Kinetic Immunochemical Method for the Simultaneous Quantification of Creatine Kinase Isoenzymes

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We describe the development and evaluation of a kinetic immunochemical method for the simultaneous quantification of isoenzymes. Specifically, we use the inhibition of the M subunit of creatine kinase (CK; EC 2.7.3.2) by antibodies to quantify isoenzymes CK-MM and CK-MB in serum matrices. Nonlinear least-squares data-processing is used to compute the enzyme activities from the time-dependent response of absorbance vs. time. Variables affecting the method, namely temperature, substrate concentration, and antibody concentration as well as their interactions, are evaluated by using response-surface methodology. For several concentrations of CK-MM and CK-MB in the range of diagnostic significance, least-squares fits of computed (y) vs. expected (x) values yielded equations of \( y = 0.98x + (2.0 \times 10^{-5}) \) \( \text{s}^{-1} \) for CK-MM and \( y = 1.04x - (4.6 \times 10^{-5}) \) \( \text{s}^{-1} \) for CK-MB for rates between 0 and 3.5 \( \times \) \( 10^{-5} \) \( \text{s}^{-1} \) (CK-MM) and 0.5 \( \times \) \( 10^{-5} \) \( \text{s}^{-1} \) (CK-MB). The equations for comparison of kinetic results (y) with results by a kit method (x) were \( y = 0.97x + (6.5 \times 10^{-5}) \) \( \text{s}^{-1} \) for CK-MM and \( y = 1.02x + (4.3 \times 10^{-5}) \) \( \text{s}^{-1} \) for CK-MB. Pooled day-to-day relative standard deviations (CVa) for the kinetic method were 4.1% and 2.8% for CK-MM and CK-MB, respectively.

In a previous paper (1), we described a kinetic approach for the simultaneous quantification of isoenzymes based on the inhibition of enzyme activity by antibodies. We used synthetic mixtures of the muscle (M) and brain (B) subunits of the creatine kinase (CK; ATP:creatinine N-phosphotransferase, EC 2.7.3.2) isoenzymes as a model system to evaluate the feasibility of the approach. In that work, we assumed that the antibody used inhibited nearly 100% of CK-MM activity and virtually none of the CK-BB activity. Because of the very promising results obtained in that study, we wanted to evaluate more completely the potential of the kinetic method for the quantification of CK isoenzymes in sera. Specifically, we evaluated (a) additional experimental variables and their interactions that would affect the method; (b) the potential for using more commonly available antibodies, which do not completely inhibit CK-MM and may inhibit CK-MB by >50%; and (c) the applicability of the method for serum samples.

We established conditions for successful quantification of the subunits with use of antibodies that inhibited CK-MM by approximately 95% and CK-MB by about 64%. Here we report our evaluation of the experimental conditions for the simultaneous quantification of the subunits and of the quantitative performance features thereof, and compare the results of this kinetic approach with those of an equilibrium method based on a commercially available reagent kit system.

Mathematical Description

We have derived an expression for the time-dependent product concentration for a situation involving two enzymes, one of which is inhibited in a first-order fashion. For such a situation, in which the course of the reaction is monitored by changes of absorbance, we showed that the time-dependent absorbance \( A_t \) is described by

\[
A_t = A_i + \frac{V_{i1}}{k_1} (1 - e^{-k_1t}) + V_{i0}t
\]

where \( A_i \) is the initial absorbance; \( V_{i1} \) and \( V_{i0} \) are the initial velocities, expressed as rates of absorbance change (\( \text{d}A/\text{dt} \)), for the inhibited and uninhibited species, respectively; \( k_1 \) is a first-order rate constant for the inhibition process; and \( t \) is time. Experimental data for \( A_t \) are fitted to this equation to compute the initial velocities \( (V_{i1} \text{ and } V_{i0}) \) of the two subunits of the enzyme. For this system, the reaction is monitored by absorbance changes at 340 nm, which results from the production of NADPH by the coupled reaction system (2). Velocities obtained from the fitting process can be converted to enzyme activity, either by calculation with the molar absorptivity of NADPH or by calibration with enzyme preparations; we used the latter approach in this study.

