactone had a slightly more powerful effect than before.

The observation that enrichment of β -fucosidase activity and removal of more than 90% of the β -galactosidase activity led to a similar enrichment of β -glucosidase strongly suggests the identity of β -glucosidase and β -fucosidase and their difference from β -galactosidase. The unchanged effect of all the lactones after almost complete removal of β -glucosidase activity (and of β -D-fucosidase activity; Levy & McAllan, 1963a) is strong confirmation that in the limpet neither the β -glucosidase nor the β -D-fucosidase hydrolyses β -galactosides.

Barley. An enzyme preparation from barley could be enriched with respect to β -glucosidase activity by fractionating with ammonium sulphate as outlined in Table 2, the fraction precipitated between 50% and 80% saturation limits showing considerable enrichment of β -glucosidase. In a

Table 2. *Fractionation of a barley preparation with ammonium sulphate*

The enzyme in a barley extract obtained after homogenization, autolysis and centrifugation as described in the Experimental section was precipitated with $(\text{NH}_4)_2\text{SO}_4$ between the limits of saturation shown. Assays were done in 0.2M- Na_2HPO_4 -0.1M-citric acid buffer, pH 4.5, and results are expressed as $\mu\text{g. of } o\text{-nitrophenol liberated/g. of dry barley in 1 hr.}$

Saturation with $(\text{NH}_4)_2\text{SO}_4$ (%)	β -Glucosidase	β -Galactosidase	Glucosidase/Galactosidase ratio
0-25	573	286	2.0
25-35	1114	878	1.3
35-50	17480	6370	2.8
50-80	8750	1037	8.5
Whole extract	31250	8600	3.6

similar preparation, the ratio of β -glucosidase to β -galactosidase rose from 3.3 in the original preparation to 7.8, and the ratio of β -D-fucosidase to β -galactosidase rose from 1.1 to 2.7. This similarity in rise of β -glucosidase and β -D-fucosidase activities with respect to β -galactosidase again suggests the identity of the first two enzymes. When the enriched preparation was tested with glucono-(1 $\rightarrow$ 5)- and galactono-(1 $\rightarrow$ 5)-lactone, the only appreciable difference observed, compared with the original preparation, was the less powerful effect of galactonolactone on β -galactosidase (Table 1).

Conditions for the production of a 'galactosidase-enriched' preparation were determined. A study of the stabilities of β -galactosidase and β -glucosidase after incubation for 20 min. at 37° over a pH range 3 to 7 showed a pH value of 3.75 to be the most suitable for separation of the enzyme activities. The effect of heating a 20-80%-saturated ammonium sulphate fraction at 37° and pH 3.75 for various times is shown in Fig. 1. After 2 hr. almost all β -glucosidase activity was destroyed, whereas 60% of the β -galactosidase activity remained. Heating at 37° at pH 3.75 for 2 hr. was therefore adopted as a standard procedure for making a 'galactosidase-enriched' preparation. There was no appreciable difference between the 50%-inhibition values for glucono-(1 $\rightarrow$ 5)- and galactono-(1 $\rightarrow$ 5)-lactone for this preparation (Table 1) and for the original. As with the limpet, these unchanged values confirm that β -glucosidase and β -galactosidase are separate entities.

Emulsin β -glucosidase and β -galactosidase

It has been demonstrated that the β -glucosidase and β -galactosidase activities of sweet-almond emulsin are due to one enzyme (Heyworth & Walker,

Fig. 1. Effect of incubation at 37° for various times on the β -galactosidase (○) and β -glucosidase (Δ) activities of a barley preparation [20–80% saturated $(\text{NH}_4)_2\text{SO}_4$ fraction] adjusted to pH 3.75 with HCl. The pH was brought to 4.5 and assays were done in 0.2M- Na_2HPO_4 –0.1M-citric acid buffer, pH 4.5. Results are expressed as percentages of the control (zero time). In the control 100 μg . of *o*-nitrophenol was liberated by β -glucosidase and 104 μg . of *o*-nitrophenol by β -galactosidase.

1962). Further confirmation of this has been obtained from some enzyme-stability experiments. When β -glucosidase and β -galactosidase activities in an emulsin preparation were compared before and after incubation for 20 min. at 37° in 0.2M-disodium hydrogen phosphate–0.1M-citric acid and in 0.05M-sodium butyrate–hydrochloric acid buffers of pH values ranging from 3.5 to 7.0, the activities of the two enzymes were found to vary in parallel, with the greatest stability at pH 5.5. Comparison of activities before and after heating at 37° and 55° for various times in phosphate–citrate buffers at pH 5 and 7 again showed parallel variation.

It was observed that the sodium butyrate–hydrochloric acid buffer used by Heyworth & Walker (1962) did not allow the enzymes to display maximal activity. Compared with their activities in 0.2M-disodium hydrogen phosphate–0.1M-citric acid buffer, pH 5.5, there was about 20% inhibition of β -galactosidase and about 3% inhibition of β -glucosidase activity in 0.05M-sodium butyrate–

Fig. 2. Effect of pH on the rate of hydrolysis by an emulsin preparation of 5 mM-*o*-nitrophenyl β -glucoside in 0.2M- Na_2HPO_4 –0.1M-citric acid buffer (○) and in 0.05M-sodium butyrate–HCl buffer (●), and of 5 mM-*o*-nitrophenyl β -galactoside in 0.2M- Na_2HPO_4 –0.1M-citric acid buffer (Δ) and in 0.05M-sodium butyrate–HCl buffer (▲). For activities of enzyme preparation see the Experimental section.

hydrochloric acid buffer, pH 5.5. Acetate buffer resembled butyrate in this respect. A similar effect could be observed on introducing butyrate into an assay done in phosphate–citrate buffer.

