Communications to the Editor

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A NEW ASSAY FOR α-AMYLASE ISOZYMES USING γ-CYCLODEXTRIN AS SUBSTRATE

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Our previous finding that γ-cyclodextrin is hydrolyzed by human pancreatic α-amylase 3 times more rapidly than by human salivary α-amylase led us to the development of a new differential assay for human α-amylase isozymes. The assay is based on the measurement of α-amylase activity in a sample by the use of two α-amylase assay methods, one of which utilizes γ-cyclodextrin as substrate.

KEYWORDS —— human salivary α-amylase; human pancreatic α-amylase; α-amylase isozymes; γ-cyclodextrin; differential α-amylase assay

Measurements of α-amylase are very important clinically, for example in the diagnosis of pancreatic and salivary diseases. The differential determination of pancreatic and salivary amylases enhances the diagnostic value of serum and urinary amylase assay. The two types of amylase can be separated by electrophoretic,[2] electrofocusing,[3] chromatographic,[4] and radioimmunological[5] methods, but nearly all of them are semiquantitative, insensitive, or time-consuming. A relatively simple method,[6] which is based on the use of a selective inhibitor for salivary amylase and is marketed as the Phadebas IsoAmylase Test kit (Pharmacia Diagnostics), has also been proposed. However, the method has not yet been fully evaluated.

Recently we reported[7] that human pancreatic amylase hydrolyzes γ-cyclodextrin about 3 times more rapidly than human salivary amylase if the activities of the two enzymes were of the same value when assayed by the Nelson method.[8] This finding prompted us to develop a new method for distinguishing between salivary and pancreatic amylases. We briefly report here the principle of this method and the preliminary experiments with partially purified human amylases, but not serum or urine.

Preparations of α-amylase-free glucoamylase (from Aspergillus niger), maltodextrin-free γ-cyclodextrin, and partially purified human salivary and pancreatic amylases were described previously.[7] All assays were performed at 37°C. The amylase activity was standardized by the Nelson method on 0.5% soluble starch as substrate, and 1 unit of activity was the amount that produced 1 μmol reducing group per min at pH 6.9. The amylase assay using γ-cyclodextrin was previously described in detail.[7] This is based on the measurement of glucose produced by hydrolysis of γ-cyclodextrin to maltodextrin with amylase, followed by hydrolysis of the maltodextrin with glucoamylase.

The principle of the present method is as follows: if a linear relationship between the amount of enzyme and the activity holds for each of salivary and pan-

creatic amylases within a limited range of activity when assayed by the Nelson method, the activity (A) of a sample containing both amylases is represented by

$$A = s + p \quad \text{--------(1)},$$

where s and p are the activities of salivary and pancreatic amylases, respectively. This applies to the assay using γ-cyclodextrin. Thus, when the same sample as assayed by the Nelson method is determined by the γ-cyclodextin method, its activity (A') is to be expressed by

$$A' = s' + p' \quad \text{--------(2)},$$

where s' and p' are the activities of salivary and pancreatic amylases. Besides, the following equations are to be given under the conditions specified above.

$$\frac{s'}{s} = a \quad \text{--------(3)} \quad \text{and} \quad \frac{p'}{p} = b \quad \text{--------(4)}$$

The values, a and b, are constants. From the four equations, 1,2,3 and 4, the activities of salivary (s) and pancreatic (p) amylases in the sample are given by

$$s = \frac{A' - bA}{a - b} \text{--------(5)} \quad \text{and} \quad p = \frac{A' - aA}{b - a} \text{--------(6)}$$

We can thus quatitate both amylases by measuring amylase activity in a sample with two different assay methods, one of which utilizes γ-cyclodextrin as substrate.

It was actually found that the Nelson method gives a linear relationship between the amount of each amylase and the activity (less than 70 mU) as shown in Fig. 1. Linearity was tested for the method using γ-cyclodextrin with the same enzyme

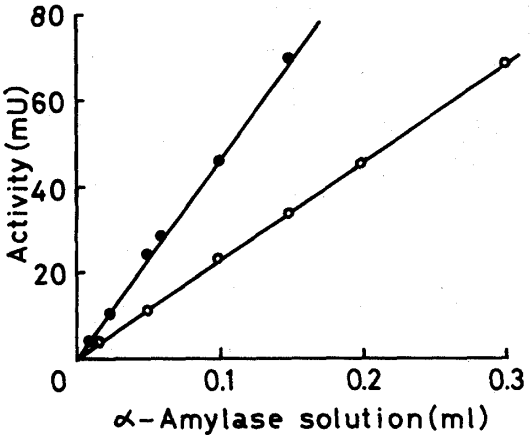

Fig. 1. Relationship between the Amount of α-Amylase and the Activity in the Nelson Method.
The assay mixture consisted of 0.5% soluble starch, 50 mM β-glycerophosphate buffer (pH 6.9) and 5 mM $CaCl_2$. Upon addition of the enzyme the total volume was 1 ml. After incubation for 15 min at 37°C, the reducing potency generated was measured by the Nelson method using D-glucose as standard. ●: salivary α-amylase (470 mU/ml); o: pancreatic α-amylase (230 mU/ml).

