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Enzymes and Protein Markers

# Total and Pancreatic Amylase Measured with 2-Chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosylmaltoside

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***Background:*** **Many different methods have been used to assay amylase activity, using nitrophenylated oligosaccharides as substrate; however, the hydrolysis steps in these methods are complex.**

***Methods:*** **We developed a new continuously monitoring assay for amylase activity in biological fluids, using 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosylmaltoside (GalG2CNP) as the substrate; this assay was used with anti-human salivary amylase monoclonal antibodies for specific determination of the pancreatic isoenzyme. Amylase converted GalG2CNP into β-D-galactopyranosylmaltose and 2-chloro-4-nitrophenol, which was measured at 405 nm.**

***Results:*** **GalG2CNP was cleaved between 2-chloro-4-nitrophenol and β-D-galactopyranosylmaltose and did not undergo transfer reactions. The within-assay CVs (n = 20) for total amylase (T-AMY) and pancreatic amylase (P-AMY) were 0.6–1.6% and 0.5–2.5%, respectively; and day-to-day CVs (n = 10) for T-AMY and P-AMY were 0.8–3.7% and 0.6–4.1%, respectively. T-AMY and P-AMY activities in serum or urine obtained by the proposed method correlated well with those determined by the 2-chloro-4-nitrophenyl 4-*O*-β-D-galactopyranosyl-β-maltotetraoside method or the modified IFCC method.**

***Conclusions:*** **This novel assay for T-AMY and P-AMY measures both activities stoichiometrically, directly, and easily, and may be suitable for routine procedures.**



Assaying the activity of α-amylase (1,4-α-D-glucan-4-glucanohydrolase; EC 3.2.1.1), especially that of pancreatic amylase, in human serum is important for accurate diagnosis of pancreatic disorders. Methods using various oligosaccharides covalently bound to 2-chloro-4-nitrophenol (CNP)[3] or 4-nitrophenol as substrate and auxiliary enzymes such as α-glucosidase or glucoamylase for the measurement of total (T-AMY) and pancreatic (P-AMY) amylase activities have been investigated *(1–6)*. Most of the substrates need a bulky modification of the glucose residue at the nonreducing end to prevent substrate degradation by the auxiliary enzymes. The main problem with many of these methods is multiple hydrolysis. Recently, methods using 2-chloro-4-nitrophenyl-α-D-maltotrioside (G3CNP) as a substrate have been developed *(7–10)*; in these methods, the chromophore is released without auxiliary enzymes. However, partial polymerization of liberated glucose with the substrate complicates the reaction *(8)*.

A currently available immunoinhibition method that uses specific monoclonal antibodies *(11–13)* against salivary amylase (S-AMY) shows no cross-reactivity with P-AMY. Thus, we used 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosylmaltoside (GalG2CNP; Fig. 1), which has D-galactose at the nonreducing end of the D-glucosyl group, as the substrate and two anti-human S-AMY monoclonal antibodies, Tu66C7 and Tu88E8, for the isoenzyme determination. GalG2CNP requires no auxiliary enzymes and is cleaved only at the aglycone bond by amylase. Transfer of glucose to GalG2CNP does not

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[3] Nonstandard abbreviations: CNP, 2-chloro-4-nitrophenol; S-, P-, and T-AMY, human salivary, pancreatic, and total amylase; G3CNP, 2-chloro-4-nitrophenyl-α-D-maltotrioside; GalG2CNP, 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosylmaltoside; G2CNP, 2-chloro-4-nitrophenyl-α-D-maltoside; G1CNP, 2-chloro-4-nitrophenyl-α-D-glucoside; and GalG4CNP, 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosyl-β-maltotetraoside.

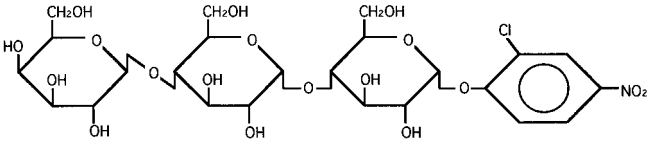

Fig. 1. Structural formula of GalG2CNP.

occur; therefore, the hydrolysis of the substrate proceeds stoichiometrically.

