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ACTIVITY AND POLYMORPHISM OF THE COBALT-ACTIVATED
ACYLASE IN TISSUES OF RODENTS DURING DEVELOPMENT*

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The activity of cobalt-activated acylase, measured towards N-chloroacetyl- and
N-butryryl-γ-L-glutamyl-β-naphthylamide, was found in all tissues of the adult animals.
In the kidney, liver and small intestine of adult guinea-pig and rat two fractions
differing in electrophoretic mobility (fractions 1 and 2) were present. The early foetus
contained fraction 2, sometimes accompanied by fraction 3 which later disappeared;
on further development of the foetus, fraction 1 appeared.
Fraction 1 was distinctly activated by cobalt ions; fractions 2 and 3 were strongly
inhibited by deaminated leucylphenylalanine. In the guinea-pig, the molecular weight
of the three fractions ranged from 43 000 to 59 000.

The presence in human tissues of two fractions of the Co⁴⁺-acylase¹ was de-
monstrated in the accompanying paper (Ziomek & Szewczuk, 1978); the fractions
differ in substrate specificity, effector susceptibility, thermal stability and molecular
weight. In the present work, changes are described in the activity of Co⁴⁺-acylase
and its polymorphic forms during development of rodents. Some properties of
the enzyme are also reported.

MATERIALS AND METHODS

The following animals of either sex were used: three-coloured guinea-pigs,
Wistar rats, Porton mice, syrian golden hamsters, and rabbits (white Polish crossed

¹ Abbreviations used: Co⁴⁺-acylase, cobalt-activated acylase; ClAc-Glu-BNA, N-chloro-
acetyl-γ-L-glutamyl-β-naphthylamide; Bu-Glu-BNA, N-butryryl-γ-L-glutamyl-β-naphthylamide;
GGTP, γ-glutamyltranspeptidase.
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[15]
with White Danish). Foetuses of guinea-pigs and rats were obtained after decapitation of the pregnant females. Time of gestation was calculated since the date of mating.

Freshly isolated tissues were homogenized with 4 vol. of 0.15 M-NaCl in a Potter-Elevehjem glass homogenizer cooled with ice; the sediment was removed by centrifugation at 10,000 g for 10 min, and the supernatant used for the experiments.

Reagents and the method used for determination of Co^{2+}-acylase activity were the same as described earlier (Szewczuk, 1971). Deamination of Leu-Phe was performed by treatment of the dipeptide with nitrite in acid medium (Szewczuk et al., 1974). Zymograms of Co^{2+}-acylase were obtained after electrophoresis in polyacrylamide gel by the procedure described in the accompanying paper (Ziomek & Szewczuk, 1978).

RESULTS

Activity of Co^{2+}-acylase in animal tissues

It was previously demonstrated (Ziomek & Szewczuk, 1978) that in human liver the activity towards CIAc-Glu-BNA and Bu-Glu-BNA as substrates is characteristic for fraction 2 of Co^{2+}-acylase, and that fraction 1 decomposes only CIAc-Glu-BNA. In the present work, the activity towards both these substrates was found in all tissue extracts from the adult animals tested (Table 1). The activity towards CIAc-Glu-BNA was the highest in kidneys, small intestines and livers. In the kidneys of all animals (except the rabbit), and in the liver of hamster and mouse, the activity against Bu-Glu-BNA was about a half that against CIAc-Glu-BNA. In other tissues, the activities with either substrate were closely similar.