Materials and Methods

Instrumentation and Software

We used a centrifugal mixing system (Rotochem IIIA; Deerfield, IL) to mix samples and reagents and to measure time-dependent absorbances at 340 nm. Software, data transfer, and curve-fitting procedures have been described previously (1, 3–6).

Reagents

All solutions were prepared with distilled de-ionized water.

Isoenzymes. The CK-MM (lyophilized powder) was obtained from Cambridge Medical Diagnostics, Billerica, MA. The human CK-MB isoenzyme was obtained in a pH 7 solution containing (per liter) 5 mmol of Tris succinate, 150 mmol of NaCl, 1 mmol of EDTA, and 1 mmol of 2-mercaptoethanol, from both Cambridge Medical Diagnostics and Scripps Laboratories, San Diego, CA. The powders were stored at −20°C, the liquid at 5°C.

Antibodies. Antibodies to human CK-MM were obtained as undiluted antisera from Cambridge Medical Diagnostics and Diagnostics Systems Laboratory, Webster, TX. Lyophilized antisera were obtained as powders from Pel-Freeze Biologicals, Rogers, AR, and monoclonal antibodies from Hybritech, San Diego, CA. All antibody solutions were diluted with imidazole buffer, described below.

Buffer. We prepared imidazole buffer to contain, per liter, 0.100 mol of imidazole (Boehringer Mannheim Biochemi-
cals, Indianapolis, IN), 10 mmol of magnesium acetate (MCB, Cincinnati, OH), 2 mmol of EDTA (Fisher Chemical Co., Fair Lawn, NJ), and 1 g of bovine serum albumin (cat. no. A-4503; Sigma Chemical Co., St. Louis, MO). The solution was adjusted to pH 6.7 with a 1 mmol/L solution of acetic acid and stored frozen until needed.

Stock enzyme solutions. All enzyme solutions were prepared in a diluent solution containing, per liter, 30 g of bovine serum albumin, 20 mmol of N-acetylcysteine (Sigma), 100 mmol of magnesium acetate, 2 mmol of EDTA, and 5 mol of glycerol, all adjusted to pH 6.7 with 1 mol/L acetic acid solution. After refrigeration overnight, the solution was filtered with a 7-μm (pore size) filter and stored at -20°C.

Enzyme samples. For the initial experiments, we prepared enzyme solutions by diluting the enzyme stock with the imidazole diluent described above. For method-comparison and day-to-day variation studies, we prepared enzyme samples in pooled, heat-inactivated (56°C, 30 min) human serum. The residual activity of the pooled serum was approximately 1 U/L at 25°C. Purity of the single-component stock enzyme solutions was confirmed by ion-exchange chromatography (7). The CK-MB isoenzyme used in the initial inhibition studies was isolated as the sixth and seventh fractions from the ion-exchange chromatographic procedure.

Reagent kit. We used kits containing the reagents for the quantification of total CK (CK-NAC Single Vial and CK-NAC Reagent Set; Boehringer Mannheim Diagnostics Division, Indianapolis, IN). We compared results by the kinetic method with those by the "CK-MB Reagent Set" (Boehringer Mannheim Diagnostics). All kits were used according to the manufacturer's directions.

Temperature. Except for the optimization studies, all reactions were run at 25.0 ± 0.1°C. All reagents were warmed in a water bath for at least 20 min before being mixed.

Procedure

Because the antibody/antigen reaction occurs within the first few seconds after mixing, an extended lag phase in the indicator reaction would obscure the kinetics of the inhibition reaction. To avoid this problem, we mixed enzyme samples with the reagent 5 min before antibody was added, so that the reaction mixture was at steady-state before the antibody was added. Details of the procedure have been described previously (1).

Results and Discussion

For convenience, we report activities as rates of absorbance change per second (s⁻¹); comparisons with international enzyme-activity units are discussed later in this paper. All uncertainties are reported as ±1 SD.

