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Comparison of Control Materials Containing Animal and Human Enzymes

Comparison of Enzymes of Human and Animal Origin, III

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Summary: Highly purified enzymes of diagnostic interest from human and animal organs, dissolved in pooled human serum and in bovine serum albumin solution, were compared with respect to their response to alterations in routine clinical chemical assay conditions. Their response to changes in temperature, substrate concentration and pH-value was the same. In addition, the storage stability in each matrix was identical in the lyophilized and the reconstituted state, whereas some enzymes were remarkably less stable in the pooled human serum than in bovine serum albumin. This better stability, the better availability and decreased infectious nature of the material lead to the conclusion that animal enzymes in bovine serum albumin matrix are the material of choice for the quality control of enzyme activity determinations in clinical chemistry.

Vergleichende Untersuchungen an Kontrollproben, aufgestockt mit tierischen und humanen Enzymen. Vergleich humaner und tierischer Enzyme, III. Mitteilung

Zusammenfassung: Hoch gereinigte humane und tierische Enzyme von diagnostischem Interesse, gelöst in gepooltem Humanserum und in Rinderserumalbumin-Lösung wurden in Bezug auf ihr Verhalten gegenüber Änderungen der Reaktionsbedingungen bei klinisch-chemischen Routine-Methoden verglichen. Ihre Aktivitätsänderung bei Veränderung der Reaktionstemperatur, der Substrat-Konzentrationen und des pH-Wertes waren gleich. Auch die Lagerungsstabilität im lyophilisierten und im rekonstituierten Zustand war für jede Matrix gleich. Einige Enzyme erwiesen sich aber im gepoolten Humanserum als erheblich weniger stabil als im Rinderserumalbumin. Diese bessere Stabilität, die bessere Verfügbarkeit und die geringere Infektiösität des Materials empfehlen den Gebrauch von tierischen Enzymen in Rinderserumalbumin-Matrix als geeignetstes Material zur Qualitätskontrolle für Enzymaktivitätsbestimmungen in der klinischen Chemie.

Introduction

It has been known for many years that the determination of the activity of some cellular enzymes in patients' sera is extremely valuable for diagnostic purposes. Equally well known is the importance of quality control for the improvement of the accuracy and precision of enzyme assays. Many different quality control materials are now available, ranging from special control samples for a single enzyme to universal serum based materials used for quality control or even for calibration (e.g. of continuous flow analyzers) of any constituent of diagnostic interest.

For the control of the suitability of reagents and instrumentation and of performance, enzyme activities in a range of convenient signal height, where meaningful precision can be reached, are commonly preferred. This range is for most enzymes an elevated one, as compared to normal human sera. The elevation of enzyme activity is most commonly obtained by the addition of more or less purified enzyme material to serum or serum-like matrices.

For quality control in clinical chemistry it is of prime importance that the parameter to be assayed behaves similarly in the control sample and in the test sera. Of course the most similar control material for enzyme activity determinations would be the same enzymes isolated from human organs. But, for mainly ethical reasons, these resources are limited.

We therefore undertook this study to check whether enzymes isolated from animal organs are sufficiently similar to human enzymes to be used as substitutes in quality control materials.

In two preceding papers (1, 2) we have shown a high degree of similarity, with respect to substrate affinities, pH-optima and immunogenicity, between several human and animal enzymes, that were purified in exactly the same manner. Preliminary results were also reported elsewhere (3, 4, 5). In this publication we present further work with these enzyme preparations, dissolved in bovine serum albumin solution and in human pool serum, the most widely used matrices. Routine methods were used for enzyme activity determinations, altering single reaction parameters in different experiments and comparing the response of the human and animal enzyme controls to these alterations.

Materials and Methods

The enzymes used in our experiments were purified from human and animal tissues by the same methods to the same state of purity as described in l.c. (1):

aspartate aminotransferase (EC 2.6.1.1):	heart (pig)
alanine aminotransferase (EC 2.6.1.2):	heart (pig)
lactate dehydrogenase (EC 1.1.1.27):	heart (pig)
glutamate dehydrogenase (EC 1.4.1.3):	liver (beef)
creatine kinase (EC 2.7.3.2):	muscle (rabbit)
γ-glutamyl transferase (EC 2.3.2.2):	kidney (pig)

α-hydroxybutyrate dehydrogenase (LDH-1) was the naturally occurring isoenzyme activity of lactate dehydrogenase.

Using these we prepared four types of samples:

1) bovine serum albumin solution (60 g/l) + animal enzymes
2) bovine serum albumin solution (60 g/l) + human enzymes
3) pooled normal human serum + animal enzymes
4) pooled normal human serum + human enzymes

In the pooled human serum we did not inactivate the endogenous enzymes. In the case of sample 3) total enzyme activity is therefore a combination of activities of endogenous human enzyme plus added animal enzyme.
The enzyme control samples of all four types were bottled in 3 ml quantities, lyophilized and stored at 4 °C. For reconstitution 3 ml distilled water were added.
For the determination of enzyme activities we used Test Combinations or Monotests of BOEHRINGER MANNHEIM GMBH with alterations of different parameters as stated in section results. The concentrations in the assay solutions were according to the Recommendations of the German Society for Clinical Chemistry (6).

