Histochemical Demonstration of α -Glucan Phosphorylase Activity in the Human Axillary Apocrine Sweat Gland

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The effect of glycogen and glycogen metabolites on the histochemical reaction of α -glucan phosphorylase was examined. The addition of glycogen, dextrin, amylopectin or dextran with a higher molecular weight to the substrate solution was scarcely effective in shortening incubation time necessary for the appearance of a positive reaction. In contrast, when dextran with a lower molecular weight was introduced into the substrate solution, the incubation time necessary for the appearance of the reaction was shortened. By the use of dextran-containing substrate solution, the activity of α -glucan phosphorylase in the human axillary apocrine sweat gland could be demonstrated.

α -Glucan phosphorylase known to be capable of cleaving the glucosidic bonds of glycogen exists in two different forms, phosphorylase *a* and phosphorylase *b*. Phosphorylase *a* does not require the presence of any co-factor for its activity, whereas phosphorylase *b* is inactive unless 5'-adenylic acid is added. The interconversion of these two forms is the result of a specific phosphatase activity which splits phosphate from phosphoserine groups, and of a specific kinase activity which transfers phosphate from adenosine triphosphate to serine. The former is suppressed by sodium fluoride, and the latter by ethylenediaminetetraacetic acid.

The histochemical method for demonstrating α -glucan phosphorylase was first devised by Yin and Sun,¹ and was placed on a stable footing by the studies of Takeuchi and his co-workers.²⁻⁴ With the method proposed by them, several workers⁵⁻⁹ surveyed the localization and the relative concentration of α -glucan phosphorylase in human skin. It has been shown that the secretory portion of the eccrine sweat gland is rich in α -glucan phosphorylase, while the secretory portion of the apocrine sweat gland has no activity of the enzyme. However, the fact that the apocrine secretory portion rarely contains glycogen in appreciable amounts¹⁰ suggests the presence of α -glucan phosphorylase in it. The present paper is concerned with the histochemical method for the demonstration of α -glucan phosphorylase activity in the human axillary apocrine sweat gland.

MATERIALS AND METHODS

Biopsy skin specimens were obtained from the axillae of healthy males at the age of

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18 to 25 years. As soon as the specimens were excised, they were frozen in small blocks by being immersed in isopentane chilled with liquid nitrogen. Sections were cut at 8μ in a cryostat maintained at -20°C , and were mounted on coverslips. They were dried for 5 minutes in air and incubated at 37°C for predetermined periods in Coplin jars, each of which contained 15 mg of sodium fluoride, 20 mg of ethylenediaminetetraacetic acid, 10 ml of 0.05 M acetate buffer (pH 5.8) and glucose-1-phosphate in a final concentration of 0.1 to 100 mM. At the end of each incubation period some sections were taken out, and after briefly washed in 40% ethanol, they were immersed for 30 minutes in Lugol's iodine-iodide solution, which was diluted 10 times with distilled water. They were dried in air and mounted with glycerin. The observations of the slides thus prepared were carried out by continuous use of the same binocular microscope, and the time when a positive reaction was first discernible was recorded as the end point of α -glucan phosphorylase reaction on a given slide. The control substrate solution contained no glucose-1-phosphate. All the control tests were negative.

The dipotassium salt of glucose-1-phosphate and the sodium salt of 5'-adenylic acid from muscle were obtained from Sigma Chem., Co., St. Louis. Glycogen, amylopectin, dextrin, phlorizin glucose and α -amylase were supplied from Wako Pure Chem. Indust., Osaka. Dextran was obtained from Seikagaku Kyogo Co., Tokyo.

OBSERVATIONS

1) *Effect of glucose-1-phosphate concentration on α -glucan phosphorylase reaction*

In the secretory portion of the eccrine sweat gland, α -glucan phosphorylase reaction appeared in 5 minutes of incubation at a substrate concentration of 25

TABLE 1. *Relation of time of appearance of α -glucan phosphorylase reaction with substrate concentration*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	—	—	+	+	+	+
5	—	—	+	+	+	+
2.5	—	—	—	+	+	+
1.0	—	—	—	—	—	+
0.5	—	—	—	—	—	—
0.25	—	—	—	—	—	—
0.1	—	—	—	—	—	—
Apocrine secretory portion						
100	—	—	—	—	—	—
50	—	—	—	—	—	—
25	—	—	—	—	—	—
10	—	—	—	—	—	—
5	—	—	—	—	—	—
2.5	—	—	—	—	—	—
1.0	—	—	—	—	—	—
0.5	—	—	—	—	—	—
0.25	—	—	—	—	—	—
0.1	—	—	—	—	—	—