Table 1

Co^{2+}-acylase activity in tissue extracts from adult animals

The incubation mixture contained in 0.5 ml: 2 μmoles of substrate, 25 μmoles of Tris/HCl buffer, pH 7.4, 1 μmole of CoCl_2, 5 μmoles of Gly-Gly, 0.2 unit of GGTP and 0.2 mg of protein. The activity was determined against CIAc-Glu-BNA (CIAc) or Bu-Glu-BNA (Bu). Figures in parentheses represent numbers of investigated animals.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>guinea-pig (4)</th>
<th>rabbit (3)</th>
<th>rat (3)</th>
<th>hamster (2)</th>
<th>mouse (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIAc</td>
<td>Bu</td>
<td>CIAc</td>
<td>Bu</td>
<td>CIAc</td>
</tr>
<tr>
<td>Liver</td>
<td>74.3</td>
<td>71.2</td>
<td>8.3</td>
<td>8.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>119.3</td>
<td>41.4</td>
<td>32.5</td>
<td>32.0</td>
<td>31.7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>63.8</td>
<td>59.9</td>
<td>18.6</td>
<td>16.1</td>
<td>10.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>21.1</td>
<td>16.5</td>
<td>—</td>
<td>—</td>
<td>10.6</td>
</tr>
<tr>
<td>Lung</td>
<td>18.2</td>
<td>15.3</td>
<td>4.4</td>
<td>3.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Heart</td>
<td>13.7</td>
<td>10.2</td>
<td>0.9</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Brain</td>
<td>17.0</td>
<td>18.0</td>
<td>7.0</td>
<td>6.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Fig. 1. Changes in Co$^{2+}$-acylase activity in guinea-pig tissues during development. Conditions of assay as given in Table I. Substrates: CIAc-Glu-BNA, outlined columns; Bu-Glu-BNA, hatched columns. Mean activity values from 3 - 4 separate determinations are given. Vertical bars denote the range of the obtained values. B, Birth; A, adult.

Changes in enzyme activity during development

In the guinea-pig tissues distinct changes in Co$^{2+}$-acylase activity were observed during development (Fig. 1). In foetal liver, the activity against both substrates was practically unchanged from day 35 to day 60 of gestation, was raised on the first day after birth, then showed a temporary decrease and rose again to reach the adult values. In kidney, the activity towards Bu-Glu-BNA resembled that in liver, whereas the activity towards CIAc-Glu-BNA rose in a continuous manner. In foetal intestine, both enzyme activities increased with the gestational age, reached the highest values in the newborn animals, and then decreased. The developmental changes of both enzyme activities in the guinea-pig spleen, lung, heart and brain (not shown) were similar to those observed in liver.

Polymorphism of Co$^{2+}$-acylase

In the guinea pig foetus on day 35 of gestation, only one fraction of the enzyme ($R_m$ 0.21), active against both substrates was found on the zymograms of liver and kidney. In 42-day foetuses and the newborn and adult animals, two fractions were found (Fig. 2): both were active towards CIAc-Glu-BNA but only the slower-migrating fraction showed also the activity towards Bu-Glu-BNA. By analogy with the two polymorphic forms of the Co$^{2+}$-acylase found in human liver (Ziomek & Szewczuk, 1978), the faster-migrating fraction ($R_m$ 0.37) was designated fraction $I$, and the less mobile one ($R_m$ 0.21) fraction 2.
Fig. 2. Zymograms of Co²⁺-acylase in tissue extracts from the guinea-pig and rat at different stages of development. Polyacrylamide-gel electrophoresis and staining for enzyme activity were as described by Ziomek & Szewczuk (1978). The electrophoretic mobility (Rm) is expressed as the ratio of the distance migrated by the Co²⁺-acylase fraction to that migrated by bromophenol blue (Rodbard & Chrambach, 1971). F, Foetus (guinea-pig, 42 days; rat, 18 days); N, newborn; A, adult.

In the intestine of the guinea-pig foetuses on days 35, 42 and 50 of gestation, in addition to fraction 2, a still slower migrating fraction (Rm 0.14) was present; it was active with both substrates, and was designated fraction 3. Its contribution in the total enzyme activity in the small intestine extracts of the foetuses was, respectively, 60, 58 and 67%. On the intestine zymograms from the 60-day foetuses and the newborn and adult animals, only fractions 1 and 2 were present; the ratio of their activity measured against CIα-Glu-BNA was 1:4. In the kidney of the 42-day foetus and the newborn and adult guinea-pig, the activity ratio of fractions 1 and 2 was, respectively, 1:1.5, 1:1.5 and 1:0.7, whereas in the liver it was 1:4 and was independent of the development stage. Fraction 3, accompanying fraction 2, was found also on zymograms of placentae taken from female guinea-pigs on the 35th day of pregnancy.