Biochemica Test Combination GOT opt.	(order no. 15923)
Biochemica Test Combination GPT opt.	(order no. 15925)
Biochemica Test Combination LDH	(order no. 15977)
Biochemica Test Combination α-HBDH LDH-1-isoenzyme	(order no. 15953)
Monotest GlDH	(order no. 15883)
Biochemica Test Combination CPK act.	(order no. 15926)
Monotest γ-GT	(order no. 15885)

Results

Thermal stability of the enzymes in the lyophilized state

Samples of the control materials type 1–4 were stored for three weeks at 33 °C. Enzyme activities were assayed 30 min after reconstitution. We did not find any loss of enzyme-activity in the bovine serum albumin-based samples type 1 and 2; whereas the enzymes aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase were less stable in the samples type 3 and 4, which contained pooled human serum. In both samples 10–20% of the activity of these enzymes was lost.

Stability of the enzymes in the reconstituted sample

After reconstitution we stored samples type 1–4 at 4 °C, 25 °C and – 20 °C. Enzyme activities were determined at fixed time intervals. Our results can be summarized as follows:
At *4 °C* the enzymes aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, α-hydroxybutyrate dehydrogenase and γ-glutamyl transferase were stable for two days in all 4 types of samples with apparent activity changes below ± 10%. Glutamate dehydrogenase and creatine kinase were only stable in samples of type 1 and 2, based on bovine serum albumin. In the samples of type 3 and 4, based on pooled normal human serum, both enzymes showed a loss of activity of about 25% after storage for two days. This matrix effect was shown by glutamate dehydrogenase and creatine kinase only, but the animal and human enzymes behaved similarly.
At *25 °C* the behaviour of all 7 enzymes was just the same as observed at 4 °C: aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, α-hydroxybutyrate dehydrogenase and γ-glutamyl transferase were stable for 6 hours in the reconstituted sample, as well as glutamate dehydrogenase and creatine kinase in the samples of type 1 and 2. The loss of activity of glutamate dehydrogenase and creatine kinase was up to 30% in the samples of type 3 and 4 after a storage of 6 hours. Again, no difference in behaviour between human and animal enzymes could be found.
At *– 20 °C* the enzymes alanine aminotransferase, lactate dehydrogenase, α-hydroxybutyrate dehydrogenase, glutamate dehydrogenase and γ-glutamyl transferase were stable for 4 weeks in all 4 types of samples with apparent activity changes of less than ± 10%; aspartate aminotransferase and creatine kinase were stable in the samples of type 1 and 2. In the reconstituted and frozen samples of type 3 and 4 aspartate aminotransferase and creatine kinase lost about 15% of their activity. It could be stated again that no difference in behaviour of enzymes of human and animal origin exists.

Temperature conversion factors

Temperature conversion factors derived from our experiments are summarized in table 1. The differences found between samples of type 1–4 are statistically not significant. This means that when animal enzymes in a bovine serum albumin-based matrix are used as controls, they are just as sensitive to deviations in reaction temperature as human sera with added human enzymes.

Tab. 1. Temperature conversion factors

Enzyme	Sample No.	Temperature conversion factors for 30 °C (F_{30}) and 37 °C (F_{37}) referred to 25 °C	
		F_{30}	F_{37}
aspartate aminotransferase	1	1.42	2.09
	2	1.42	2.03
	3	1.40	2.11
	4	1.35	2.13
alanine aminotransferase	1	1.34	1.87
	2	1.28	1.76
	3	1.34	1.75
	4	1.38	1.85
lactate dehydrogenase	1	1.37	1.97
	2	1.39	1.99
	3	1.34	2.05
	4	1.33	2.00
α-hydroxybutyrate dehydrogenase	1	1.33	1.62
	2	1.37	1.64
	3	1.30	1.55
	4	1.34	1.51
creatine kinase	1	1.38	2.01
	2	1.43	1.86
	3	1.40	1.99
	4	1.44	1.83
γ-glutamyl transferase	1	1.33	1.81
	2	1.39	1.77
	3	1.37	1.82
	4	1.39	1.82
glutamate dehydrogenase	1	1.18	1.52
	2	1.18	1.45
	3	1.19	1.49
	4	1.13	1.51

Substrate concentration

For the enzymes under investigation activity determinations were performed in the four sample types, using defined suboptimum substrate concentrations. The results are summarized in table 2. Again there are no significant differences between animal and human enzymes in the matrices used.