There was also a clear separation of the pH values for optimum activity when phosphate–citrate buffer was used. β -Glucosidase displayed optimum activity at pH 5.1, and β -galactosidase at pH 4.6 in this buffer, whereas in butyrate buffer both enzymes showed optimum activity at pH 5.3–5.5 (Fig. 2). As can be seen, the inhibitory effect of butyrate on β -galactosidase was even greater when tested at pH 4.5 than at pH. 5.5. Similar inhibitory effects with butyrate were observed in tests on β -glucosidase and β -galactosidase in a barley preparation: there was greater inhibition of β -galactosidase than of β -glucosidase. The effect of Triton X-100 on the enzyme activities in emulsin, noted by Heyworth & Walker (1962), was found to be unimportant.

K_m and K_i values for glucoside and fucoside substrates and glucono- and fucono-lactone with limpet, barley and emulsin preparations

The mean values for K_m for glucoside and fucoside substrates, and the corresponding values for K_i for glucono-(1→5)- and fucono-(1→5)-lactone, with enzyme preparations from limpet, barley and emulsin

Table 3. K_m values for *p*-nitrophenyl β -D-fucoside and for *o*-nitrophenyl β -glucoside with enzymes from limpet, emulsin and barley, and the corresponding K_i values for glucono-(1 $\rightarrow$ 5)-lactone and fucono-(1 $\rightarrow$ 5)-lactone

Substrate concentrations were 0.1–10 mM for the fucoside and 0.5–10 mM for the glucoside. For description of enzyme preparations and activities see the Experimental section: Assays were done in Na_2HPO_4 -citric acid buffer, pH 4.5. Values for K_m and K_i were calculated by the method of Lineweaver & Burk (1934).

Enzyme	Substrate	K_m (mM)	K_i (mM)	
			Gluconolactone	Fuconolactone
Limpet	<i>p</i> -Nitrophenyl β -D-fucoside	0.60	0.014	0.0027
	<i>o</i> -Nitrophenyl β -glucoside	0.89	0.015	0.0039
Emulsin	<i>p</i> -Nitrophenyl β -D-fucoside	1.8	0.042	0.013
	<i>o</i> -Nitrophenyl β -glucoside	6.0	0.036	0.012
Barley	<i>p</i> -Nitrophenyl β -D-fucoside	1.5	0.0075	0.023
	<i>o</i> -Nitrophenyl β -glucoside	2.9	0.0076	0.024

are shown in Table 3. Both lactones acted competitively throughout. With all three enzyme preparations there is agreement in the K_i values for each inhibitor when compared against the two substrates, providing further confirmation that the same enzyme is responsible for the hydrolysis of both substrates. It has already been shown by Heyworth & Walker (1962) that with an emulsin preparation there is good agreement in the K_i values for glucono- and galactono-lactone when measured against glucoside and galactoside substrates, suggesting the identity of β -glucosidase and β -galactosidase from this source.

The affinities of the limpet and emulsin enzymes for fuconolactone were greater than for gluconolactone, whereas for the barley enzymes the converse was true. The remarkably high β -D-fucosidase activity of emulsin (see the Experimental section) is worthy of note.

DISCUSSION

Four enzyme preparations were examined, two plant and two animal. In the extract of almond emulsin there appeared to be a single enzyme that hydrolysed β -glucosides, β -galactosides and β -D-fucosides, thus displaying a lack of specificity for both C-4 and C-6 in the substrate. The rat enzyme also was unspecific for C-6, but was specific for C-4, in that the preparation had little action on β -glucosides. Lack of specificity for C-6 in mammalian β -galactosidase is known to extend to α -L-arabinosides (Conchie *et al.* 1967).

The remaining preparations, from the limpet and from barley, were alike in that each contained a clearly distinguishable β -glucosidase and β -galactosidase, indicating that both enzymes were specific for C-4 in the substrate. It was therefore contrary to all expectations to find that in both preparations the β -glucosidase, and not the β -galactosidase, hydro-

lysed β -D-fucosides. This demands a coupled lack of specificity for C-4 and C-6, and introduces a new factor into the interpretation of glycosidase-specificity studies.

The only explanation we can offer for the present findings is one based on steric hindrance in the combination of β -glucosidase with the substrate. If inversion of the configuration at C-4 in glucose leads to interference by the hydroxyl group in galactose, it is possible that the interfering group can be accommodated when the hydroxyl group on C-6 is removed, as in fucose. If one starts by regarding the enzyme primarily as a β -D-fucosidase, the argument is reversed.

On the other hand, results obtained in a few preliminary experiments done in collaboration with Dr R. Begbie are in apparent conflict with this theory. When 4-deoxyglucose (a gift from Dr E. J. Hedgley) was subjected to catalytic oxidation so as to form the aldono-(1 $\rightarrow$ 5)-lactone (Conchie *et al.* 1967), the product caused 40% inhibition of limpet β -galactosidase at 0.22 mM, but had no effect on the

β -glucosidase in the preparation. The lactone solution had no significant effect on either enzyme activity in almond emulsin.

Further evidence was incidentally obtained in these preliminary experiments on the glycosidase-inhibitor reaction. Three (1 $\rightarrow$ 5)-anhydrohexitols (presented by Dr N. K. Richtmyer) were tested as inhibitors of appropriate glycosidases. The mannose and glucose derivatives had no effect on rat-epididymal α -mannosidase and limpet β -glucosidase respectively, and (1 $\rightarrow$ 5)-anhydrogalactitol caused barely significant inhibition of rat-epididymal β -galactosidase (19% at 1.4 mM). It is evident that the C-1 carbonyl group in lactones is necessary for combination with glycosidases, perhaps in the form of the hydrate.

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