performance of blood glucose estimation outside the laboratory. A similar policy should be applied to patient-operated apparatus whenever possible; this can be done when the patient comes to the hospital for periodic visits or by using blood collected on filter paper and mailed in. Personnel and patients should be periodically retrained at intervals depending on their performance.

The technical assistance of Ms. Liliane Crawford is gratefully acknowledged. We thank Ms. Huguette Lachance and Violette Jompe for secretarial assistance. We also express our thanks to Ms. Françoise Ancil for her collaboration.

References


Differential Assay of Salivary and Pancreatic α-Amylase in Serum and Urine, with Use of Monoclonal Antibody to Human Salivary Amylase Immobilized on Bacterial Cell Wall

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We previously reported (Clin Chim Acta 1986;159:89) that bacterial cell wall chemically coated with a monoclonal antibody specific to human salivary (S) amylase (EC 3.2.1.1) could be successfully used to separate S and pancreatic (P) amylase in solution. We have now applied this method to serum and urine samples and found that the activities of S and P amylases so measured correlated well with those measured by the isocarboxylase inhibitor method. The present method is simple and reliable for routine clinical tests.

Additional Keyphrases: isoenzymes • enzyme activity • enzyme inhibition assay compared • pancreatitis • cancer

Differential assay of pancreatic (P) and salivary (S) amylase is important in clinical diagnosis, P amylase being increased in sera of patients with acute pancreatitis, S amylase in sera of patients with parotitis or some cancers. Therefore, several methods for differential assay of amylases have been developed based on electrophoresis (1–5), column chromatography (6, 7), radioimmunoassay (8–10), and amylase inhibitor from wheat germ (11–13). All these involve some problems. Electrophoresis and chromatography are time consuming and are inappropriate for prompt assay of many samples. The radioimmunoassay (10) has restrictions for practical use and does not separate the two isoenzymes completely. The amylase inhibitor method (11–13) is easy, but the inhibitor does not totally differentiate the isoenzymes, S amylase and P amylase being inhibited by 80–83% and 12–20%, respectively. Mifflin et al. (14) also have recently described a method for differential assay of the amylases with use of a monoclonal antibody immobilized on poly(vinylidene fluoride), and they showed its good differentiating ability.

In a previous study, we reported establishing a hybridoma that produced a monoclonal antibody specifically reactive with S amylase, but not with P amylase (15). The antibody, immobilized on bacterial cell wall, showed excellent differentiation of S and P amylase isoenzymes. Here we describe further applications of the method to serum or urine from patients or normal subjects. Values for amylase activity as measured by this method are compared with those measured by the amylase inhibitor method.

Materials and Methods

Serum (n = 78) and urine (n = 25) specimens were collected in Kohka Hospital.

Immobilized monoclonal antibody was prepared as described previously (15) and used for separating the amylase isoenzymes in these samples as follows: Incubate 40 μL of amylase solution with 20 μL of monoclonal antibody preparation for 5 min at 37 °C and centrifuge for 1 min at 1000 × g. Then add 20 μL more antibody preparation and re-centrifuge. Remove 20 μL of the supernate to another test tube and incubate with 1 mL of amylase substrate solution according to the directions for the amylase assay kit (Hoechst Japan, Tokyo, Japan).

For differential assay of the amylases by the amylase inhibitor method, we used Phadebas Amylase Inhibitor A

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Results and Discussion

We determined P and S amylase activities in 78 sera by the present method and the amylase inhibitor method, and calculated the P amylase ratio. Results by the present method (y) correlated well with those by the amylase inhibitor method (x), both for amylase activities and P amylase ratio: \( r = 0.999 \) for P amylase activity (y = 1.04x - 6.91 U/L, range 0-8000 U/L, \( S_y = 27.5 \)) ; \( r = 0.989 \) for S amylase activity (y = 0.984x + 2.90 U/L, range 0-2100 U/L, \( S_y = 45.9 \)); and \( r = 0.977 \) for P amylase ratio (y = 0.987x + 1.415%, range 5-95%, \( S_y = 5.32 \)). Results for urine samples also correlated well by the two methods: \( r = 0.971 \) for P amylase activity, \( r = 0.998 \) for S amylase activity, and \( r = 0.989 \) for P amylase ratio.

The within-run CVs for our method were low for all three sera, but the CVs were comparatively high for day-to-day reproducibility for sera with low enzyme activities (Table 1).

The double-diffusion technique and enzyme immunoassay showed that the immunoglobulin subclass of MA19A was IgG2 and the light chain of the antibody was \( \kappa \).

Monoclonal antibodies recognizing the different isoamylases have been developed by Ito et al. (16) and Mifflin et al. (14). The former showed that S amylase antibody to the bacterial immobilized on polystyrene spheres was active by enzymoimmunoassay but an analysis for resolution of the two types of amylase was not yet done when the serum contained both, nor was the usefulness of their method for clinical diagnosis demonstrated. The latter group (14), however, showed that antibody immobilized on poly(vinylidene fluoride) carrier could separate P amylase from the solution containing two types of amylase and might be useful for clinical diagnosis.

Our monoclonal antibody bound to bacterial cell-wall material also removed S amylase almost completely from solutions containing two types of amylase without diminishing P amylase activity (15). Moreover, as shown here, results by the present method correlated well (\( r = 0.999 \)) with those by the conventional amylase inhibitor method. In comparison, the coefficient of correlation between P amylase activity as determined by the method of Mifflin et al. and by conventional electrophoresis was 0.946 or 0.990; that between amylase inhibitor method and electrophoresis, obtained by Okabe et al. (13), was \( r = 0.993 \).

Table 1. Reproducibility of Immobilized Monoclonal Antibody Method

<table>
<thead>
<tr>
<th>Serum 1</th>
<th>Within run</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
<th>Day to day</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
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<tbody>
<tr>
<td>Total</td>
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<td>233</td>
<td>3.5</td>
<td>1.3</td>
<td>229</td>
<td>12</td>
<td>5.6</td>
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<tr>
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<td></td>
<td>221</td>
<td>1.5</td>
<td>1.5</td>
<td>209</td>
<td>12</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>12</td>
<td>2.2</td>
<td>3.2</td>
<td>15</td>
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<td>P ratio, %</td>
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<td>3.3</td>
<td>1.3</td>
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<td>2.4</td>
<td>10</td>
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<tr>
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<tr>
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<td>10</td>
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<td>1.9</td>
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References