We describe here the measurement of total and pancreatic amylase activity. This proposed method has also been evaluated and compared with other widely established methods. The proposed method is based on the reaction:

$$\text{GalG2CNP} + \text{H}_2\text{O} \xrightarrow{\text{amylase}} \text{GalG2} + \text{CNP}$$

where GalG2 is β-D-galactopyranosylmaltose. The rate of CNP release is followed at 405 nm. P-AMY activity is measured by the same method as T-AMY after S-AMY activity is inhibited by anti-human S-AMY monoclonal antibodies.

## Materials and Methods

### APPARATUS

The proposed method and the conventional methods were performed with a Hitachi 7170 automated analyzer. Each aglycone fragment generated from GalG2CNP by amylase hydrolysis was examined with a Shimadzu SPD-M6A HPLC equipped with a Tosoh TSKgel ODS-120T (4.5 × 250 mm) column.

### CHEMICALS

GalG2CNP was purchased from Yoshitomi Fine Chemicals. NaCl, $CaCl_2$, and KSCN were from Nacalai Tesque, and MES buffer was from Dojindo Labs. G3CNP was from Oriental Yeast, and 2-chloro-4-nitrophenyl-α-D-maltoside (G2CNP) and 2-chloro-4-nitrophenyl-α-D-glucoside (G1CNP) were from Toyobo. Anti-human S-AMY monoclonal antibodies Tu88E8 and Tu66C7 were purchased from Nippon Roche. Calibzyme AMY [P] and [S] came from International Reagents. L-Ascorbic acid, D-glucose, sucrose, and α-D-fructose were purchased from Wako Pure Chemical Industries; bilirubin was from Sigma; and Intra-lipid 10% was from Kabi Vitrum.

### REACTION REAGENTS, ASSAY PROCEDURE, AND CALCULATION

Reagent 1 (R1-TAMY and R1-PAMY) and reagent 2 for assaying T- and P-AMY activities are given in Table 1 *(11–13)*. The assay protocol is shown in Table 2. The activities obtained from R1-TAMY are the T-AMY activity (*T*). The catalytic concentrations after the antibody reaction using R1-PAMY, which contained anti-human S-AMY antibodies, indicate the residual amylase activity (*R*), i.e., 100% of P-AMY and 5% of S-AMY activity because 95% of pure human salivary amylase (Calibzyme AMY [S]) activity is inhibited and pure human pancreatic amylase (Calibzyme AMY [P]) is not. Therefore, P-AMY activity is calculated by using the equations: P-AMY activity = $[R - (0.05 \times T)]/0.95$; and S-AMY = T-AMY − P-AMY.

### CHROMATOGRAPHIC ANALYSIS

Calibzyme AMY [P] or [S] (0.03 U) was added to 1.0 mL of reagent solution (750 μL of R1-TAMY + 250 μL of reagent 2), and the mixture was incubated for 0, 15, 30, 60, 120, 180, or 360 min at 37 °C. Ten microliters of the mixture was then injected onto the HPLC column; each sample was eluted with methanol-acetic acid-water (30:1:69, by volume) at a flow rate of 1 mL/min, and monitored at 280 nm. Each peak on the chromatogram was identified by comparison with reference materials.

### COMPARISON METHOD

We also measured the activities of T-AMY and P-AMY in patients' sera and urines by 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosyl-β-maltotetraoside (GalG4CNP) methods [Ref. *(2)*; Diacolor Liquid AMY kit and Diacolor Liquid P-AMY kit from Toyobo] and the modified IFCC method *(6)*, which uses 4, 6-ethylidene-4-nitrophenyl-α-D-maltoheptaoside (Liquitec AMY EPS kit and Liquitec P-AMY EPS kit from Nippon Roche).

**Table 1. Reagents and final composition of the reaction mixture.**

| | R1-TAMY[a] | R1-PAMY[a] | R2[b] | Final composition |
|---|---|---|---|---|
| MES buffer | 50 mmol/L | 50 mmol/L | 50 mmol/L | 50 mmol/L |
| pH | 6.0 (37 °C) | 6.0 (37 °C) | 6.0 (37 °C) | 6.0 (37 °C) |
| KSCN | 200 mmol/L | 200 mmol/L | | 150 mmol/L |
| $CaCl_2$ | 6.7 mmol/L | 6.7 mmol/L | | 5.0 mmol/L |
| NaCl | 400 mmol/L | 400 mmol/L | | 300 mmol/L |
| Tu88E8 | | 15.0 mg/L | | 11.3 mg/L |
| Tu66C7 | | 2.0 mg/L | | 1.5 mg/L |
| GalG2CNP | | | 20.0 mmol/L | 5.0 mmol/L |
| Volume fraction of sample | | | | 0.0164 |

[a] R1-TAMY and R1-PAMY are reagent 1 for T-AMY and P-AMY, respectively.
[b] Reagent 2.

