The Comparative Enzymology of Creatine Kinases

I. ISOLATION AND CHARACTERIZATION FROM CHICKEN AND RABBIT TISSUES

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SUMMARY

The two chicken creatine kinases from brain and from muscle have been purified as well as the chicken hybrid enzyme. The brain and muscle enzymes from the rabbit have also been purified and compared to the chicken enzymes. There are significant differences in amino acid composition between the brain and muscle types; the hybrid enzyme has an intermediate composition. Peptide maps of the muscle and brain types further indicate that the sequences of the two types are considerably different. Antibodies prepared against the chicken muscle type enzyme do not cross-react, as measured by complement fixation methods or by Ouchterlony tests, with the chicken brain type enzymes; antibodies against the purified brain type enzyme do not react with the muscle type enzyme. However, the hybrid enzyme reacts with both antibodies, although quantitatively less than with the pure enzymes. The two brain type enzymes from the chicken and rabbit, or the two muscle enzymes, are more similar in their properties than are the brain and muscle forms of one species.

EXPERIMENTAL PROCEDURE

Materials

Chicken breast muscle, heart, and brain were obtained from commercial sources. Frozen rabbit brains were obtained from the Pel-Freez Company. Rabbit muscle creatine kinase was purchased from Boehringer Mannheim Corporation. Diethylaminoethyl cellulose type 11 was from Brown Company, and Sephadex G-100 from Pharmacia. Creatine phosphate and triethanolamine-HCl were purchased from Calbiochem, and creatine from Eastman Kodak. ATP, ADP, AMP, hexokinase, and glucose 6-phosphate dehydrogenase were from Boehringer Mannheim Corporation. Guanidine, urea, glucose, and Tris were from Fisher. TPN was obtained from P-L Biochemicals, Inc.

Methods

The standard enzyme assay was similar to that of Oliver (10) and Nielsen and Ludvigsen (11); the enzyme reaction was ADP + creatine phosphate → ATP + creatine. The assay medium in a final volume of either 3 or 1 ml contained 0.1 M Tris HCl or triethanolamine-HCl buffer, pH 7.5; 0.15 mM TPN; 3.3 mM MgCl₂; 3.3 mM glucose; 0.5 mM ADP; 5 μg of glucose 6-phosphate dehydrogenase; 5 μg of hexokinase; 8 mM creatine phosphate; and an appropriate amount of enzyme. An enzyme unit is defined as that amount of enzyme which converts 1

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μmole of substrate in 1 min at 25°. The reaction is started by
addition of creatine phosphate and the change in absorbance
at 540 μμ is followed. There was no reduction of TPN in the
absence of creatine phosphate with pure enzymes; in impure
fractions reduction of TPN due to competing reactions (e.g.
adenylic kinase) was compensated for. The reverse reaction
(ATP + creatine → creatine phosphate + ADP), when used,
was carried out according to Tanzer and Gilvarg (12) and its
modification by Burger, Richterich, and Aebi (2).

The colorimetric assay of Kuby, Noda, and Lardy (13) was
occasionally used without major modifications.

Protein Determination

The biuret procedure described by Gornall, Bardawill, and
David (14) was used with bovine serum albumin as standard.
Protein concentration in column effluents was measured by
determining the absorbance at 280 μm.

Purification of Enzymes

Chicken (MM)1 Creatine Kinase from Breast Muscle—The
initial steps of the purification followed those of Kuby et al.
(13).

Step 1: Chicken breast muscle, 36 pounds, was ground in a
meat grinder and extracted overnight at 4° in 80 liters of 0.01
M MgSO4. The suspension was centrifuged and the precipitate
was discarded.

Step 2: To the supernatant, totaling 66.4 liters, solid amon-
monium chloride was added to a concentration of 0.1 M, and
the pH was adjusted to 8.5 with 5 M NH4OH.

Step 3: At a temperature of −10° 99 liters of ethanol were
slowly added with stirring, producing a dense white precipi-
tate. The solution was brought to 20° in a large water bath and stirred
for 2 hours. The solution was centrifuged, the enzyme remain-
ing in the supernatant. The recovery of enzyme activity was
greater than 100%, presumably because of the absence of com-
peting reactions present in the crude extract.

Step 4: To the slightly cloudy colorless supernatant at 4°,
2.0 M MgSO4 was added with stirring to a final concentra-
tion of 0.03 M. A dense white precipitate formed. The solution was
stirred at 4° for 4½ hour and then centrifuged. This step removed
95% of the activity from the supernatant, which was discarded.