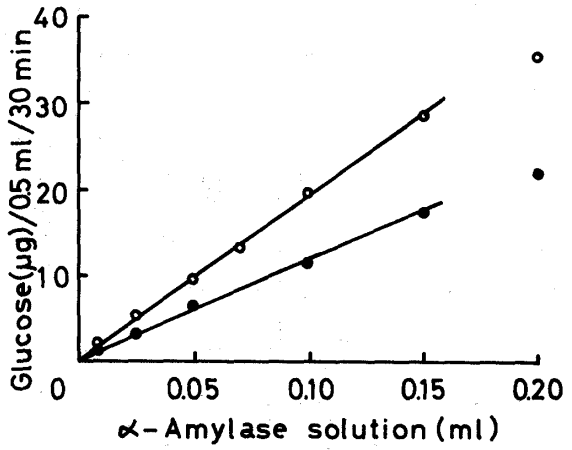

Fig. 2. Relationship between the Amount of α-Amylase and the Rate of D-Glucose Formation in the γ-Cyclodextrin Method.
See the test for the assay method. ●: salivary α-amylase (470 mU/ml); o: pancreatic α-amylase (230 mU/ml).

solutions as used for the Nelson method. A linear relationship was observed for each amylase also in the γ-cyclodextrin method; within a range below 70 mU for salivary amylase and within 34 mU for pancreatic amylase (Fig. 2). From Figs. 1 and 2,

the ratio b/a is calculated to be 3.2, indicating that the hydrolysis of γ-cyclodextrin by pancreatic amylase is 3.2 times more rapid than that by salivary amylase when the activities of both enzymes were standardized by the Nelson method. This value (around 3) of the ratio b/a seems to be apt for the present method based on the principle described above.

The validity of the present differential method for amylase isozymes was tested by assaying the isozymes in mixtures of partially purified human salivary and pancreatic amylases. The mixtures were prepared so as to contain both amylases in various ratios. All of the obtained values were in good agreement with the theoretical values as shown in Fig. 3. This result suggests that the presence of salivary amylase does not interfere with the reactions of pancreatic amylase on both starch and γ-cyclodextrin, and vice versa.

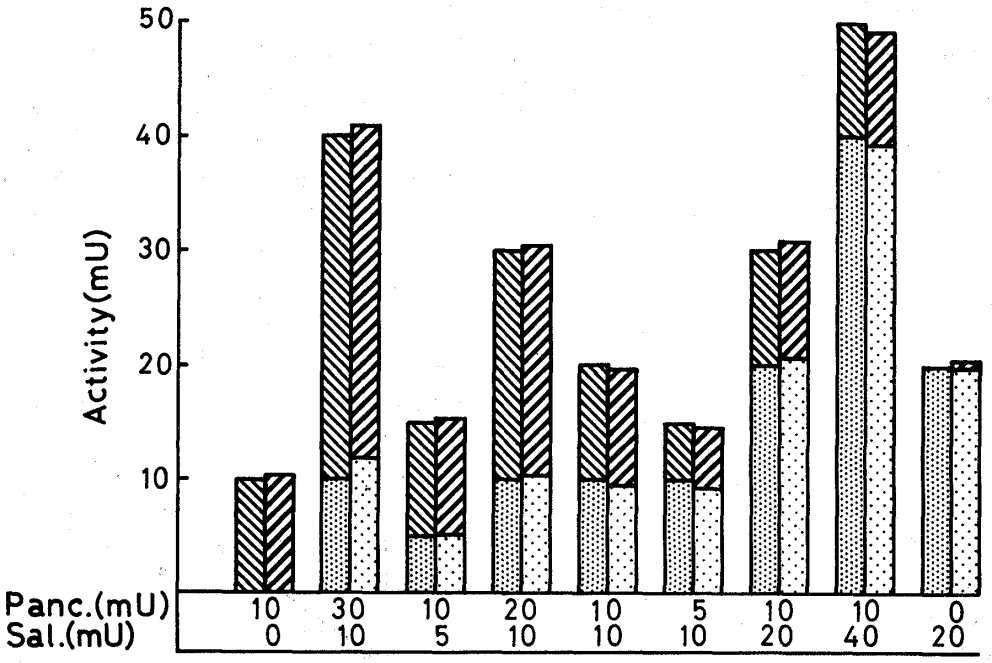

Fig. 3. Differential Assay of α-Amylase Isozymes in Mixtures of partially Purified Salivary and Pancreatic α-Amylases.
Each mixture was assayed for α-amylase activity by both the Nelson and γ-cyclodextrin method, and the amounts of salivary and pancreatic α-amylases were calculated according to equations 5 and 6, respectively. The left coulmn in each pair represents the theoretical value, while the right column shows the observed value. The lower and upper parts of each column represent salivary and pancreatic α-amylase activities, respectively. The amounts of both α-amylases present in mixtures are shown at the bottom of each pair of the columns.

We can not apply the present differential assay of amylase isozymes directly to serum and urine, because both of the two methods used here for determining amylase activity are susceptible to interference by proteins and reducing substances. We are now in the process of developing a differential amylase assay applicable to serum and urine. The principle of this method may possibly hold in isozyme quantitation of enzymes other than amylase.

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