SHORT COMMUNICATIONS

Wenn es die leichtere Zugänglichkeit eines e-Wasserstoffatoms ist, welche die Oxydationsgeschwindigkeit von Derivaten von sowohl Cyclohexan als von Tetrahydropyran bestimmt, müssen β-Verbindungen der Zucker am glycosidischen Kohlenstoffatom ein e-Wasserstoffatom tragen, da β-Verbindungen ja weit schneller oxydiert werden als die anomeren α-Verbindungen. β-D-Glucose und β-Methylglucosid haben demnach eine axiale Hydroxygruppe oder Methoxygruppe bei C1.


Column Electrophoresis of Nucleotides from Ribonucleic Acid of Brain Microsomes

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Analysis of the nucleotide composition of ribonucleic acids (RNA) is usually accomplished by ion exchange chromatography or by paper electrophoresis. This latter technique is extremely simple and quick, but it allows the handling of only limited amounts of material. In the present work column electrophoresis has been used, and has proved a good tool for this purpose. It has been employed for analysing the nucleotide composition of RNA from brain microsomes, which are similar to liver and pancreas microsomes in showing a high content of RNA.

The separations were performed on a cellulose powder column according to Porath, and with elution beginning before the end of the electrophoretic run for better resolution. Samples of hydrolysates from about 6 mg RNA were handled in the present experiments, but at least four times higher amounts can be as well separated on the same column (2 cm diameter, 70 cm length). Microsomes were prepared by

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differential centrifugation from a homogenate of rat brain in 0.25 M sucrose. The fraction sedimenting between 18 000 × g (15 min) and 105 000 × g (60 min) was immediately precipitated with trichloroacetic acid (10 % final concentration). The precipitate was washed with trichloroacetic acid and extracted thoroughly with organic solvents. All these operations were performed keeping the temperature as close as possible to 0°C, in order to minimize any possible enzymic RNA hydrolysis. The dry precipitate was hydrolysed with 0.3 M KOH at 37°C for 18 h. The hydrolysate was neutralized with perchloric acid, and deproteinized by adding more perchloric acid (0.4 M final concentration). The supernatant after centrifugation was neutralized with KOH, and the perchlorate which precipitated overnight at −5°C was discarded. This supernatant was used for electrophoresis, either as such or after freeze-drying.

The results of one electrophoretic separation with 0.05 M ammonium formate buffer pH 3.5 are shown in Fig. 1. The peaks of the four nucleotides, which have been identified from their relative mobilities in comparison with pure compounds, and from their spectra, are completely separated under these conditions. There is in addition an incomplete separation of the two isomers of guanylic acid, as has already been observed by paper electrophoresis at the same pH 4°C. The small peaks are probably formed by a small aliquot of oligonucleotides.

The spectrophotometric measurement of the optical densities at 260 mμ of the fractions collected permitted the calculation of the relative amounts of the four nucleotides, as reported in Table 1. The extinction coefficients given by Beaven et al.3 were used. The total sum accounts for 90 % of the total nucleotide content of the original hydrolysate, calculated according to Scott et al.4 If the minor peaks are also taken into account, and their amount approximately calculated with the same factor, then the recovery is 94 %. The table reports for
Table 1. Nucleotide composition (in moles per 100 moles) of RNA of brain microsomes, compared with data on other tissues.

<table>
<thead>
<tr>
<th></th>
<th>cat calf rat brain thymus liver microsomes</th>
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</thead>
<tbody>
<tr>
<td>Uridylic acid</td>
<td>19.7 20.6 19.3 20</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>32.5 31.8 33.6 32</td>
</tr>
<tr>
<td>Adenylic acid</td>
<td>18.1 21.6 19.1 21</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>29.7 26.0 28.0 28</td>
</tr>
</tbody>
</table>

* These data have been recalculated, in order to render them comparable.

comparison the only available data on brain RNA. They concern a purified preparation of RNA, obtained from slices of tissue after prolonged incubation. The way of preparation, besides the difference in species, can be the reason for the difference in nucleotide composition. The table reports also some recent data obtained by other authors who have performed, as we have done, direct hydrolysis of the RNA-protein. Although they concern different tissues, they show a close similarity with the present data on brain microsomes.