Step 5: The precipitate from Step 4 was suspended in 6.6
liters of 0.07 M magnesium acetate, and stirred by a magnetic
stirrer overnight at 4°. It was then centrifuged at 30,000 × g
for 1 hour. The supernatant contained approximately 80% of
the enzyme activity, but only insignificant quantities of enzyme
could be obtained from the precipitate of Step 4 by further ex-
tractions.

Step 6: The extract from Step 5 was dialyzed at 4° for 24
hours against 0.01 M Tris-HCl, pH 8.0, containing 10−4 M EDTA.
To the extract solid ammonium sulfate was added to a final con-
centration of 70% (calculated from the saturation at 25°, even
though the solution was kept throughout at 4°). The enzyme
was precipitated without change in specific activity. The sus-
pension was centrifuged and the supernatant was discarded.

Step 7: The precipitate from Step 6 was dissolved in 230 ml
of 0.01 M Tris-HCl, pH 8.0, and dialyzed for 2 days against
this buffer.

Step 8: The enzyme solution, containing 27 mg per ml of
protein, was placed on a column, 15 × 150 cm, of DEAE-cellu-
lose, prepared as described elsewhere (15) and eluted at 25°
with a linear concentration gradient of Tris-HCl, pH 8.0, of
0.09 to 0.1 M; each reservoir contained 6 liters of buffer. The
enzyme eluted as a single peak near the end of the gradient.

Step 9: The active fractions were pooled, and at 4° solid
ammonium sulfate was added to give a final concentration of
65%. With slow additions of ammonium sulfate the enzyme
precipitated. It was then dissolved in 0.05 M Tris-HCl, pH 8.0,
or was stored as the ammonium sulfate suspension. In either
form it was stable for many months in the cold. The final yield
was 2.3 g, and the over-all recovery was 25%. The enzyme
had a specific activity of 720 μmole per min per mg of protein.

Chicken (BB) Creatine Kinase from Heart—Step 1: Chicken
hearts, 1500 g, were ground in a Waring Blender together with
twice the volume of cold 0.01 M KCl and 0.001 M EDTA.
The homogenate was stirred at 4° for 2 hours and squeezed through
cheesecloth. The suspension was centrifuged and the precipi-
tate was discarded.

Step 2: The supernatant, 2410 ml, was made 10−3 M with
respect to 2-mercaptoethanol and this was maintained through-
out the purification. By addition of solid salt the supernatant
was brought to a 45% ammonium sulfate concentration. After
centrifugation there was no enzyme activity in the precipitate.
The pH was kept above 7.5 by addition of 5 M NH4OH.

Step 3: The supernatant, 2700 ml, was brought up to a 60%
ammonium sulfate concentration. The precipitate was dissolved
in 0.05 M Tris buffer, pH 8.0, and dialyzed for 24 hours against
the same buffer. The resulting 195 ml contained all the re-
mainder activity. A 5-fold increase in specific activity was
obtained.

Step 4: The dialyzed enzyme solution, containing 37.7 mg of
protein per ml, was placed on a DEAE-cellulose column (120 ×
5 cm). The column was washed through with 3700 ml of 0.05
M Tris buffer, pH 8.0. A considerable amount of protein which
had no creatine kinase activity was removed in one major peak.
Further elution took place with a linear concentration gradient
at pH 8.0 of 0.05 M Tris-HCl to 0.5 M NaCl made up in 0.05 M
Tris-HCl. Each reservoir contained 2 liters of buffer. The protein,
containing the enzyme activity, was eluted at a con-
centration of about 0.2 M NaCl in a single peak.

Step 5: The active fractions were pooled, and at 4° solid
ammonium sulfate was added to give a final concentration of
70%. The precipitate was collected, redissolved in 0.1 M Tris,
pH 8.0, and dialyzed against the same buffer. The increase in
specific activity was 22-fold at this stage.

Step 6: The enzyme solution was concentrated and placed
on a column of Sephadex G-100 (120 × 4 cm) and eluted with 0.1
M Tris-HCl buffer, pH 8.0. The fractions containing approxi-
mately the same specific activity were pooled. The increase in
specific activity was 20-fold. The yield was 340 mg, and the
over-all recovery was 17%. The enzyme had a specific activity
of 480 μmole per min per mg of protein.