TABLE 2. *Relation of time of appearance of α -glucan phosphorylase reaction to substrate concentration in the presence of 5'-adenylic acid in 3 mM*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	+	+	+	+	+	+
5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.0	+	+	+	+	+	+
0.5	-	+	+	+	+	+
0.25	-	-	-	+	+	+
0.1	-	-	-	-	+	+
Apocrine secretory portion						
100	-	-	-	-	-	-
50	-	-	-	-	-	-
25	-	-	-	-	-	-
10	-	-	-	-	-	-
5	-	-	-	-	-	-
2.5	-	-	-	-	-	-
1.0	-	-	-	-	-	-
0.5	-	-	-	-	-	-
0.25	-	-	-	-	-	-
0.1	-	-	-	-	-	-

mM or in 60 minutes at 2.5 mM. In contrast, no reaction was found in the apocrine secretory portion (Table 1).

2) *Effect of 5'-adenylic acid on α -glucan phosphorylase reaction*

The addition of 5'-adenylic acid in a concentration of 3 mM or over was effective for the reaction of the eccrine secretory portion and in shortening incubation time necessary for reaching the reaction end point. However, the apocrine secretory portion showed no reaction even when 5'-adenylic acid was added in 10 mM to the substrate solution series (Table 2).

3) *Effect of glycogen and glycogen metabolites on α -glucan phosphorylase reaction*

Two substrate solution series were employed: (1) Containing 5'-adenylic acid in 3 mM, and (2) 5'-adenylic acid-free series. Glycogen or several glycogen metabolites such as dextrin, dextran and amylopectin, in a final concentration of 1, 10, 50, 100 or 200 mg per ml, were added to the parallel series of substrate solutions.

By the addition of glycogen, dextrin, dextran with an average molecular weight of 2,000,000 or amylopectin, no conspicuous effect was obtained on the incubation time necessary for reaching the reaction end point or on the distribution of the reaction (Tables 3-5, Fig. 1). However, the addition of dextran with

TABLE 3. *Effect of glycogen (50 mg per ml) on α -glucan phosphorylase reaction in the presence of 5'-adenylic acid in 3 mM*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	+	+	+	+	+	+
5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.0	+	+	+	+	+	+
0.5	—	+	+	+	+	+
0.25	—	—	+	+	+	+
0.1	—	—	—	—	+	+
Apocrine secretory portion						
100	—	—	—	—	—	—
50	—	—	—	—	—	—
25	—	—	—	—	—	—
10	—	—	—	—	—	—
5	—	—	—	—	—	—
2.5	—	—	—	—	—	—
1.0	—	—	—	—	—	—
0.5	—	—	—	—	—	—
0.25	—	—	—	—	—	—
0.1	—	—	—	—	—	—

an average molecular weight of 500,000 to 5,000,000 was effective in shortening the incubation time necessary for the appearance of the reaction in the eccrine secretory portion, and made it possible for the reaction to appear in the apocrine secretory portion (Table 6). The most prominent effect was obtained by the addition of dextran with an average molecular weight of 40,000 to 110,000 (Fig. 2).

The above results were obtained in either substrate solution series.

4) Specificity test

In this experiment, the substrate solution was composed of 100 mg of glucose-1-phosphate, 15 mg of 5'-adenylic acid, 15 mg of sodium fluoride, 20 mg of ethylenediaminetetraacetic acid, 1,000 mg of dextran with an average molecular weight of 40,000 and 10 ml of 0.05 M acetate buffer at pH 5.8.

The addition of phlorizin in a final concentration of 10 mM to the substrate solution completely inhibited the reaction in the apocrine and eccrine secretory portions. When glucose was added in 20 mM, a competitive inhibition was observed.

On the other hand, serial sections were incubated in the substrate solution for 90 minutes at 37°C, and then immersed in 0.5% α -amylase solution buffered at pH 5.8 for 60 minutes at 37°C. The reaction product was digested.

TABLE 4. *Effect of dextrin (50 mg per ml) on α -glucan phosphorylase reaction in the presence of 5'-adenylic acid in 3 mM*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	+	+	+	+	+	+
5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.0	+	+	+	+	+	+
0.5	—	+	+	+	+	+
0.25	—	—	—	+	+	+
0.1	—	—	—	—	+	+
Apocrine secretory portion						
100	—	—	—	—	—	—
50	—	—	—	—	—	—
25	—	—	—	—	—	—
10	—	—	—	—	—	—
5	—	—	—	—	—	—
2.5	—	—	—	—	—	—
1.0	—	—	—	—	—	—
0.5	—	—	—	—	—	—
0.25	—	—	—	—	—	—
0.1	—	—	—	—	—	—

Fig. 1. α -Glucan phosphorylase reaction of an apocrine sweat gland from a 21-year-old male. Section was incubated for 90 min at 37°C in the substrate solution, which contained glycogen in 100 mg per ml. No reaction is found in the apocrine secretory portion. $\times 120$.