Antibody Selection

We evaluated several lots of antibodies from different suppliers; the inhibition characteristics for single-component CK-MM samples are summarized in Table 1. Because the products from Pel-Freez and Diagnostic Systems Laboratories were most effective in inhibiting CK-MM, we used them for further evaluation. Additional experiments showed that the latter antibodies had the most desirable characteristics: nearly 100% inhibition of CK-MM and about 50% inhibition of CK-MB.

Table 1. Inhibition Characteristics of Different Antisera* for the CK-MM Isoenzyme

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Lot no.</th>
<th>Uninhibited</th>
<th>Inhibited</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMD</td>
<td>GA1346F</td>
<td>1.48</td>
<td>0.068</td>
<td>95.4</td>
</tr>
<tr>
<td>CMD</td>
<td>GA1544F</td>
<td>1.48</td>
<td>1.25</td>
<td>15.4</td>
</tr>
<tr>
<td>Hybteck</td>
<td>360631</td>
<td>1.48</td>
<td>1.40</td>
<td>5.5</td>
</tr>
<tr>
<td>CMD</td>
<td>GA1415F</td>
<td>1.48</td>
<td>0.79</td>
<td>48.8</td>
</tr>
<tr>
<td>Pel-Freez</td>
<td>1465</td>
<td>1.44</td>
<td>0.020</td>
<td>98.6</td>
</tr>
<tr>
<td>DSL</td>
<td>01934</td>
<td>1.46</td>
<td>0.065</td>
<td>95.5</td>
</tr>
</tbody>
</table>

* All antisera were diluted fivefold.

CMD, Cambridge Medical Diagnostics; DSL, Diagnostics Systems Laboratories.

A broad range of enzyme activities. Figure 1 compares the activities with (y) and without (x) antibodies present. The range of rates corresponds to an activity range of 235 to 1191 U/L at 25°C; the average inhibition, calculated by comparing the residual inhibited rate (ordinate) with the uninhibited rate (abscissa), was 92.7 (SD 0.5%). These results were sufficiently encouraging for us to attempt to optimize reaction conditions.

Reaction Conditions

Because the coupled-indicator reaction system for CK has been so well optimized (8, 9), there were few variables that we could study without significantly affecting the coupled sequence. Nevertheless, we chose to examine effects of temperature and antibody concentration on measured rates and the inhibition properties of the antibodies. Also, because creatine phosphate may influence the rate of inhibition of CK-MM (10), we also studied the effects of creatine phosphate concentration on the inhibition and response of the coupled reaction system.

We used a full-factorial design to evaluate the effects of three values each of temperature, volume of antibody, and substrate (creatine phosphate) concentration (see Table 2). Experiments were done in duplicate with a single concentration of the CK-MM isozyme. Data for A vs t from experiments with antibodies present were processed with the curve-fitting method described in equation 1, with use of all 200 data points. We processed data from experiments without antibodies present by using a zero-order model. All velocities were normalized to a fixed volume (50 μL) of added antibody solution.

Ideally, for a given set of conditions, the sum of initial rates computed with equation 1, $V_* = V_{l,1} + V_{l,0}$, should be
equal to the zero-order rate, \( V_n \), obtained for the same conditions but without antibody. Therefore, we used the ratio \( V_s/V_n \) as a performance indicator. To quantify interactive effects among the variables studied [temperature (T), substrate (creatinine phosphate) concentration (C), and volume of antibody (v)], we computed values of \( V_s/V_n \) for each set of conditions examined and fit these results to a quadratic model of the form

\[
V_s/V_n = b_0 + b_T T + b_C C + b_v v + b_{TT} T^2 + b_{CC} C^2 + b_{TV} v^2 + b_{TC} TC + b_{TV} Tv + b_{CV} Cv
\]

where the b’s are coefficients that give the best fit of the several values of the \( V_s/V_n \) ratio to this equation. [Numerical values for the coefficients obtained for the 27 different sets of conditions in the three-variable factorial design, along with uncertainties and Student’s t-ratios, are available upon request from the authors or from the Editorial Office of this journal.] We then used these coefficients to compute response surfaces, to help in visualizing the interactions among the variables studied.