**Table 2. Protocol of the assay for T- and P-AMY activities on the Hitachi 7170 automated analyzer.[a]**

| | T-AMY[b] | Residual-AMY[b] |
|---|---|---|
| Analysis mode | Rate A | Rate A |
| Wavelength (sub/main) | 700/405 | 700/405 |
| pH | 6.0 | 6.0 |
| Temperature | 37 °C | 37 °C |
| Serum (urine) volume | 4 μL | 4 μL |
| R1-TAMY volume | 180 μL | |
| R1-PAMY volume | | 180 μL |
| Incubation time after reagent 1 added | 5.0 min | 5.0 min |
| Reagent 2 volume | 60 μL | 60 μL |
| Incubation time after reagent 2 added | 5.0 min | 5.0 min |
| Measuring interval after start (measurement point) | 7.35–10.0 min (25–34 points) | 7.35–10.0 min (25–34 points) |
| K-factor | 4552 | 4552 |

[a] Amylase activity was calculated by the equation: activity (U/L) = $[(\Delta A/\text{min}) \times V]/(\varepsilon \times l \times v) = \Delta A/\text{min} \times 4552$ [$\varepsilon = 13.4 \times 10^6$ cm$^2$/mol at pH 6.0 (37 °C); $l$ = light pathlength (0.01 m); $V = 244$ μL; $v = 4$ μL].

[b] Activities obtained from R1-PAMY or R1-TAMY, with and without antibodies, are the residual-AMY and T-AMY activities, respectively.

### SPECIMENS

Serum specimens were collected from patients in Nagoya University Hospital. We obtained informed consent from patients for this study.

## Results

### PRODUCT ANALYSIS BY HPLC

The release of CNP from GalG2CNP hydrolyzed by P-AMY over 6 h is shown in Fig. 2. The rate of increase of CNP corresponded to the decrease of GalG2CNP; the other aglycone fragments (G2CNP and G1CNP) did not appear. Polymerized products of GalG2CNP produced by the enzyme-catalyzed hydrolytic cleavage were not observed. The products from S-AMY-hydrolyzed GalG2CNP were similar to those of P-AMY.

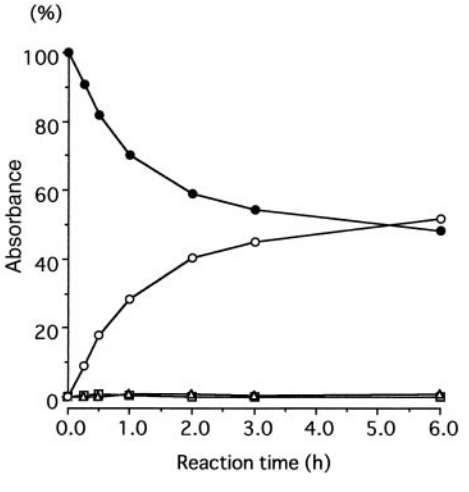


Fig. 2. Time course of the release of aglycone fragments from GalG2CNP by P-AMY.

The absorbances (%) at 280 nm of aglycone fragments were examined. ●, GalG2CNP; □, G2CNP; △, G1CNP; ○, CNP.

### OPTIMIZATION STUDIES

Two pooled human sera with T-AMY activities of 95 and 356 U/L, respectively, and Calibzyme AMY [P] (P-AMY, 258 U/L), [S] (S-AMY, 212 U/L) were used for the optimization studies of this proposed method.

*Effect of pH.* The effects of pH on T-AMY activity were examined in 50 mmol/L MES buffer at various pH values (Fig. 3A). The maximum activity of T-AMY was observed at pH 5.50, decreasing at pH 6.0 or above. The reaction of S-AMY with anti S-AMY antibody was inhibited at a pH <6.0; therefore, we chose MES buffer, pH 6.0.

