SUBSTRATE SPECIFICITY FOR PANCREATIC AMYLASE

Tadashi TAKEUCHI, M.D., Ph. D., Tadahiko KOZU, M.D., Shinichiro WATANABE, M.D., Masako MORITA, M.D., Keiko SHIRATORI, M.D., and Izumi SHIBATA, M.D.

Departments of Medicine and Gastroenterology, Tokyo Women's Medical School
10, Kawadacho, Shinjuku-ku, Tokyo, Japan

Summary

Substrates commonly used for the determination of amylase activity include potato starch, corn starch and dye-labeled starch. Determination of the amylase activity of serum using these different starches has shown that the measured value varies depending upon the ratio of isoamylases present, namely between pancreatic amylase (P-type) and salivary amylase (S-type), contained in the serum.

With corn starch as substrate, the P-type dominant serum exhibited an apparently higher value than the S-type dominant serum.

In the use of blue-starch* which is employed as a chromogenic method, the P-type dominant serum gave a higher value than the S-type dominant serum. Red-starch** which is also used as a chromogenic method, however, did not cause the P-type dominant serum to show such a high level of amylase activity as blue-starch.

These differences in amylase activity can be also shown by determining the Km values of pancreatic amylase and salivary amylase using these substrates. Thus, corn starch and blue-starch showed smaller Km values to pancreatic amylase than to salivary amylase. They were thus proved to have a strong affinity for pancreatic amylase. In contrast, potato starch, red-starch and glycogen had good affinity for salivary amylase.

In pancreatic disease in which pancreatic amylase is increased without much elevation in the total amylase level in the serum, it might be possible to detect the abnormality of pancreatic amylase activity if either corn starch or blue-starch is used as a substrate for measurement of the serum amylase activity.

Key Words: salivary amylase, pancreatic amylase, dye-labeled starch, Km value.

Introduction

As an enzymatic diagnosis for pancreatic disease, determination of amylase activity has been utilized for more than half a century. Saccharogenic and amyloclastic methods have been most popularly employed in clinical practice. These methods, however, have some drawbacks such as a narrow range of measurement and poor reproducibility.

The chromogenic methods, which have been frequently employed in routine laboratory tests in recent years, are a new approach for measuring amylase activity. In these methods, the substance which is produced by binding a blue or red dye to amylose and/or amylopectin and is made insoluble, is hydrolyzed by the action of amylase, so that the substance containing the dye becomes soluble and

* Blue-starch
** Red-starch
eluated and is then determined spectrophotometrically. The amount of the eluted dye is proportional to the activity of amylase. This offers many advantages such as simplicity, good reproducibility, utilization of substrate in a stable form and a wide range of measurement of amylase activity1,2).

In the present study, starch, dye-labeled starch and glycogen were used as substrates and their affinities to amylase isozymes, i.e. pancreatic amylase and salivary amylase, was investigated. The clinical application of the findings will be described in this paper.

Materials and Methods

Amylase samples: The serum samples were obtained from patients admitted to Tokyo Women’s Medical School Hospital. The total amylase activity was found normal in some cases and abnormal in other cases. As a source of salivary amylase for measurement of the Km value, the saliva of healthy subjects was centrifuged at 3,000 rpm for 5 minutes and the supernatant used. Pancreatic juice, which was collected by intubating endoscopically into the papilla of Vater under stimulation by secretin was used as the enzyme source of pancreatic amylase. These samples were frozen for storage before use.

Amylase assay: The amylase activity of each serum sample was measured using different substrates such as solubilized corn starch (Dade Co., Miami, Florida) and solubilized potato starch (Sigma, St. Louis) in accordance with the Van-Loon Method3, i.e., one of the amylolytic methods. The dinitrosalicylic acid method4) which is a saccharogenic method was used for the measurement of the Km values with corn starch, potato starch and glycogen (Merck Co.) of pancreatic and salivary amylases. For determination of amylase activity in other cases a chromogenic method was employed using blue-starch or red-starch.

Isoamylase separation: For separation of P-type and S-type amylases in serum, electrophoresis was performed on a cellulose acetate membrane using the blue-starch agar-plate method4,5). In this way the amylase in serum is separated into two main peaks of P-type and S-type and each peak can be separately assayed by a densitometer5).

Results

Fig. 1 represents the ratio of the amylase activity which was determined for serum containing S-type and P-type amylases in various proportions by the use of corn starch and potato starch as substrates by the Van-Loon method. Serum containing 90% or more P-type amylase (P-type dominant) showed a higher amylase activity than serum containing 90% or more S-type amylase (S-type dominant) of pancreatic and salivary amylases. For determination of amylase activity in other cases a chromogenic method was employed using blue-starch or red-starch.

Fig. 1. The same test sample was measured with a different substrate, namely corn starch and potato starch, by Van-Loon method, and the ratio of the obtained values is indicated in the above figure. A higher value is obtained for P-type amylase dominant serum when measured by the use of corn starch as a substrate (p<0.01).