Figure 2 (A–C) shows response surfaces of \( V_s/V_n \) for two variables at a fixed value of the third. At a fixed temperature (Figure 2A) and a low antibody concentration, \( V_s/V_n \) increases with substrate concentration. However, at medium to high antibody concentrations (50–75 \( \mu \)L added), the ratio changes little with substrate concentration (Figure 2C). Because substrate concentration has little effect on the inhibition reaction, we chose the 30 mmol/L concentration recommended for the coupled reaction system.

The velocity ratio increases as the antibody concentration is increased (Figure 2A). Relatively large amounts of antibody are required to ensure 100% inhibition of the CK-MM isoenzyme. As a compromise between complete inhibition and cost, we chose a concentration corresponding to 50 \( \mu \)L of antibody solution added to the reaction mixture. Also, lower temperatures tend to favor more complete inhibition; we chose 25 °C as a reasonable compromise between more complete inhibition and convenience. Under these conditions (25 °C, 50 \( \mu \)L antibody, 30 mmol/L substrate concentration) about 96% of the CK-MM activity is inhibited.

We also evaluated the effects of the same three variables on the total absorbance change produced by the inhibited component. Figure 3 summarizes results of this study. The observed dependencies for temperature and antibody concentration are as expected. Except at very low antibody concentrations, substrate concentration has little effect on the absorbance change, but increased temperature increases the enzyme reaction rate and decreases the extent of inhibition (Figure 2B), and therefore increases the absorbance change resulting from the inhibited component. On the other hand, increased antibody concentration increases the extent of inhibition and decreases the absorbance change. Although we expected that substrate concentration would have little effect on the absorbance change, Figure 3C shows that it has a significant (but unexplained) effect. We did not use this information explicitly in selecting the reaction conditions, but did use it implicitly to ensure that assay sensitivity for the conditions selected on the basis of velocity ratios remained adequate.

Because the fitting model is completely empirical, it is improbable that any physical significance can or should be associated with the coefficients determined. Indeed, almost certainly, these coefficients will be different for other preparations of antibody. However, they may be useful to other investigators, to predict general trends as a starting point for future studies with this system.

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Table 2. Values of Variables Used in the Factorial-Design Study

<table>
<thead>
<tr>
<th>Factor</th>
<th>Values used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>37.0</td>
</tr>
<tr>
<td>Substrate concn, mmol/L</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
</tr>
<tr>
<td>Volume of antibody, ( \mu )L</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>70.0</td>
</tr>
</tbody>
</table>

We used a full-factorial design, with each experiment performed in duplicate (n = 27).

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\( \) Numerical values for the various b coefficients are available upon request from the authors or from the Editorial Office of this journal.

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Fig. 2. Response surfaces for \( V_s/V_n \) (see text) at (A) fixed temperature, 30 °C; (B) fixed substrate concentration, 20 mmol/L; and (C) fixed antibody volume, 50 \( \mu \)L.
Inhibition Properties of CK-MB

We used freshly separated (7) fractions of CK-MB to prepare samples that produced total uninhibited rates \( V_0 \) from \( 2 \times 10^{-4} \) to \( 9 \times 10^{-4} \) \( \text{s}^{-1} \). Values for \( V_{i0} \) and \( V_{i1} \) obtained by the curve-fitting method were used to evaluate the inhibition properties of these fractions. On the basis of results for the CK-MM isoenzyme, we had expected that the sum of velocities obtained with the curve-fitting method would correlate well with the total uninhibited zero-order rate, but, surprisingly, that was not the case. Also, we expected that the CK-MB would be slightly less than 50% inhibited; again, that was not the case.

