Table 2. 0.02 M \( \text{K}_2\text{CO}_3 \) in 0.50 M \( +\text{H}_2\text{NCH}_2\text{COO}^-/0.05 \text{ M H}_4\text{NCH}_2\text{COO}^- \).

<table>
<thead>
<tr>
<th></th>
<th>( \text{C}_\text{metal glycinat} )</th>
<th>( \text{C}_\text{CO}_3 \text{ total millimole} )</th>
<th>% carb-</th>
<th>( k\text{amate} + k\text{onate} )</th>
<th>( k\text{amate} )</th>
<th>( k\text{onate} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycinate</td>
<td>0</td>
<td>21.3</td>
<td>33.5</td>
<td>0.0047</td>
<td>0.0016</td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>18.3</td>
<td>39.3</td>
<td>0.0058</td>
<td>0.0023</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>18.0</td>
<td>34.9</td>
<td>0.0065</td>
<td>0.0023</td>
<td>0.0042</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>18.2</td>
<td>36.7</td>
<td>0.0085</td>
<td>0.0031</td>
<td>0.0054</td>
</tr>
<tr>
<td>Cupric glycinate</td>
<td>0.01</td>
<td>18.0</td>
<td>35.6</td>
<td>0.0048</td>
<td>0.0017</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

and cupric cyanide ion, are not titrated

together with excess barium ion.

The details are as follows:

20.00 ml of the specimen are run into a

flask containing 2.00 ml of a mixture that

is 6.4 M in sodium hydroxide and 2 M in

potassium cyanide. 8.00 ml of this mixture

are precipitated with barium chloride and

analyzed as described by Jørgensen. It

was found that a faint blue colour appeared

before the equivalence point was reached,

but at the end point a distinct jump in

colour intensity was observed.

The complexometric titration gives re-

sults which are identical to those obtained

by the method previously used.

The experiments are listed in Table 2.

It is seen, that the catalytic action of

zinc glycinate is significant but it can not

be compared in order of magnitude with

the catalytic action of zinc tetrammine.

A corresponding experiment with 0.01 M

cupric glycinate gave no significant effect.

Acid Soluble Cytidine Nuclotide

Linked Amino Acids in Extracts

of Rabbit Liver

GUNNAR ÅGREN

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University of Uppsala, Sweden

In recent papers \(^1,^2\) it was reported that

\( ^{32}\text{P} \)-labeled trichloroacetic acid (TCA)

extracts of liver and kidneys of the rat

and rabbit contained an ultra violet (UV)

absorbing peak (\( X_4 \)) which contained nin-

hydrin positive substances. After purifica-

tion by ionophoresis and paperchromato-

graphy the material from the peak still

gave a ninhydrin positive reaction. A

labeled UV-peak in the same position of

the elution curve could also be observed

in TCA extracts of \( ^{32}\text{P} \)-labeled tissue homog-

enates of rat kidneys. The nucleotide-

amino acid complex exhibited changes in

UV-absorption spectrum characteristic of

cytidine nucleotide (CMP). Since CMP-

linked amino acids do not seem to have

been observed in animal cells previously

some properties of the fraction will be

described briefly.

In Fig. 1 a section of the elution diagram

of the TCA extract is presented consisting

of the peaks immediately before and after

AMP (adenylic acid). The TCA extract of

rabbit livers was eluted according to Hurl-

bert \( et \ al. \)^\(^3\) The two first labeled UV-peaks

(\( X_4 \)) appearing with formic acid con-

centration between 0 and 0.23 M were taken to

dryness. At paper electropherograms in


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Fig. 1. Acid soluble nucleotides from 7 rabbit livers. The nucleotides were separated on a 6.5 x 45 cm Dowex 1 formate column by gradient elution with reservoir content changed at the tubes numbered as follows: 92, 1 N formic acid; 250, 4 N formic acid. The continuous line represents radioactivity and the broken line represents optical density values at 260 μm.

1 M acetic acid most of the free amino acids were carried away leaving a labeled UV-spot which gave a positive ninhydrin reaction. In one dimensional paper chromatography with isobutyric acid and ammonia materials from both fractions behaved differently. Only with material from the second X₅ peak one sharp spot with congruent UV, ³²P-labeling and positive ninhydrin reaction was obtained together with a ninhydrin negative UV-spot. All of the material from the second X₅ peak was purified in this way. When this fraction was again run through a Dowex 1 formate column with a gradient 0 → 1 M formic acid concentration a sharp peak giving UV-absorption, activity and positive ninhydrin reaction was observed. Both compounds from X₅ exhibited changes in their UV-absorption spectra characteristic of cytidine nucleotides; namely an absorption maximum in acid medium at 280 μm with an absorption minimum at 240 μm. The absorption maximum in neutral solution was located at 270 μm and the minimum at 250 μm⁴. Following hydrolysis in 0.1 N HCl for 3 min⁵ the hydrolysate of the purified material from the second X₅ fraction showed the following properties. In electrophorograms in 1 N acetic acid a sharp, labeled ninhydrin positive spot with UV-absorption was still observed together with three ninhydrin positive spots slowly moving towards the anode and one spot towards the cathode. At pH 5 in 0.1 N pyridine acetate buffer a labeled, ninhydrin positive spot with UV-absorption could be observed together with one ninhydrin positive spot moving towards the anode and one to the cathode. After hydrolysis in sealed tubes for 20 h at 120°C the compound was completely hydrolyzed. Electrophorograms in acetic acid showed at least seven ninhydrin positive spots all moving towards the cathode and one or two which did not move. Papers run at pH 5 showed two ninhydrin positive spots moving against the anode and several to the cathode. A two dimensional electrophero-chromatogram⁶ showed spots corresponding to aspartic acid, glutamic acid, at least six neutral and one basic amino acid. Following hydrolysis in 1 N HCl for 7 min electrophorograms in pyridine acetate showed four sharp, labeled spots giving UV-absorption and positive ninhydrin reaction. Two spots corresponding to one acid and one basic amino acid were also observed.

All these data taken together seem to prove that extracts from animal tissues contain amino acids linked to CMP-nucleotides probably in peptide combinations. The results with 7 min hydrolysis are rather complicated and do not exclude the possibility that the material may consist of a complex of CMP-nucleotides with adjacent amino acids linked to each other. The partial acid hydrolysis may produce free nucleotides, nucleotides with amino acids, peptides and free amino acids. So far we have not been able to isolate a substance with quite similar properties from L. casei.

This investigation was supported by grants from the Swedish Medical Research Council.


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