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Determination of α-Amylase Activity: Methods Comparison and Commutability Study of Several Control Materials

Gemma Gubern,[1] Francesca Canalias, and F. Javier Gella

Six different methods for α-amylase determination were compared by assaying human serum samples covering a wide range of α-amylase values. All the methods studied use as substrate a maltooligosaccharide with a chromophore group at the reducing end; some are chemically blocked at the nonreducing end. Intermethod comparison by regression and correspondence analyses showed significant differences for two methods. The commutability of 12 commercial control materials containing α-amylase was also assessed by the different methods in comparison with human serum specimens containing the pancreatic and salivary isoenzymes. We also studied the behavior of pancreatic and salivary materials prepared in our laboratory. Control materials with α-amylase of nonhuman origin were not commutable with the enzyme in human sera and should not be used for intermethod calibration.

Indexing Terms: *intermethod comparison/isoamylases/calibration/synthetic substrates*

α-Amylase (EC 3.2.1.1) is probably the enzyme with the most published methods for assay of its catalytic activity (*1*). Early procedures used as substrate starch, amylose, amylopectin, or some chemically modified derivatives of polymers. More recently, maltooligosaccharides of defined chain length (3 to 7 glucosyl units) have been introduced as substrates for α-amylase, and especially those having a 4-nitrophenyl or 2-chloro-4-nitrophenyl group attached to the reducing end of the chain are currently in use. In these methods, α-amylase splits the substrate into fragments, which are in turn hydrolyzed by the auxiliary enzyme α-glucosidase (EC 3.2.1.20) to smaller fragments, glucose, and free chromophore. A further improvement in methodology for determining α-amylase was the introduction of 4-nitrophenyl oligosaccharides chemically blocked at the nonreducing end. The presence of the blocking group allows the use of glucoamylase (EC 3.2.1.3) as an auxiliary enzyme, in addition to α-glucosidase.

Although its composition was described in 1988 (*2*), only recently has a "direct" substrate for the determination of α-amylase become commercially available. The 2-chloro-4-nitrophenyl-maltotrioside is cleaved by α-amylase to yield free chromophore without the need for ancillary enzymes.

Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Edifici M, 08193-Bellaterra (Barcelona), Spain. Fax Int + 34 35 81 15 73.
[1] Author for correspondence.
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The continual introduction of new methods for determining the catalytic concentration of serum α-amylase is leading to wide interlaboratory dispersion of values, as demonstrated by external quality-assurance surveys. The Scandinavian Committee on Enzymes suggested the use of a common calibrator to which serum α-amylase values would be referred, irrespective of the method used (*3*). Such calibrators should behave in each method in a way that closely mimics the endogenous α-amylase activity in human serum. The ability of a material to show interassay properties comparable with those of human serum has been termed "commutability" (*4*) and depends on the methods tested, the nature and source of the enzyme, and the matrix in which the enzyme is dissolved (*5*). We stress that not only the calibrator but also the control materials should be commutable to monitor properly intermethod comparability. Several authors have previously shown the lack of commutability of the α-amylase that is contained in many control materials (*5–9*).

Here we have compared six methods for determining serum α-amylase and studied the intermethod relationships of several materials containing α-amylase.

Materials and Methods

α-Amylase Methods

Commercial kits containing six different substrates were used in the intermethod comparison: 4-nitrophenyl-α-maltoheptaoside (method A) and 4,6-ethylidene-4-nitrophenyl-α-maltoheptaoside (method B) from Boehringer Mannheim, Mannheim, Germany; 4,6-benzylidene-4-nitrophenyl-α-maltoheptaoside (method C) from BioMérieux, Marcy-l'Etoile, France; 2-chloro-4-nitrophenyl-β-maltoheptaoside (method D) from Merck, Darmstadt, Germany; 6-benzyl-4-nitrophenyl-α-maltopentaoside (method E) from Wako, Osaka, Japan; and 2-chloro-4-nitrophenyl-α-maltotrioside (method F) from Genzyme, Kent, UK. The reagent preparation and the assay procedures were performed according to the manufacturers' instructions adapted to a Cobas Fara centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland). Assay temperature was 37°C. The calculation factor was obtained from the sample and reagent volumes, the molar absorptivity of the measured product, and the stoichiometric coefficient; this did not always coincide with that given by the manufacturer, which is often obtained by intermethod calibration.

Specimens

Human serum samples (n = 94) covering a wide range of α-amylase values were selected (44 to 1100 U/L by method B, upper reference limit, 220 U/L): 28 samples had α-amylase activity <220 U/L, 39 samples were between 220 and 500 U/L, and 27 samples had >500 U/L. Pancreatic and salivary α-amylase were measured in each serum sample by using the Pancreatic α-Amylase EPS kit from Boehringer Mannheim. Only samples containing >70% pancreatic isoenzyme (P group) or >70% salivary isoenzyme (S group) were finally selected for the methods comparison study. Each serum sample was aliquoted and stored at −20°C.