*Effect of KSCN.* The T-AMY activities of specimens increased with increasing concentrations of KSCN, almost reaching the maximum at 150 mmol/L or above (Fig. 3B),

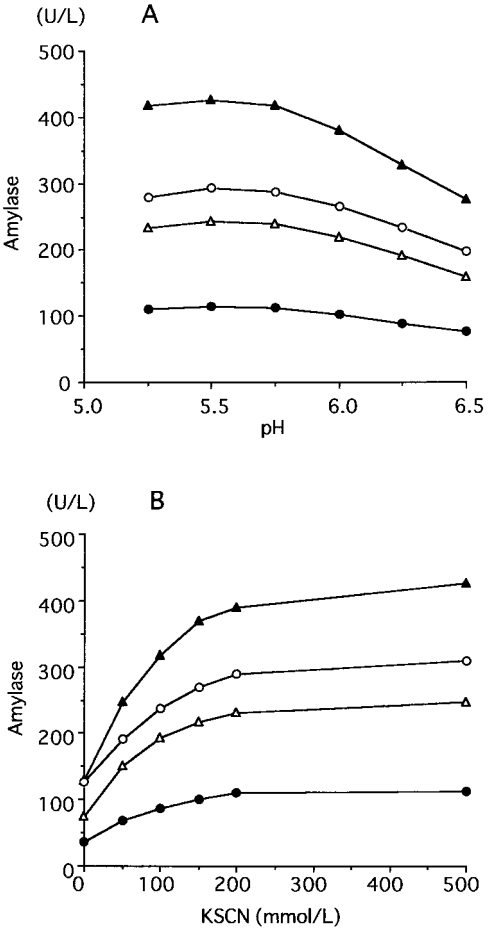


Fig. 3. Effects of pH (at 37 °C; *A*) and KSCN concentration (*B*) on T-AMY activity.

Two pooled human sera (▲, 356 U/L T-AMY; ●, 95 U/L T-AMY), Calibzyme AMY [P] (○; 258 U/L P-AMY), and Calibzyme AMY [S] (△; 212 U/L S-AMY) were used. (*A*), 150 mmol/L KSCN, 300 mmol/L NaCl, 5.0 mmol/L $CaCl_2$, 5.0 mmol/L GalG2CNP. (*B*), pH 6.0 (37 °C), 300 mmol/L NaCl, 5.0 mmol/L $CaCl_2$, 5.0 mmol/L GalG2CNP.

whereas the reactivity of anti-S-AMY antibody decreased with increasing KSCN concentrations: 95.4%, 94.4%, 89.0%, and 77.8% of S-AMY activity was inhibited at 150, 300, 600, and 900 mmol/L KSCN, respectively. Therefore, we chose 150 mmol/L KSCN.

*Effects of $CaCl_2$, NaCl, and GalG2CNP.* We examined the effects of 0.5–10.0 mmol/L $CaCl_2$ and 50–500 mmol/L NaCl, as activators, and 2.4–12.0 mmol/L GalG2CNP on T-AMY activity. The T-AMY activities of the specimens increased with increasing concentrations of $CaCl_2$, NaCl, and GalG2CNP. We chose 5.0 mmol/L $CaCl_2$, 300 mmol/L NaCl, and 5.0 mmol/L GalG2CNP to obtain the maximum T-AMY activity.

*Effect of anti-S-AMY antibody.* S-AMY activity decreased with increasing concentrations of Tu88E8 and Tu66C7 antibodies, whereas the P-AMY activity was not affected (Fig. 4). We added 15.0 mg/L Tu88E8 antibody and 2.0 mg/L Tu66C7 antibody to reagent 1 for P-AMY measurement because S-AMY was inhibited ≥95% at these concentrations.

*Measurement interval.* After reagent 2 was added, the reaction was linear with time for sera, whereas the reagent blank did not increase (Fig. 5). We chose the measurement interval from 7.35 to 10.0 min after start.

### ASSAY EVALUATION

*Precision.* The within-run and day-to-day CVs of the proposed assay were established with sera of low (serum L; 38.2 U/L T-AMY, 24.7 U/L P-AMY), middle (serum M; 99.7 U/L T-AMY, 56.0 U/L P-AMY), and high (serum H; 398.0 U/L T-AMY, 173.2 U/L P-AMY) amylase concentrations. The data (Table 3) showed excellent reproducibility.