The fractions corresponding to each peak were pooled and lyophilized twice, in order to remove the ammonium formate. The dry powders, redissolved in a small volume, were used for further analysis. The four recovered nucleotides were submitted to chromatography with various solvents, in order to test their homogeneity. With the solvent isobutyric acid-ammonia the uridylic acid gave rise to an additional spot. Its spectrum and chromatographic behaviour correspond to those of the "fifth nucleotide" described by Davis and Allen.

The same nucleotides were submitted to electrophoresis on paper at pH 2.5. One new spot separated ahead of guanylic acid. When a sample of guanylic acid was submitted to electrophoresis at similar conditions on a small cellulose column, a small faster migrating peak separated, as can be seen in Fig. 2. Its amount, calculated with the same extinction coefficient as guanylic acid, is 2.3% of the total guanylic acid, and its spectrum is similar to that of the unknown component 1 B obtained by Kemp and Allen by paper electrophoresis at pH 2.5 of guanylic acid from pancreas RNA.

Paper electrophoresis of the four nucleotides was performed in borate buffer pH 9.2. One slower migrating compound separated from uridylic acid. It was present also in uridylic acid already deprived from the "fifth nucleotide" by chromatography.

The author wishes to express her gratitude to Dr. J. Porath for constant interest and advice.


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investigated plant, this activity is found in the mitochondria, which can be removed. In the photosynthetic bacteria, those fractions which show LIP have not yet been obtained free from the respiratory system. Thus the ATP-ase activity found in these bacteria $^{**}$ may reflect, at least partly, the OP rather than the LIP system.

A study was made of the ATP-ase activity in isolated spinach chloroplasts. They were prepared $^{**}$ by the method of Allen, Whatley and Arnon. Chloroplasts, freed of mitochondria, do not respire, according to these authors. In agreement with this, the oxygen uptake was zero with succinate as substrate and very low with DPNH. Arnon $^8$ has reported that the chloroplasts seem to be rather free of ATP-hydrolyzing enzymes. We obtain, however, a considerable ATP-ase activity in the presence of added Mg$^{++}$.

The Mg$^{++}$-dependent ATP-ase activity of whole chloroplasts and chloroplast fragments, prepared as in Ref. $^9$, and the strong inhibition caused by atebrin are shown in Table 1. Löw $^{11}$ has shown that atebrin and chlorpromazine inhibit mitochondrial Mg$^{++}$-activated ATP-ase and that some other ATP-hydrolyzing enzymes, which are not connected with electron transport systems, are unaffected. 1 mM chlorpromazine gave about 85% inhibition with chloroplast fragments.

<table>
<thead>
<tr>
<th>$\mu$moles P</th>
<th>liberated/h/mg chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole chloroplasts</td>
<td>9</td>
</tr>
<tr>
<td>$\uparrow$ $\uparrow$ + atebrin</td>
<td>0.7</td>
</tr>
<tr>
<td>Chloroplast fragments</td>
<td>9</td>
</tr>
<tr>
<td>$\uparrow$ $\uparrow$ + atebrin</td>
<td>0.4</td>
</tr>
</tbody>
</table>

$^{**}$ Many thanks are due to Dr. M. B. Allen for showing the author how to isolate spinach chloroplasts.

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### Adenosine Triphosphatase in Chloroplasts

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The following two hypotheses are strongly supported by experimental evidence. 1. Light-induced phosphorylation (LIP) is an electron transport phosphorylation $^{12}$. 2. The ATP-ase reactions in mitochondria mirror oxidative phosphorylation (OP) reactions, in the reverse direction, going from ATP towards the electron transport chain $^4$.

Assuming that hypothesis 1 is correct, the question arises as to what extent similar or identical mechanisms are operating in the generation of ATP in OP and in LIP. Assuming that hypothesis 2 is correct, an investigation of the ATP-ase reactions in LIP systems should give information about the possible similarity between the two phosphorylation mechanisms.

Of the two known LIP systems, plant chloroplasts and bacterial chromatophores, the former have been obtained free from respiratory activity. In spinach, the most

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* Abbreviations: P, orthophosphate; ATP, adenosine triphosphate; ATP-ase, adenosine triphosphatase; DPNH, reduced diphosphopyridine nucleotide; M, moles per liter.