Step 7: Electrophoresis showed a trace of more slowly migrat-
ing contaminating protein. The protein was placed on a second
DEAE-cellulose column (42 × 2 cm). The column had been
equilibrated with 0.05 M Tris-HCl buffer, pH 8.6. The elution
by a concentration gradient from 0.05 M Tris-HCl buffer to
0.5 M NaCl made up in 0.05 M Tris-HCl buffer removed this
Chicken MM enzyme

Ox brain (21)

and extracted for 3 hours in 8 liters of 0.01 M Tris-HCl, pH 8.0, and was dialyzed against this buffer for 2 days.

The precipitate was removed by centrifugation at 100,000 X g for 1 hour.

The extract from Step 5 was dialyzed against 0.01 M Tris-HCl, pH 8.0, containing 1 × 10⁻³ M 2-mercaptoethanol, and was stirred overnight.

The precipitate was removed by centrifugation at 100,000 × g for 1 hour.

The enzyme was checked for purity in the ultracentrifuge, and it was found contaminated by approximately 10% of material of a slightly lower s value. A 10-ng portion was placed on a Sephadex G-100 column (0.8 × 50 cm) and eluted with 0.1 M KCl-0.05 M Tris, pH 7.5. The resulting peak fractions were free of contaminants.

**Immunological Studies**

Antibodies to the two purified forms of creatine kinase from chick tissues were prepared in the rabbit by the method of Plescia, Braun, and Palzuc (16). Microcomplement fixation studies were performed according to the method of Wasserman and Levine (17).

**Amino Acid Analysis**

Amino acid analyses were carried out with a Beckman/Spinco analyzer. The procedure of Spackman, Stein, and Moore (18) was followed for quantitative amino acid analysis on ion exchange resins. The acid hydrolysis was carried out for 24 hours.

**Tryptic Digestion and Fingerprints**

MM and BB type creatine kinases (10 mg each) were treated according to Harris and Poham (19). The digestion with 1% trypsin was carried out at 37°C for 4 hours. The digests were freeze-dried and dissolved in a small amount of 0.05 M NH₄OH. Samples of the digests were applied to Whatman No. 3MM paper and subjected to electrophoresis (at 60 volts per cm) at pH 6.5 (pyridine-acetic acid buffer) for 70 min. A guidestrip was developed with ninhydrin reagent. According to this guidestrip the basic, neutral, and acidic peptides were distinguished. The neutral peptides were submitted to re-electrophoresis at pH 3.5 for 55 min (at 60 volts per cm) to get sufficient separation. Then a strip of each of the three fractions was cut out, sewn onto fresh sheets of paper, and submitted to chromatography in 1-butanol-acetic acid-water-pyridine (30:6:24:20) to develop the second dimension. After dipping in ninhydrin reagent, the peptide maps were obtained.

**RESULTS**

**Catalytic Properties**—The four purified enzymes differed substantially in their catalytic characteristics. The two enzymes of brain type (the chicken enzyme was derived from heart, but is identical with that found in chicken brain) showed slightly lower Kₘ values for both ADP and creatine phosphate than did the muscle type enzymes. The Kₘ values (Table I) for creatine were assayed in the direction of creatine phosphate synthesis. They were also lower for the brain type enzymes from both species by a factor of 2. Similar values given in the literature are those given by Kuby, Noda, and Lardy (20) for the rabbit muscle enzyme, and those given by Wood (21) for the ox brain enzyme; these are also listed in Table I.

In addition to a difference in Kₘ values, there was a moderate degree of inhibition by excess creatine phosphate for the brain enzymes, amounting to a reduction from maximum activity to 70% in the presence of 33 mM creatine phosphate. Accordingly a comparison of the reaction rates in the presence of high concentrations of both ADP and creatine phosphate versus those with low concentrations of both substrates emphasizes the catalytic differences between the enzymes. These values are given in Table II.