Changes in pH-value

The dependence of the activity of the enzymes on changes in pH-values, which might occur in routine, is shown in table 3. Again the dependence is the same for animal and human enzymes in both matrices. For aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase we could find no change of activity in the pH-range studied, due to their broad pH-optimum.

Activation of transaminases with pyridoxal phosphate

For the activation of transaminase we incubated 1 ml reconstituted sample from each of the four different types with 0.1 ml of pyridoxal phosphate solution (3.75 mmol/l). A slight activation could be shown only for aspartate aminotransferase in the human serum based sample types 3 and 4, probably due to the activation of endogenous enzyme.

Effect of NADH-inhibitors on lactate dehydrogenase

The appearance of lactate dehydrogenase inhibiting substances in NADH solutions upon storage is well known. It depends on the quality of the NADH used and on the storage conditions. We used a special batch of NADH, which showed a relatively high degree of lactate dehydrogenase inhibition after 4 weeks storage of the solution. As summarized in table 4 lactate dehydrogenase in all sample types shows the same degree of inhibition.

Tab. 2. Changes in substrate concentration

Enzyme	Substrate	Standard concentration (mmol/l) (= 100 % activity)	Suboptimal concentration (mmol/l)	Activity mean (%)		range (%)	
aspartate aminotransferase	*L*-aspartate	200	25	67		61–71	
alanine aminotransferase	*L*-alanine	800	100	76		68–83	
lactate dehydrogenase	pyruvate	0.6	0.2	91		89–92	
α-hydroxybutyrate dehydrogenase	2-oxobutyrate	3.0	1.0	60		59–60	
creatine kinase	creatine phosphate	35	8.75	72		66–76	
γ-glutamyl-transferase	γ-glutamyl-*p*-nitranilide	4	2				
	glycylglycine	40	20	41		40–43	
glutamate dehydrogenase	ammonium acetate	100	25	A	B	A	B
	2-oxoglutarate	7	A 7 B 3	63	71	55–71	68–79

Tab. 3. Changes in pH-value

Enzyme	Standard pH-Value (= 100% activity)	pH-Value	Activity mean (%)	range (%)
aspartate aminotransferase	7.4	7.0	96	96–97
		8.0	100	96–103
alanine aminotransferase	7.4	7.0	98	94–99
		8.0	98	96–101
lactate dehydrogenase	7.5	6.5	104	101–107
		8.0	96	91–99
α-hydroxybutyrate dehydrogenase	7.5	7.0	114	106–122
		8.0	82	80–87
creatine kinase	7.0	7.5	79	73–83
γ-glutamyl transferase	8.25	7.5	73	67–78
		8.75	95	92–98
glutamate dehydrogenase	8.0	7.5	81	74–88
		8.5	82	79–85

Tab. 4. Effect of NADH-inhibitors on lactate dehydrogenase activity

Enzyme	Sample No.	Activity NADH solution freshly prepared U/l	NADH-solution stored at 4°C/ 4 weeks U/l	%
lactate dehydrogenase	1	261	102	39
	2	205	84,5	41
	3	180	77.9	43
	4	180	73.5	41

Discussion

The reasons for incorrect enzyme activity determinations in clinical chemistry can be listed as follows:

1. assay temperature incorrect
2. performance inadequate (e.g. pipetting, mixing, measurement, calculation)
3. substrate concentrations false
4. pH-value wrong
5. inhibitors or activators present in the assay mixture
6. photometer defect
7. sample-specific difficulties (e.g. turbidity, abnormally high concentrations of metabolites, therapeuticals or interfering enzymes)

It is a well established fact today that the accuracy and precision of enzyme activity determinations can be largely enhanced by quality control, the assay of enzyme activities in control samples in series with the unknowns, which allows the detection of most sources of error. The sources of error listed above are all detectable with suitable control samples, except the last group of difficulties specific for single problematic samples, which are not generally susceptible to quality control.

It is clear that a control material suitable for the detection of aberrations in the assay must respond to these alterations in the same way and with the same sensitivity as the samples under investigation. It is shown in this study that animal enzymes, added to bovine serum albumin matrices fulfill this requirement very well. They respond to alterations in assay temperature, substrate concentrations, pH-values, inhibitors and activators in exactly the same way as human enzymes in a pooled human serum matrix. Of course, performance errors and photometer defects can also be detected with both kinds of material. In addition, the use of animal enzyme in bovine serum albumin material has some advantages:

- enzyme activities adjustable to the preferred range
- reproducible and versatile material
- diminished danger of infections (e.g. free of Australia Antigen)
- very good enzyme stabilities obtainable
- no additional difficulties by batch-specific unknowns (e.g. turbidities, metabolites).

This leads to the conclusion that animal enzymes in a bovine serum albumin matrix are very convenient materials for enzyme quality controls in clinical chemistry.

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