α-Amylase activity was also determined in 12 commercially available control serum materials: Precipath U (Boehringer Mannheim), Liotrol P (BioMérieux), Validate A (Organon Teknika, Eppelheim, Germany), Serodos Plus (Human, Taunusstein, Germany), Qualitrol H (Merck), Randox Elevated (Randox, Crumlin, N. Ireland), Control Serum II (BioSystems, Barcelona, Spain), Biotrol 33 Plus (Biotrol, Paris, France), Lyphocheck 2 (Bio-Rad, Anaheim, CA), Control Serum P (Roche, Basel, Switzerland), Accutrol Abnormal (Sigma, St. Louis, MO), and Decision Level 3 (Beckman, Fullerton, CA).

α-Amylase activity was also determined in two materials prepared in our laboratory. Both materials were prepared in a matrix containing 20 mmol/L 1,4-piperazine-diethanesulfonic acid (PIPES) buffer, pH 7.4, 15 mmol/L sodium azide, 50 mmol/L sodium chloride, 0.5 mmol/L EDTA, 1.5 mmol/L calcium chloride, and 5 g/L human albumin. Purified α-amylases from human pancreas or saliva were added to this matrix. The liquid materials were stored at 4°C and were used within 2 months. Stability of the materials was tested in accelerated studies at 37°C; there was no detectable loss of α-amylase activity after 20 days of storage at this temperature. Pancreatic α-amylase was purified by the procedure of Sampson et al. (*5*). Salivary α-amylase was prepared as described by Bernfeld (*10*).

Statistical Analysis

Methods comparison and commutability of materials were calculated by regression analysis according to Passing and Bablok (*11*), and by a multivariate statistical technique described by Bretaudiere et al. (*8, 12*). The data matrix was studied directly by correspondence analysis with use of a Système Portable pour l'Analyse de Données (Cisia, Sèvres, France).

Results

Characteristics of the Methods

Lag phase. The incubation time required to allow reading of constant reaction rates was studied with human serum samples containing salivary and pancreatic α-amylase. Methods A, B, and D required >4 min to reach linearity, methods C and E required ~1 min, and method F showed an almost constant reaction rate from the beginning of the incubation.

Precision. Precision was estimated by analyzing pancreatic material as a sample four times on four different days. The most precise method was method C (CV 0.89%), followed by methods B (CV 1.00%), E (CV 1.01%), and A (CV 1.13%). Methods D (CV 2.07%) and F (CV 2.10%) showed greater variation.

Methods Comparison

α-Amylase was measured in duplicate by each method in serum samples selected as described in *Materials and Methods* and classified into the S and P groups. Methods comparison showed homogeneous results. Correlation coefficients were close to 1.0 and almost all the intercepts were close to 0. Table 1 includes the results of the Passing–Bablok regression analysis. Each sample was assayed by all the methods during the same day in a randomized order.

Table 1. Passing–Bablok regression analysis of pancreatic (P) group and salivary (S) group sera.[a]

Pairs of methods	Intercept		Slope	
	P group	S group	P group	S group
A/B	−0.09 (−4.26 to 4.26)	0.34 (−3.28 to 2.64)	0.39 (0.38 to 0.40)	0.42 (0.41 to 0.43)
A/C	15.60 (11.10 to 20.00)	12.50 (5.88 to 16.70)	0.40 (0.39 to 0.41)	0.43 (0.42 to 0.46)
A/D	16.10 (6.73 to 23.30)	8.99 (−4.49 to 17.80)	0.88 (0.86 to 0.91)	0.90 (0.87 to 0.94)
A/E	−0.03 (−3.66 to 3.74)	4.79 (−0.51 to 8.42)	0.52 (0.52 to 0.53)	0.50 (0.49 to 0.52)
A/F	7.36 (1.96 to 14.80)	0.24 (−12.60 to 10.80)	0.60 (0.59 to 0.62)	0.62 (0.58 to 0.66)
B/C	16.90 (11.70 to 20.80)	10.88 (2.12 to 15.50)	1.01 (0.97 to 1.04)	1.05 (1.02 to 1.12)
B/D	14.60 (−0.58 to 28.60)	8.81 (−5.47 to 18.90)	2.29 (2.19 to 2.38)	2.15 (2.06 to 2.24)
B/E	−1.33 (−7.43 to 5.11)	3.62 (−3.47 to 7.86)	1.36 (1.31 to 1.40)	1.19 (1.16 to 1.24)
B/F	4.95 (−4.96 to 10.90)	−2.65 (−9.85 to 9.44)	1.56 (1.51 to 1.62)	1.49 (1.39 to 1.56)
C/D	−18.20 (−28.80 to 0.43)	−13.70 (−32.30 to 0.67)	2.24 (2.16 to 2.30)	2.02 (1.95 to 2.15)
C/E	−22.10 (−30.10 to −13.90)	−12.50 (−21.70 to −4.20)	1.33 (1.29 to 1.38)	1.15 (1.10 to 1.21)
C/F	−13.80 (−24.50 to −3.99)	−12.70 (−25.20 to −1.14)	1.52 (1.47 to 1.58)	1.40 (1.30 to 1.47)
D/E	−11.80 (−16.80 to −4.52)	−0.28 (−6.11 to 6.65)	0.60 (0.58 to 0.61)	0.56 (0.53 to 0.58)
D/F	−2.57 (−16.20 to 5.39)	−4.54 (−15.80 to 9.53)	0.68 (0.65 to 0.71)	0.67 (0.63 to 0.71)
E/F	9.29 (2.09 to 16.60)	−8.91 (−22.10 to 10.60)	1.14 (1.11 to 1.18)	1.25 (1.13 to 1.34)