Some assays have been carried out with nucleoside diphosphates other than adenosine, with the use of a less accurate

### Table I

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Kₘ, ADP</th>
<th>Kₘ, creatine phosphate</th>
<th>Kₘ, creatine</th>
<th>Conditions</th>
</tr>
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<tbody>
<tr>
<td>Chicken MM enzyme</td>
<td>3.3 × 10⁻⁶</td>
<td>3.3 × 10⁻⁶</td>
<td>3.3 × 10⁻⁶</td>
<td>See text</td>
</tr>
<tr>
<td>Rabbit MM enzyme</td>
<td>3.3 × 10⁻³</td>
<td>3.3 × 10⁻³</td>
<td>3.3 × 10⁻³</td>
<td>See text</td>
</tr>
<tr>
<td>Chicken BB enzyme</td>
<td>3.3 × 10⁻⁶</td>
<td>3.3 × 10⁻⁶</td>
<td>3.3 × 10⁻⁶</td>
<td>See text</td>
</tr>
<tr>
<td>Rabbit BB enzyme</td>
<td>3.3 × 10⁻³</td>
<td>3.3 × 10⁻³</td>
<td>3.3 × 10⁻³</td>
<td>See text</td>
</tr>
<tr>
<td>Rabbit muscle (20)</td>
<td>6.5 × 10⁻⁵</td>
<td>6.5 × 10⁻⁵</td>
<td>6.5 × 10⁻⁵</td>
<td>Glycylglycine, pH 7.0, or glycine, pH 9.0, 30°C</td>
</tr>
<tr>
<td>Ox brain (21)</td>
<td>10.0 × 10⁻⁵</td>
<td>10.0 × 10⁻⁵</td>
<td>10.0 × 10⁻⁵</td>
<td>V-Ethylmorpholine, pH 7.9, or glycine, pH 9.0, 30°C</td>
</tr>
</tbody>
</table>

impurity as a small peak eluting ahead of the creatine kinase peak.

**Rabbit (BB) Creatine Kinase from Brain**—Step 1: Five pounds of frozen rabbit brains were thawed and ground in a meat grinder, and extracted for 3 hours in 8 liters of 0.01 M KCl containing 1 × 10⁻³ M 2-mercaptoethanol. The suspension was filtered through cheesecloth.

Steps 2 to 4: The procedure given above for the chicken muscle enzyme was followed except for appropriate changes in volumes, and the inclusion of 1 × 10⁻³ M 2-mercaptoethanol throughout.

Step 5: The precipitate from Step 4 was resuspended in 800 ml of 0.05 M Tris-HCl, pH 8.0, containing 1 × 10⁻³ M 2-mercaptoethanol, and 1 × 10⁻³ M EDTA, and was stirred overnight. The precipitate was removed by centrifugation at 100,000 × g for 1 hour.

Step 6: The extract from Step 5 was dialyzed against 0.01 M Tris-HCl, pH 8.0, containing 1 × 10⁻³ M 2-mercaptoethanol, and was precipitated by the addition of solid ammonium sulfate to a concentration of 70%.

Step 7: The precipitate was dissolved in 100 ml of 0.1 M Tris-HCl, pH 8.0, and was dialyzed against this buffer for 2 days.

Step 8: The enzyme solution was placed on a column of DEAE-cellulose, 4.8 × 48 cm, and was eluted with a gradient consisting of 1500 ml of 0.1 M Tris-HCl, pH 8.0, to which were continuously added 1500 ml of 0.1 M Tris-HCl containing 1.0 M KCl, pH 8.0. The enzyme was eluted as a single peak, contaminated by approximately 10% of material of slightly lower s value as judged by ultracentrifugation.

Step 9: The active fractions were pooled and precipitated with ammonium sulfate at 70% saturation. The precipitate was dissolved in and dialyzed against 0.05 M Tris-HCl, 0.1 M KCl, pH 7.5.

Step 10: The enzyme solution was placed on a column of Sephadex G 100, medium grade, 0.1 × 50 cm, and was eluted with 0.05 M Tris-HCl, 0.1 M KCl, pH 7.5. The active fractions were again precipitated with ammonium sulfate, and redissolved in buffer. The final yield was 55 mg, and the overall recovery was 20%. The enzyme had a specific activity of 530 μmoles per min per mg of protein.

**Rabbit (MM) Creatine Kinase**—The crystalline enzyme was purchased from Boehringer Mannheim Corporation. The enzyme was checked for purity in the ultracentrifuge, and it was found contaminated by approximately 10% of material of a slightly lower s value. A 10-ng portion was placed on a Sephadex G-100 column (0.8 × 50 cm) and eluted with 0.1 M KCl-0.05 M Tris, pH 7.5. The resulting peak fractions were free of contaminants.
colorimetric assay (13) for disappearance of creatine phosphate. These results are given in Table III. The brain enzymes seem slightly less specific in their nucleotide requirement but the disparity is not great. In both cases the rates with deoxy-ADP approached those with ADP as substrate. As far as the experimental conditions can be compared, these results correspond to those found by O'Sullivan and Cohn (22).