Fractions 2 and 3 (Rm 0.21 and 0.12, respectively) were found to be present on the zymograms of liver and intestine of rat foetus on the 18th day of gestation. Both fractions were active with the two substrates tested, and their activity ratio in the liver and small intestine was, respectively, 1:5.7 and 1:2.3. Fraction 2 was the only fraction present in rat foetal kidney, in liver and intestine of the neonates, and in the intestine of adult animals. In the kidney of newborn and adult rats, both fraction 1 (Rm 0.31) and 2 (Rm 0.21) were found, and the ratio of their activities was, respectively, 1:2.3 and 1:1; in adult rat liver this ratio was 1:5.7. On zymograms of the Co²⁺-acylase from heart, lung, spleen and brain of both adult guinea-pig and adult rat, only fraction 2 was present.
The zymograms of tissue extracts from other adult animals showed the presence of only fraction 2 in rabbit liver and intestine, and of two fractions in rabbit kidney (R_m, respectively, 0.27 and 0.20). In hamster liver, kidney and intestine two fractions (R_m 0.31 and 0.21) were demonstrated, whereas the corresponding mouse tissues contained only fraction 2 (R_m 0.21).

Some properties of the polymorphic forms of the Co^{2+}-acylase in the guinea-pig

The influence of Co^{2+} ion and deaminated Leu-Phe on the activity of the Co^{2+}-acylase fractions resolved by polyacrylamide-gel electrophoresis, was studied in the foetal intestine, and in the liver, kidney and intestine of adult guinea-pig (Table 2). When the zymograms were developed by incubation in the reaction mixture containing phosphate buffer, Co^{2+} ion activated fraction 1 but had little, if any, effect on the activity of fraction 2 and fraction 3. On the other hand, in Tris/HCl buffer both fraction 2 and 3 of foetal intestine were strongly activated by Co^{2+}. Deaminated Leu-Phe inhibited foetal fractions 2 and 3, as well as fraction 2 in adult liver, kidney and intestine, but had no effect on fraction 1. In all the tissues studied preincubation with 5 mM-EDTA for 15 min at 37°C of the acylase fractions separated by electrophoresis in polyacrylamide gel resulted in a complete loss of enzyme activity.

Table 2

Effect of Co^{2+} and deaminated Leu-Phe on the activity of the Co^{2+}-acylase fractions in guinea-pig tissues, measured on zymograms

The tissue extract was submitted to polyacrylamide-gel electrophoresis, then the gels were incubated in a mixture containing 0.8 mM-CoCl_2, 120 mM-phosphate buffer, pH 7.0, and 2 mM-ClAc-Glu-BNA (Ziomek & Szewczuk, 1978). From the densitometric tracings of the zymograms, the relative activity was calculated and expressed as percentage of the activity obtained without the addition of CoCl_2.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fraction 1</td>
</tr>
<tr>
<td>Liver (adult)</td>
<td>172</td>
</tr>
<tr>
<td>+de-Leu-Phe, 0.04 mM</td>
<td>172</td>
</tr>
<tr>
<td>Kidney (adult)</td>
<td>145</td>
</tr>
<tr>
<td>+de-Leu-Phe</td>
<td>137</td>
</tr>
<tr>
<td>Intestine (adult)</td>
<td>270</td>
</tr>
<tr>
<td>+de-Leu-Phe</td>
<td>270</td>
</tr>
<tr>
<td>Intestine (42-day foetus)</td>
<td>0</td>
</tr>
<tr>
<td>+de-Leu-Phe</td>
<td>0</td>
</tr>
</tbody>
</table>

* In parentheses is given the activity in Tris/HCl buffer, pH 7.4.