[a] Human serum samples containing >70% pancreatic or >70% salivary α-amylase, respectively. Values are estimated (and confidence region); n = 94.

Table 2. Commutability study.[a]

Control materials[b]	Compared methods														
	A/B	A/C	A/D	A/E	A/F	B/C	B/D	B/E	B/F	C/D	C/E	C/F	D/E	D/F	E/F
PP-U	S			S				S		S		S		SP	
LT-P	SP	P	SP	S	S	S	SP	S	P	SP	SP		SP	S	S
VD-A	P			S						S		P			
SD-P	S							P		S					
QT-H	P				S	SP			S			S			
RA-E	P	P				P			S			S			
CS-II	P							P				S			
BT-33	P			SP		S		P	S	S		SP			S
LC-2			SP				P				S	P			
CS-P			S		SP				S	SP	S	P		SP	
AT-A	S			P				P		S					
DE-3	P			S						S		SP			
P material	P	P	SP		SP	SP	P	P	SP	P	P	P		SP	S
S material	S	S	SP	S		S	S	S	P		S	SP	SP		S

[a] S: commutability with salivary (S) serum group, P: commutability with pancreatic (P) serum group.

[b] PP-U, Precipath U; LT-P, Liotrol P; VD-A, Validate A; SD-P, Serodos Plus; QT-H, Qualitrol H; RA-E, Randox Elevated; CS-II, Control Serum II; BT-33, Biotrol 33 Plus; LC-2, Lyphocheck 2; CS-P, Control Serum P; AT-A, Accutrol Abnormal; and DE-3, Decision Level 3.

Commutability of Materials

The catalytic concentration of α-amylase was measured by the various methods in the control materials and in the prepared materials containing salivary and pancreatic α-amylase purified from human sources. Commutability of the materials with the human serum specimens for each couple of methods was judged by comparing the result from one method with the result from the other. The materials were considered commutable if the results were within the 95% confidence region of the respective regression equations. Table 2 summarizes the commutability study. For the majority of methods pairs, only the pancreatic material and the control material Liotrol P were found to be commutable with human sera containing the pancreatic isoenzyme.

The behavior of the materials was further studied with correspondence analysis. Calculation of the three factorial axes (1, 2, and 3) obtained by correspondence analysis showed that they account for, respectively, 61%, 14%, and 12% of the total variance of the system. Fig. 1 shows the distribution map of human sera groups S and P, control materials, and pancreatic and salivary materials for axes 1, 2, and 3. The projection of two specimens in proximity indicates that they possess a similar intermethod behavior. Axis 1, the most significant axis, describes differences between the behavior of the human α-amylase isoenzymes. Along axis 2 we found a great difference in the projection of specimens with α-amylase of human origin and of nonhuman origin. Separation of specimens according to their α-amylase origin was also observed along axis 3. Several human sera, two from the S group and six from the P group, were clustered in the positive direction along axis 1 and far from the other human sera. Those sera were not commutable for four or more pairs of methods compared, perhaps because of the presence of unknown interferents.