TABLE 5. *Effect of dextran with an average molecular weight of 2,000,000 (50 mg per ml) on α -glucan phosphorylase reaction in the presence of 5'-adenylic acid in 3 mM*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	+	+	+	+	+	+
5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.0	+	+	+	+	+	+
0.5	-	+	+	+	+	+
0.25	-	-	+	+	+	+
0.1	-	-	-	-	+	+
Apocrine secretory portion						
100	-	-	-	+	+	+
50	-	-	-	-	-	-
25	-	-	-	-	-	-
10	-	-	-	-	-	-
5	-	-	-	-	-	-
2.5	-	-	-	-	-	-
1.0	-	-	-	-	-	-
0.5	-	-	-	-	-	-
0.25	-	-	-	-	-	-
0.1	-	-	-	-	-	-

Fig. 2. α -Glucan phosphorylase reaction of an apocrine sweat gland from a 21-year-old male. Section was incubated for 90 min at 37°C in the substrate solution, which contained dextran with an average molecular weight of 40,000 in 100 mg per ml. The apocrine secretory portion is intensely reactive. $\times 120$.

TABLE 6. *Effect of dextran with an average molecular weight of 40,000 (50 mg per ml) on α -glucan phosphorylase reaction in the presence of 5'-adenylic acid in 3 mM*

Substrate concentration (mM)	Time of incubation (min)					
	5	15	30	60	90	120
Eccrine secretory portion						
100	+	+	+	+	+	+
50	+	+	+	+	+	+
25	+	+	+	+	+	+
10	+	+	+	+	+	+
5	+	+	+	+	+	+
2.5	+	+	+	+	+	+
1.0	+	+	+	+	+	+
0.5	+	+	+	+	+	+
0.25	-	+	+	+	+	+
0.1	-	-	-	-	+	+
Apocrine secretory portion						
100	-	-	+	+	+	+
50	-	-	-	+	+	+
25	-	-	-	+	+	+
10	-	-	-	+	+	+
5	-	-	-	-	+	+
2.5	-	-	-	-	-	-
1.0	-	-	-	-	-	-
0.5	-	-	-	-	-	-
0.25	-	-	-	-	-	-
0.1	-	-	-	-	-	-

DISCUSSION

α -Glucan phosphorylase catalyzes the synthesis or degradation of amylose-type molecules by transglucosylation. The equilibrium constant for this reaction is given by

$$K = \frac{[(C_6H_{10}O_5)_{n+1}][HPO_4^{--}]}{[(C_6H_{10}O_5)_n][Glucose-1-phosphate^{--}]}$$

Since each molecule of polysaccharide is both a reactant and a product, K is determined by the ratio of phosphoric acid to glucose-1-phosphate. When the concentration of phosphoric acid is relatively high in relation to the concentration of glucose-1-phosphate, glycogen is broken down by the enzyme. In contrast, when the concentration of glucose-1-phosphate is relatively high as compared with the concentration of phosphoric acid, the reaction proceeds to glycogen formation. α -Glucan phosphorylase does not act upon glucose-1-phosphate except in the presence of a primer such as glycogen, dextrin, dextran or amylopectin. In other words, the enzyme needs an acceptor to which new glucosidic units can be transferred from glucose-1-phosphate.¹¹ On the basis of these biochemical data, Takeuchi and Kuriaki² devised a method for the histochemical demonstra-

tion of α -glucan phosphorylase activity. Their substrate solution contained glycogen as a primer. Erönkö and Palkama¹² stated that the addition of glycogen to the substrate solution promoted glycogenesis during incubation. With the scintillation method, Meijer¹³ showed that in the histochemical method ¹⁴C-glycogen is not used as a primer, and that the glycogen already present in the tissue serves as a primer. As shown in Tables 3-5, the addition of glycogen, dextrin, dextran with a higher molecular weight or amylopectin was scarcely effective in shortening the incubation time necessary for reaching the reaction end point. In contrast, when dextran with a lower molecular weight was added, the incubation time necessary for reaching the reaction end point was shortened. These results suggest that polysaccharides with a relatively high molecular weight cannot penetrate into tissue sections, and consequently cannot serve as a primer.

On the other hand, when phlorizin or glucose was introduced into the dextran (with an average molecular weight of 40,000)-containing substrate solution, the reaction was completely inhibited. When the control substrate solution without substrate was employed, no positive reaction was found. Accordingly, the present results indicate that the staining in sections obtained by the addition of dextran to the substrate solution is due exclusively to the activity of α -glucan phosphorylase.

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