Although the sum of computed initial rates varied linearly with the total uninhibited rate, with a slope near unity, there was a substantial additive error. Specifically, the least-squares statistics are

\[
V_s = (1.00 \pm 0.018) V_u - (6.7 \pm 1.1) \times 10^{-5} \text{s}^{-1}
\]

with \( S_{xy} = 1.3 \times 10^{-5} \text{s}^{-1} \) and \( r = 0.998 \). Similarly, a plot of the initial rate for the inhibited component vs the total uninhibited rate was linear, but neither the slope nor the intercept was as expected. Specifically, the least-squares statistics are

\[
V_{i1} = (0.64 \pm 0.018) V_u - (7.5 \pm 1.1) \times 10^{-5} \text{s}^{-1}
\]

with \( S_{xy} = 1.3 \times 10^{-5} \text{s}^{-1} \) and \( r = 0.998 \). For the uninhibited component, a similar plot was linear with least-squares statistics of

\[
V_{i0} = (0.362 \pm 0.0017) V_u + (0.81 \pm 0.11) \times 10^{-5} \text{s}^{-1}
\]

with \( S_{xy} = 1.3 \times 10^{-5} \text{s}^{-1} \) and \( r = 0.9999^+ \).

The two surprising features of these data are that the CK-MB fractions are more than 50% (~64%) inhibited, and there is a substantial intercept associated with the plot for the inhibited and total rates. This intercept is the origin of the additive error in the comparison of the sum of rates, \( V_u \), with the total zero-order rate, \( V_s \). We cannot explain this intercept.

We then used the reagent kit on the same enzyme preparations and found that, with the kit reagent, the rate was about 66% of the uninhibited rate, which is reasonably consistent with the value of 64% inhibition obtained with the other antibody. There are three possible explanations for this behavior. For example, the antibody may not be as selective as is generally assumed; however, experience with the BB isoenzyme suggests that the antibody does not inhibit that enzyme. Another possibility is that the B subunit in the MB isoenzyme does not retain its full activity when the M subunit is inhibited. The third possibility is that the fractions presumed to contain the MB isoenzyme actually contain both MB and MM isoenzymes. It would require a major effort to ascertain which of these possibilities is correct. Thus, because our primary objective in this work was to evaluate the kinetic data-processing approach, we elected to proceed, using a calibration approach that would take account of both the incomplete inhibition of the MM isoenzyme and the apparent greater than 50% inhibition of the MB isoenzyme. If other antibodies and isoenzymes behave in ideal fashion, then much simpler calibration procedures will be applicable.

Calibration Equations

For the MM isoenzyme, the total initial rate, \( V_{iMM} \), in the presence of antibody will be the sum of the uninhibited
(zero-order) rate, \(V_{0,m}\), and the inhibited (first-order) rate, \(V_{1,m}\), as follows:
\[
V_{i,m} = V_{0,m} + V_{1,m}
\]  
(3)

For the MB isoenzyme, the total initial rate, \(V_{i,b}\), will also be the sum of the uninhibited rate, \(V_{0,b}\), and inhibited rate, \(V_{1,b}\), as follows:
\[
V_{i,b} = V_{0,b} + V_{1,b}
\]  
(4)

Thus, to obtain the desired information, \(V_{i,m}\) and \(V_{i,b}\), we must obtain numerical values for the four quantities on the right-hand sides of equations 3 and 4 and solve a series of four equations in four unknowns.

The curve-fitting process applied to a mixture of the two isoenzymes yields two of the four equations needed. The computed value of the initial rate for the uninhibited, zero-order process, \(V_{0,b}\), will be the sum of the zero-order components resulting from the MM and MB isoenzymes as follows:
\[
V_{i,b} = V_{0,b} + V_{0,m}
\]  
(5)

Similarly, the computed value for the initial rate of the first-order process, \(V_{1,b}\), will be the sum of rates for the two isoenzymes as follows:
\[
V_{1,b} = V_{1,b} + V_{1,m}
\]  
(6)

where \(V_{1,b}\) and \(V_{1,m}\) are the rates for the first-order (inhibited) components of the MB and MM isoenzymes, respectively.