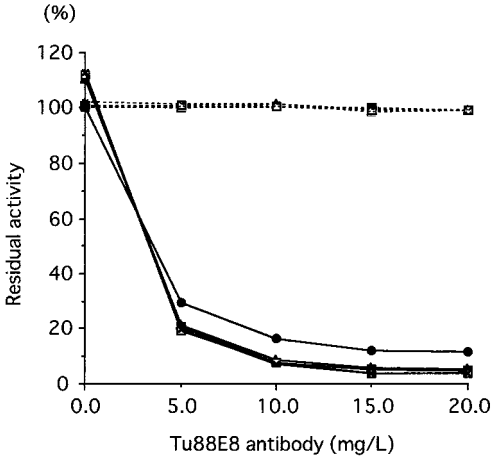


Fig. 4. Effect of various concentrations of monoclonal S-AMY antibodies Tu88E8 and Tu66C7 on P-AMY (- - - -) or S-AMY (——) activities.

Various concentrations of Tu66C7 antibody (●, 0 mg/L; ▲, 1.0 mg/L; □, 2.0 mg/L; △, 3.0 mg/L; ×, 5.0 mg/L) were added to reagent 1, which contained various concentrations of Tu88E8 antibody (0–20.0 mg/L). Residual activities (%) of S-AMY or P-AMY were calculated for Calibzyme AMY[P] (258 U/L P-AMY) or Calibzyme AMY[S] (212 U/L S-AMY), respectively.

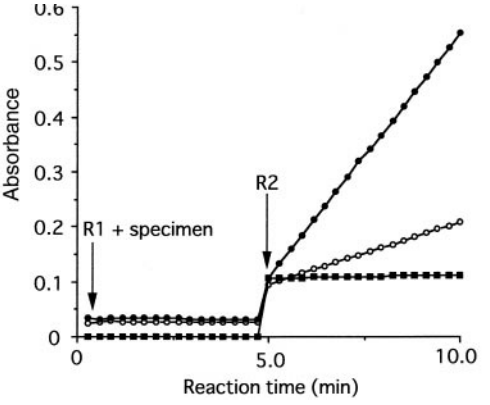


Fig. 5. Time course of the reaction.

Two patients' sera [T-AMY, 397 U/L (●) or 101 U/L (○)] and 154 mmol/L NaCl (■; reagent blank) were used. *R1*, reagent 1; *R2*, reagent 2. *Arrows* indicate the addition of the reagents.

*Analytical recovery.* Pooled human serum (55 U/L T-AMY, 28 U/L P-AMY) supplemented with 107, 215, 308, or 412 U/L Calibzyme AMY [P] and 87, 175, 253, or 338 U/L Calibzyme AMY [S] showed recoveries of 95.3–99.2% (mean, 96.7%) and 94.2–99.3% (mean, 97.3%) for T-AMY. The respective recoveries for the P-AMY assay supplemented with AMY [P] were 96.5–101.2% (mean, 98.8%).

*Linearity.* The T-AMY assay was linear up to at least 1100 U/L for serum.

*Critical limit.* We examined the critical limit of this T-AMY assay by assaying 154 mmol/L NaCl 10 times. The result

**Table 3. Precision of automated T-AMY and P-AMY assays.**

| | Pooled serum-L | Pooled serum-M | Pooled serum-H |
|---|---|---|---|
| T-AMY assay | | | |
| Within-run (n = 20) | | | |
| Mean, U/L | 38.0 | 99.0 | 397.8 |
| SD, U/L | 0.61 | 0.75 | 2.25 |
| CV, % | 1.6 | 0.76 | 0.57 |
| Day-to-day (n = 10) | | | |
| Mean, U/L | 38.2 | 99.7 | 398.0 |
| SD, U/L | 1.40 | 0.95 | 3.20 |
| CV, % | 3.7 | 0.95 | 0.80 |
| P-AMY assay | | | |
| Within-run (n = 20) | | | |
| Mean, U/L | 23.9 | 54.2 | 168.9 |
| SD, U/L | 0.61 | 0.76 | 0.91 |
| CV, % | 2.5 | 1.4 | 0.54 |
| Day-to-day (n = 10) | | | |
| Mean, U/L | 24.7 | 56.0 | 173.2 |
| SD, U/L | 1.00 | 1.94 | 4.29 |
| CV, % | 4.1 | 3.5 | 0.58 |