The effect of Co^{2+} ion in different buffer media on Co^{2+}-acylase was also tested directly in the extracts from liver, kidney and small intestine of adult guinea-pig.
Table 3

Molecular weight of Co²⁺-acylase fractions in guinea-pig tissues

Molecular weight was determined by polyacrylamide-gel electrophoresis according to Hedrick & Smith (1968). The protein standards used were: pepsin (mol. wt. 35 000), bovine serum albumin (67 000), both products of Sigma (St. Louis, Mo., U.S.A.), and human haptoglobin 1-1 (98 000), a kind gift of Prof. Dr. Wanda Dobroszycyka of the Medical School in Wroclaw.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animal age</th>
<th>Fraction</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>adult</td>
<td>1</td>
<td>50 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>45 000</td>
</tr>
<tr>
<td>Kidney</td>
<td>adult</td>
<td>1</td>
<td>54 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>58 000</td>
</tr>
<tr>
<td>Intestine</td>
<td>adult</td>
<td>1</td>
<td>43 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>43 000</td>
</tr>
<tr>
<td>Intestine</td>
<td>42-day-fetus</td>
<td>2</td>
<td>56 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>59 000</td>
</tr>
</tbody>
</table>

and 42-day foetus. The activating effect of this ion was more pronounced when the assays were performed in Tris/HCl buffer of pH 7.4 as compared with phosphate buffer, pH 7.0. The lower activation of the enzyme by Co²⁺ in phosphate buffer could be due to binding of these ions.

The molecular weight of the three guinea-pig Co²⁺-acylase fractions ranged from 43 000 to 59 000 (Table 3).

DISCUSSION

Co²⁺-acylase activity was found in all tissue extracts from adult animals belonging to five rodent species. The activity was also present in the foetus and newborn guinea-pig and rat. This wide occurrence of the Co²⁺-acylase points to its significant role in metabolism.

Two fractions differing in electrophoretic mobility were demonstrated in the liver, kidney and small intestine of adult guinea-pig, rat and hamster; the other tissues tested of these animals, as well as the tissues of the mouse and rabbit, contained only the slower-migrating fraction 2. In the guinea-pig and rat foetus, only fraction 2 was present, accompanied sometimes by fraction 3. Fraction 1 appeared in the peri-natal period. The three fractions differed in substrate specificity (fraction 1 hydrolysed only ClAc-Glu-BNA, and fractions 2 and 3, also Bu-Glu-BNA), and in susceptibility to deaminated Leu-Phe (only fractions 2 and 3 were inactivated). The three fractions had the same molecular weight, and were all inactivated by EDTA, which seems to indicate that they are metalloenzymes.

On the basis of the results presented it seems possible to assume that the three Co²⁺-acylase fractions are different molecular forms of the enzyme.
REFERENCES


AKTYWNOŚĆ I POLIMORFIZM ACYLAZY AKTYWOVANEJ PRZEZ KOBALT W TKANKACH GRYZONI PODCZAS ROZWOJU

Streszczenie

Aktywność acylazy aktywowanej przez kobalt, mierzoną wobec N-chloroacetylo- i N-butyrylo-\(\gamma\)-L-glutamylo-\(\beta\)-naftyloamidu wykazano we wszystkich badanych narządach dorosłych zwierząt.

W nerwie, wątrobie i jelście cienkim dorosłej świńki morskiej i szczura występują dwie frakcje różniące się ruchliwością elektroforetyczną (frakcje 1 i 2). U wczesnych płdów wykazano obecność frakcji 2, a niekiedy i frakcji 3, która w późniejszym okresie zanikała, a pojawiała się frakcja 1.

Jony kobaltu wyraźnie aktywują frakcję 1, natomiast deaminowana leucylo-fenyloalanina silnie hamuje frakcje 2 i 3. W narządach świńki morskiej ciężary cząsteczkowe frakcje są zawarte w granicach 43 000 - 59 000.

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