We obtained the other two equations from calibration experiments with single-component samples of the MM and MB isoenzymes. For the MM isoenzyme with conditions used in this study, we found that the zero-order rate, \(V_{0,m}\), and first-order rate, \(V_{1,m}\), components were linearly related as follows:
\[
V_{0,m} = 0.0242 V_{1,m} + 5.70 \times 10^{-6} \text{ s}^{-1}
\]  
(7)

For the MB isoenzyme, we found that the zero- and first-order rate components, \(V_{0,b}\) and \(V_{1,b}\), were related by the equation
\[
V_{0,b} = 0.565 V_{1,b} = 2.58 \times 10^{-5} \text{ s}^{-1}
\]  
(8)

Because \(V_{1,b}\) and \(V_{1,m}\) in equations 5 and 6 are experimentally determined quantities (computed via the curve-fitting process), we have four equations (equations 5–8) in four unknowns. When numerical values for those four unknowns \((V_{0,b}, V_{0,m}, V_{1,b}, \text{ and } V_{1,m})\) are obtained, they can be substituted into equations 3 and 4 to compute the desired information, namely the initial rates for the MM \((V_{i,m})\) and the MB \((V_{i,b})\) isoenzymes.

To solve the four equations, we substituted for \(V_{0,m}\) and \(V_{0,b}\) from equations 7 and 8 into equation 5, solved for \(V_{1,b}\), substituted this into equation 6, and rearranged to obtain
\[
V_{i,m} = 1.045 V_{i,b} - 1.849 V_{i,b} + 5.82 \times 10^{-5} \text{ s}^{-1}
\]  
(9)

Because \(V_i\) and \(V_{i,b}\) are both experimentally determined quantities (see equation 1), a numerical value of \(V_{i,m}\) can be computed. We then used this with equations 6 and 7 to compute \(V_{1,b}\) and \(V_{0,m}\), and used the latter with equation 5 to compute \(V_{0,b}\). We then used these values of \(V_{0,m}, V_{1,m}, V_{0,b}, \text{ and } V_{1,b}\) with equations 3 and 4 to compute the initial velocities of the MM and MB isoenzymes in mixtures.

This calibration process is not a function of the kinetic method but rather is imposed by the nonideal behavior of the antibody/isoenzyme reactions.

Quantification of Isoenzymes

We prepared 25 samples by diluting five stock solutions of CK isoenzymes with heat-inactivated, pooled serum. Two of the stock solutions contained only CK-MM or CK-MB; the other three stock solutions contained both isoenzymes. We processed four aliquots from each of the 25 samples. We ran two aliquots with the DSL antibodies, one aliquot with the reagent kit for CK-MB, and one aliquot without antibody present to get a direct determination of the total activity. We used activities obtained with the single-component samples to establish equations 7 and 8 above and to compute the initial rates for the CK-MM and CK-MB isoenzymes as described above.

Our first goal was to determine if there was consistency between the total initial rates obtained with and without antibodies present. A linear least-squares fit of total initial rates for mixtures with \(y\) and without \(x\) antibodies present yielded
\[
y = (0.97 \pm 0.014)x - (1.9 \pm 2.1) \times 10^{-5} \text{ s}^{-1}
\]

with \(S_{yx} = 5.8 \times 10^{-6}\) and \(r = 0.997\). Comparison of determined with expected (prepared) values was slightly better, with slope and intercept of 0.98 and 9 \(\times 10^{-5}\) s\(^{-1}\), respectively.

We then compared CK-MM and CK-MB values determined with the proposed kinetic method and the commercial kit method with expected values and with each other. Results obtained with the kinetic method are summarized in Figure 4 and linear least-squares statistics for these and other comparisons are summarized in Table 3. The graphical data show good agreement among determined and expected values. The tabular data show reasonable agree-

![Fig. 4. Calculated rates (kinetic inhibited) vs expected rates for (A) CK-MM and (B) CK-MB in respective mixtures of the two](CLINICAL CHEMISTRY, Vol. 33, No. 2, 1987 241)
Table 3. Linear Least-Squares Statistics for Comparison of Results for Mixtures of Isoenzymes