Wood has given results suggesting that the position of equilibrium differed in enzymes obtained from ox brain and rabbit muscle. We have found no significant differences (Table IV) nor would they be expected.

Fig. 1 shows a comparison of the pH optima of the two enzymes from chicken for the forward reaction, creatine-P + ADP → ATP + creatine. They do not differ significantly, and resemble the corresponding curve given by Ennor and Rosenberg (23) for the rabbit muscle enzyme.

**Immunological Reactions**—For these assays an artificial hybrid enzyme, chicken MB enzyme, was used, prepared as described in Paper II (24).

Ouchterlony (25) precipitation tests with equal amounts of en-

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**Table II**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ratio of high rate to low rate</th>
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<tbody>
<tr>
<td>Chicken MM enzyme</td>
<td>4.50</td>
</tr>
<tr>
<td>Rabbit MM enzyme</td>
<td>3.60</td>
</tr>
<tr>
<td>Chicken BB enzyme</td>
<td>2.28</td>
</tr>
<tr>
<td>Rabbit BB enzyme</td>
<td>2.10</td>
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</table>

**Table III**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>dADP</th>
<th>IDP</th>
<th>UDP</th>
<th>dGDP</th>
<th>GDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken MM enzyme</td>
<td>50</td>
<td>4</td>
<td>1</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Chicken BB enzyme</td>
<td>15</td>
<td>1.5</td>
<td>6.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Rabbit BB enzyme</td>
<td>82</td>
<td>17</td>
<td>6.0</td>
<td>0.0</td>
<td>0.0</td>
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</tbody>
</table>

**Table IV**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Equilibrium at pH 7.0</th>
<th>pH 7.5</th>
<th>pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken MM enzyme</td>
<td>0.0049</td>
<td>0.040</td>
<td>0.084</td>
</tr>
<tr>
<td>Rabbit BB enzyme</td>
<td>0.0056</td>
<td>0.041</td>
<td>0.084</td>
</tr>
</tbody>
</table>

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**Fig. 1.** pH Optima for chicken BB and MM creatine kinases. The buffers used were: for pH 5.5 to 6.2, Tris-acetate; for pH 5.9 to 7.3, Tris-histidine; for pH 7.5 to 8.3 Tris-HCl.

**Fig. 2.** Complement fixation of chicken creatine kinases, with antiserum to chicken MM enzyme. The numbers indicate antiserum dilutions. ○—○, brain type; Δ—Δ, muscle type; ■—■, hybrid.

**Fig. 3.** Complement fixation of chicken creatine kinases, antiserum to chicken BB enzyme. The numbers indicate antiserum dilutions. Same symbols as in Fig. 2.
zyme as antigen showed a single precipitation line with the homologous antigen, a single, but weak one with the hybrid antigen, and no cross-reaction with the heterologous antigen. Figs. 2 and 3 show the quantitative complement fixation curves of the three respective enzyme forms with antibodies to the MM and BB forms. It is evident that the hybrid MB form reacted less well with both antibodies as compared with the respective pure types. In both cases the amount of hybrid antigen as well as the BB and MM antibody concentration required to give peak fixation was higher than in the corresponding homologous type.

**Amino Acid Analysis**—Table V compares the amino acid composition of the MM and BB enzymes isolated from the chicken and the MB enzyme prepared as discussed in Paper II. In addition there are given the values we obtained from rabbit MM and BB enzyme as well as the corresponding values found by Noltman, Mahowald, and Kuby (26) for the rabbit MM enzyme. The numbers are mole residues per mole of enzyme (mol wt 81,000). Particularly interesting are the considerable differences in the basic amino acid residue contents of the two forms in chicken as well as in the rabbit. Differences between the two animal species can also be seen in the content of acidic amino acid residues. These findings correspond well to the electrophoretic behavior of the four respective enzymes (see Paper II (24)). The amino acid composition of the chicken hybrid form MB shows a close correspondence with that obtained by calculating the mean between MM and BB type enzyme.