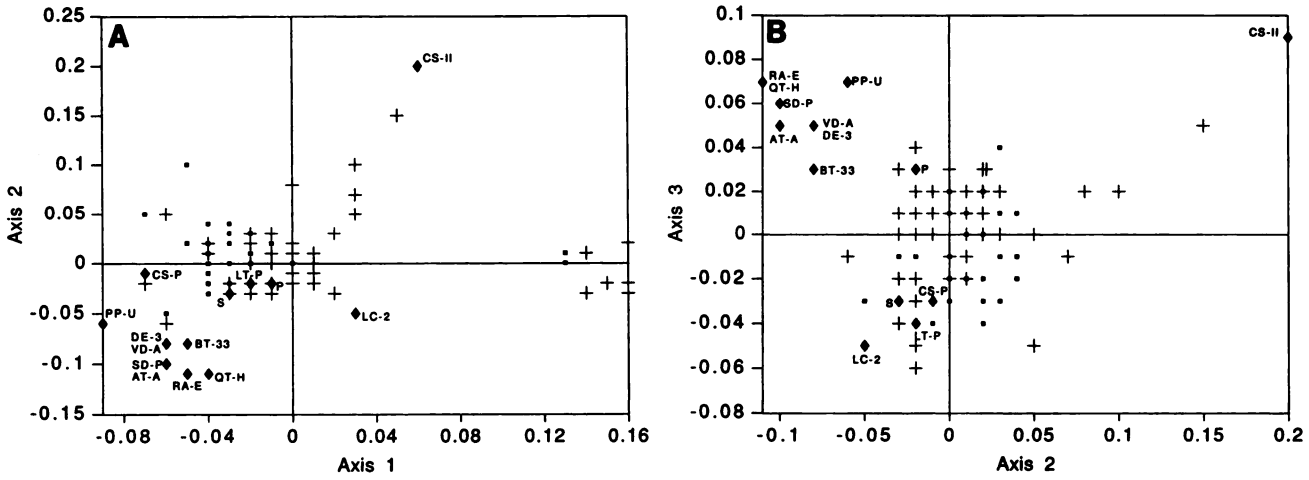

Fig. 1. Correspondence analysis of α-amylase measurements by six methods.

Projection of P and S specimens along factorial axes 1 and 2 (*A*) and along axes 2 and 3 (*B*). ◆, control materials projection; +, P-group projection; ■, S-group projection. (abbreviations as in Table 2)

Discussion

The existence of a variety of methods for α-amylase determination causes a wide interlaboratory dispersion of serum α-amylase activity values (*3*). The control materials or calibrators used in interlaboratory surveys have different behaviors, depending on the substrate, and usually show lack of commutability. We compared six methods for serum α-amylase determination and studied the commutability of several commercial control materials besides that of pancreatic and salivary materials prepared in our laboratory. All the studied methods use as substrate a maltooligosaccharide with a chromophore group (4-nitrophenyl or 2-chloro-4-nitrophenyl) at the reducing end as substrate. Some of the substrates are chemically blocked with an ethylidene (method B), benzylidene (method C), or benzyl (method E) group at the nonreducing end.

Methods comparison results were very similar for sera containing pancreatic and salivary α-amylase (Table 1). Methods A, D, E, and F showed similar activities, whereas methods B and C showed somewhat higher activities in sera with salivary α-amylase than in those with the pancreatic isoenzyme, when compared with the results of the other methods. These differences were statistically significant.

Control materials should satisfactorily simulate the intermethod behavior of patients' samples. However, almost all the control materials we tested were not commutable for the methods studied, as determined by two statistical analyses. Correspondence analysis showed different projections on the map, depending on the type of isoenzyme in serum; groups S and P were projected separately in both maps, showing a different intermethod behavior. The results for control materials showed that most of their projections fell outside the cluster determined by human sera. The materials can be classified in three groups: One includes pancreatic and salivary materials, Control Serum P, Lyphocheck 2, and Liotrol P, which were projected near the human sera. A second group contained the remaining control materials except Control Serum II and yielded projections that were close one each other and far from human sera. Control Serum II, the third group, was projected far away from human sera and the other control materials.

In conclusion, only our specially prepared pancreatic and salivary materials and Liotrol P control material were commutable for the majority of methods pairs. Differential sources of α-amylase in control materials can explain the absence of commutability (*13*). Whereas α-amylase in the Liotrol P material was of human origin, the rest of control materials were supplemented with α-amylase from bovine, porcine, or unspecified animal origin. The different behavior of Control Serum II material from human sera and the other control materials was probably due to the microbial origin of its α-amylase. Therefore, control materials supplemented with α-amylase of nonhuman origin are not commutable with the enzyme in human sera and cannot be used for intermethod calibration. Their use in external quality-assessment surveys will provide ratios between methods that will be different from the corresponding ratios involving human sera. The human-source materials most closely mimicked patients' specimens but did not show commutability across all systems evaluated. These materials may be used as intermethod calibrators only for those pairs of methods for which commutability has been demonstrated.

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