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>10^-6 s^-1</td>
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<td></td>
</tr>
<tr>
<td>Kinetic vs expected</td>
<td></td>
<td></td>
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<tr>
<td>CK-MM</td>
<td>0.98 ± 0.014</td>
<td>2 ± 2</td>
<td>6.1 0.9950</td>
</tr>
<tr>
<td>CK-MB</td>
<td>1.036 ± 0.009</td>
<td>4.6 ± 0.44</td>
<td>0.72 0.9983</td>
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<tr>
<td>Kit method vs expected</td>
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<td></td>
<td></td>
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<tr>
<td>CK-MM</td>
<td>0.997 ± 0.019</td>
<td>10.0 ± 2.5</td>
<td>7.9 0.9919</td>
</tr>
<tr>
<td>CK-MB*</td>
<td>0.806 ± 0.005</td>
<td>0.94 ± 0.13</td>
<td>0.39 0.9986</td>
</tr>
<tr>
<td>CK-MBb</td>
<td>1.020 ± 0.008</td>
<td>0.27 ± 0.22</td>
<td>0.65 0.9986</td>
</tr>
<tr>
<td>Kinetic vs kit method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MM</td>
<td>0.974 ± 0.028</td>
<td>-6.64 ± 4.0</td>
<td>12 0.9812</td>
</tr>
<tr>
<td>CK-MB*</td>
<td>1.70 ± 0.017</td>
<td>-6.2 ± 0.3</td>
<td>0.86 0.9977</td>
</tr>
<tr>
<td>CK-MBb</td>
<td>1.015 ± 0.010</td>
<td>-4.3 ± 0.3</td>
<td>0.86 0.9977</td>
</tr>
</tbody>
</table>

*Nonideal behavior (see text) not taken into account. bNonideal behavior taken into account with calibration equations. ± values are SD.

ment in most cases and, with a few exceptions, do not require further discussion. In comparisons involving the commercial kit method, failure to take the nonideal behavior of the inhibition process into account results in substantial deviations of the slope from the ideal value of unity; the problem is resolved with a calibration procedure that takes the nonideal behavior into account. Values for CK-MM by the kit method were obtained by subtracting twice the corrected value for CK-MB from the total rate.

Imprecision

We evaluated the imprecision of the method by computing the pooled standard deviations (11) for the method-comparison studies and for 10 samples run in replicate during three consecutive days. Results are summarized in Table 4, both as rates (dA/dt) and as activities (in international units). For the kinetic method, relative standard deviations for same-day studies are near 1%, day-to-day variations are in the range of 3 to 4%. By comparison, day-to-day variations for the commercial kit method were in the range of 3–6%, showing that the reproducibility of the proposed kinetic method is similar to that for the commercial kit method.

Activity Range

We included samples in this study with activities ranging from 0 to 1836 (CK-MM) and 288 (CK-MB) U/L, for a total range of activities of 0 to 2100 U/L. By comparison, the suggested linear ranges for the commercial kit procedures are 0 to 2000 U/L and 0 to 170 U/L for the CK-MM and CK-MB kits, respectively. To use the latter kit for the samples with higher activities in this study, we had to dilute the samples. Pooled standard deviations in Table 4 indicate that the detection limits (95% confidence level) are about 16 U/L for both CK-MM and CK-MB. Thus, the proposed method has a useful range for total activity of about 20 to 2000 U/L.

Discussion

The multipoint, curve-fitting method we describe here can yield individual activities of the MM and MB isoenzymes as well as total activity from a single set of measurements. Experimental results correlate well both with expected values and with results obtained with a commercial kit method, and the precision of the kinetic method is at least as good as that for the kit method. The rather involved calibration procedures used for both the kinetic and the kit methods are functions of the nonideal behavior of the antibodies or isoenzymes, or both, and not of the measurement/data-processing method. For ideal behavior (i.e., 100% inhibition of CK-MM and 50% inhibition of CK-MB), calibration procedures would be greatly simplified.

The multipoint, curve-fitting, kinetic method represents a viable alternative for quantification of CK isoenzymes. With appropriate modifications, it should be applicable to other systems in which different rates or degrees of inhibition are used to differentiate among isoenzymes or other catalytic species. We currently are studying other examples.

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References