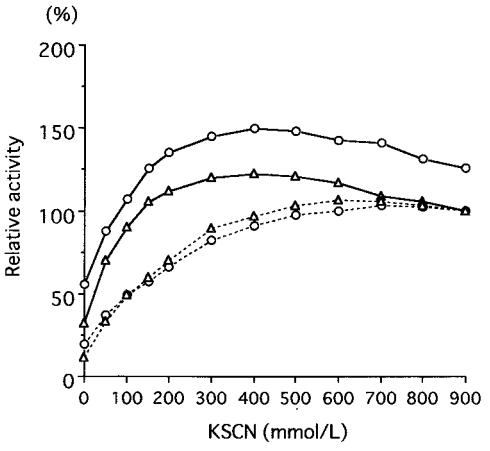


Fig. 6. Comparison of reactivities of GalG2CNP and G3CNP.

Amylase activities of Calibzyme AMY[P] (○; 258 U/L) and Calibzyme AMY[S] (△; 212 U/L) were assayed using 5.0 mmol/L GalG2CNP (——) or G3CNP (- - -) at 0–900 mmol/L KSCN [in 300 mmol/L NaCl, 5.0 mmol/L $CaCl_2$, 50 mmol/L MES, pH 6.0 (37 °C)]. Activities of P-AMY and S-AMY are shown as relative amylase activity (%) compared with P-AMY and S-AMY activities at 900 mmol/L KSCN using G3CNP, which were assigned as 100%, respectively.

was 0.40 ± 0.516 U/L (mean ± SD). The critical limit (mean + 3 SD) was 1.95 U/L.

*Interferences.* Various substances were examined for their potential effects on the T-AMY and P-AMY determination. One volume of each examined substance was mixed with nine volumes of pooled human serum (116 U/L T-AMY, 84 U/L P-AMY). We found no interference with T-AMY and P-AMY activities from bilirubin up to 0.342 mmol/L, ascorbic acid up to 1.14 mmol/L, Intra-lipid up to 0.2%, hemoglobin up to 4.0 g/L, glucose up to 10 g/L, fructose up to 1.0 g/L, and sucrose up to 1.0 g/L.

*Stability of reagents.* Reagent 1 (R1-TAMY and R1-PAMY) and reagent 2 were examined with two human pooled sera (T-AMY, 250 and 115 U/L; P-AMY, 182 and 70 U/L, respectively). Identical activities and constant reagent blanks were observed after storage for 3 months at 4 °C.

*Comparison of reactivities of GalG2CNP and G3CNP.* The amylase activity using GalG2CNP or G3CNP was compared at 0–900 mmol/L KSCN with 258 U/L Calibzyme AMY [P] and 212 U/L Calibzyme AMY [S]. Good reactivity of GalG2CNP was observed; at 0–900 mmol/L KSCN, the P-AMY and S-AMY activities obtained using GalG2CNP were higher than those using G3CNP (Fig. 6).

*Sensitivity.* The sensitivity of this T-AMY assay, although less optimal, was good, with a mean sensitivity comparable to that of the other methods: the changes in absorbance ($\Delta A$/min) for this method, the GalG4CNP method, and the modified IFCC method at an arbitrary amylase activity of 100 U/L were 0.022, 0.040, and 0.013 at uniform sample volume ratios and light pathlengths.

*Correlation.* The correlation between the proposed method and the conventional methods was examined. The T- and P-AMY activities in patients' sera and urines obtained by the proposed method correlated well with those determined by the GalG4CNP method or the modified IFCC method (Table 4).

## Discussion

Different methods have been reported for the assay of amylase activity using maltooligosaccharides of defined chain length coupled to a chromophore as substrates; however, the hydrolysis process frequently is complex *(1, 3–6)*. We developed a new continuous monitoring assay for measuring amylase activity in biological fluids with GalG2CNP as the substrate, which requires no auxiliary enzymes to release the chromophore and can be used with anti-human S-AMY monoclonal antibodies for the determination of the pancreatic isoenzyme. This new substrate, GalG2CNP, was cleaved into only two degradation products, β-D-galactopyranosylmaltose and CNP; other fragments were not produced. This is similar to G3CNP *(7–10)*, which had been considered recently as a substrate for the method by the IFCC *(10)* but was not adopted. G3CNP is polymerized to GnCNP (n >3) by a