**Peptide Maps**—Fig. 4 shows tracings of the basic, neutral, and acidic peptides obtained by tryptic digestion. The differences between the MM and BB types are apparent. The counting of the spots corresponds fairly well to the expected values from the numbers of the lysine and arginine residues. We expected 53 peptides from the MM type; we found 58. We expected from the composition 44 peptides for the BB type; we found between 42 and 44.

**DISCUSSION**

All four of the enzymes studied in this work were homogeneous on ultracentrifugation and electrophoresis (see Paper II). The chick enzymes produced a single antibody in the rabbit. We were not able to achieve crystallization, but by other criteria they were free from contamination. The isolation of the en

![Fig. 4. Drawings of tryptic peptide maps of chicken creatine kinases. For experimental conditions see text. a, basic peptides, b, neutral peptides; and c, acidic peptides.](http://www.jbc.org/)

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**Table V**

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Chick</th>
<th>Rabbit</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MM</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>Average of (BB + MM)</td>
</tr>
<tr>
<td></td>
<td>moles/81,000 g enzyme</td>
<td>moles/81,000 g enzyme</td>
</tr>
<tr>
<td>Lys</td>
<td>49</td>
<td>65</td>
</tr>
<tr>
<td>His</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>Arg</td>
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<td>40</td>
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<tr>
<td>Asp</td>
<td>87</td>
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<td>Ser</td>
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<tr>
<td>Glu</td>
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<td>Gly</td>
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<tr>
<td>Ile</td>
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<td>24</td>
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<tr>
<td>Leu</td>
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<td>71</td>
</tr>
<tr>
<td>Tyr</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Phe</td>
<td>30</td>
<td>33</td>
</tr>
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</table>

*For method of preparation see Paper II (24)*

*From Noltmann et al. (25).*
zyme from chicken muscle was relatively straightforward, because of its greater stability compared to the brain type enzymes. Milder methods and the use of a reducing agent throughout the purification were necessary to obtain reasonable yields of the brain enzymes. When the ethanol fractionation first described for the rabbit muscle enzyme (13) can be used, the higher specific activities of the ethanol extracts so obtained simplify the chromatographic steps considerably.

The peptide maps for the two enzymes from the chicken indicate many points of divergence in the primary structure of these two enzymes. Not only are there differences in the number of peptide spots, but there are different patterns for the basic, neutral, and acidic peptides. We estimate that of the 44 tryptic peptides for the chicken BB enzyme and the 56 peptides of the chicken MM enzyme, no more than 25 are common to both enzymes.

The data from the amino acid analysis of the BB and MM enzymes, including the hybrid chicken MB enzyme, suggest substantial differences in composition. More comparative data with different species are necessary to draw conclusions about similarities among different species as well as among enzyme types. We believe, however, that there are more similarities between the same enzyme types of different species than between the two enzyme types of the same species. The hybrid enzyme is intermediate in amino acid composition between its two parental forms.

The immunological reactions of the two enzymes from the chicken indicate an almost complete lack of cross-reactivity, as tested both by Ouchterlony precipitation and by complement fixation. The hybrid enzyme, chicken MB enzyme, was more or less intermediate in both systems; it would react with either antibody but required a larger amount of antigen to achieve the same amount of complement fixation.

Accordingly, it may be concluded that the creatine kinases from brain and muscle, although they catalyze the same reaction, differ considerably in their over-all amino acid composition, in their primary amino acid sequence as determined by tryptic digestion and peptide maps, and in their immunological characteristics. Differences can also be detected in the catalytic behavior of the muscle and brain enzymes, in that the brain enzymes have lower $K_m$ values for both substrates than do the muscle enzymes.

It is of interest that the multiple forms of creatine kinase are produced by the same phenomena as have been observed for the multiple forms of lactic dehydrogenases (27, 28). In lactic dehydrogenase, the enzyme exists as a tetramer; hence five possible forms of the enzyme can exist; whereas the creatine kinase is a dimeric enzyme and only three types are normally found.

Acknowledgments—We wish to thank Mr. Fred Castillo for carrying out the amino acid analyses and Dr. William S. Allison for advice with the fingerprinting procedures. We wish to thank the New England Enzyme Center for assistance in the preparation of the chicken MM creatine kinase.

REFERENCES

The Comparative Enzymology of Creatine Kinases: I. ISOLATION AND CHARACTERIZATION FROM CHICKEN AND RABBIT TISSUES
Hans M. Eppenberger, David M. Dawson and Nathan O. Kaplan


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