**Table 4. Correlation[a] between the proposed method (*y*) and the conventional methods (*x*).**

| Comparison method (*x*) | Specimen | Linear regression equation | $\gamma$ | $S_{y\|x}$ | Mean | Range | n |
|---|---|---|---|---|---|---|---|
| Assay of T-AMY activity | | | | | | | |
| Diacolor Liquid AMY kit[b] | Serum | $y = 0.649x + 2.1$ | 0.999 | 5.4 | 183.8 | 11–782 | 100 |
| Diacolor Liquid AMY kit[b] | Urine | $y = 0.642x + 1.0$ | 0.998 | 11.5 | 369.7 | 36–1600 | 80 |
| Liquitec AMY EPS kit | Serum | $y = 1.254x + 4.3$ | 0.998 | 7.2 | 93.4 | 4–411 | 100 |
| Liquitec AMY EPS kit | Urine | $y = 1.282x + 1.9$ | 0.997 | 16.0 | 184.4 | 17–800 | 80 |
| Assay of P-AMY activity | | | | | | | |
| Diacolor Liquid P-AMY kit[b] | Serum | $y = 0.620x + 1.9$ | 0.997 | 4.4 | 98.6 | 2–448 | 100 |
| Diacolor Liquid P-AMY kit[b] | Urine | $y = 0.622x - 2.3$ | 0.997 | 9.8 | 254.1 | 21–945 | 80 |
| Liquitec P-AMY EPS kit[c] | Serum | $y = 1.366x + 2.5$ | 0.996 | 5.5 | 46.9 | 1–206 | 100 |
| Liquitec P-AMY EPS kit[c] | Urine | $y = 1.360x + 1.4$ | 0.997 | 10.1 | 111.0 | 7–435 | 80 |

[a] Intermethod correlations and regression equations were calculated by least-squares method.
[b] GalG4CNP method.
[c] Modified IFCC method (4,6-ethylidene-4-nitrophenyl-α-D-maltoheptaoside method).

transfer reaction of the glucose residue liberated from G3CNP by amylase to the nonreducing end of G3CNP *(8)*. However, GalG2CNP is resistant to transglycosylation because the 4-position of the nonreducing end is modified by a D-galactose. Therefore, the amylase reaction using GalG2CNP proceeds stoichiometrically and more cleanly than that of G3CNP. On the other hand, maximum amylase activity is almost reached at KSCN concentrations ≥150 mmol/L in this method, which exceeded the amylase activity toward G3CNP, the substrate proposed by the IFCC *(10)*, at 900 mmol/L KSCN. Because the inhibitory effect of S-AMY antibodies Tu88E8 and Tu66C7 was impaired by KSCN concentrations >300 mmol/L, the GalG2CNP test is more suitable for the measurement of P-AMY than G3CNP.

We investigated the optimized conditions for assaying both T-AMY and P-AMY; however, the conditions for maximal possible release of CNP were not obtained because of the mutual dependence of pH, KSCN concentration, and antibody reaction in promoting reaction velocity. Thus, we determined the optimized conditions for the P-AMY assay with monoclonal antibodies. However, this method is also suitable for the assay of T-AMY, although less optimally, because the sensitivity of this assay is not inferior to those of other methods, such as the GalG4CNP method and modified IFCC method. Furthermore, GalG2CNP exhibited sufficient affinity and almost equal reactivity to P-AMY and S-AMY because the Michaelis-Menten ($K_m$) values for P-AMY and S-AMY were 0.86 and 0.64 mmol/L, respectively (not shown), and these $K_m$ values are similar to those for G3CNP [0.35 and 1.01 mmol/L *(7)*], 3-ketobutylidene-β-2-chloro-4-nitrophenyl-maltopentaoside [0.318 and 0.377 mmol/L *(1)*], and GalG4CNP [0.173 and 0.216 mmol/L *(2)*].

This method, which does not need auxiliary enzymes, will also lower reagent costs in contrast to conventional methods that incorporate an auxiliary enzyme. Good precision, reasonable analytical recovery, good linearity and critical limit, and good correlation with conventional methods (modified IFCC method and GalG4CNP method) were obtained with this method; in addition, various substances did not interfere with this method. This novel method for the assay of T-AMY and P-AMY activities in serum or urine can be performed stoichiometrically, directly, and easily on the Hitachi 7170 automated analyzer, which is available for routine procedures in clinical diagnosis.

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