NOTIZEN
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Experimentelles
Die Bakterien E. coli B 163 wurden zuvor durch 1-stündiges Inkubieren bei 37 °C in bezug auf K⁺-Ionen und Energiesubstrat ausgewählt. Inkubations- und Waschungszeit: 50 nM Na₂HPO₄ mit 0,23 nM HCl auf pH 9,0 eingestellt. Zur Messung wurde das zentrifugierte Zellsediment in folgender Pufferlösung suspendiert: 30,0 nM NaH₂PO₄ + 35,4 nM NaH₂PO₄, pH 6,7–6,8. Die Bakterienstamm ist auxotroph für His, Leu, Met; bezüglich Nährmedium und Züchtung siehe 12.

Effect of Triton X-100 on the Galactosyltransferase Activity of Chick Embryo Cells

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(Z. Naturforsch. 26 b, 625—626 [1971] ; received April 3, 1971)

An in vivo pilot study indicated that galactose can be incorporated into glycoproteins of chick embryo cells. We therefore attempted to develop a non-cellular system, capable of incorporating, in vitro, this carbohydrate from UDP-galactose.

All steps of the enzyme preparation were performed between 0° and 5°. Decapitated 8 day-old embryos were suspended in 0.25 M sucrose buffered with 5 x 10⁻² M Tris-HCl pH 7. The suspension was homogenized with 20 strokes in a Potter-Elvehjem homogenizer. The crude homogenate was centrifuged at 19,500 x g for 1 hour, resulting in the separation of a microsomal pellet from the cell sap. The microsomes were incubated with UDP-galactose (1³H, New England Nuclear) for 40 min at 37°, the optimum temperature. The macromolecules were precipitated with a final concentration of 10% trichloracetic acid, filtered (Whatman Glass Paper, type GF/B) and washed on the filter with a 4/1 methylal-methanol mixture. Radioactivity, measured by liquid scintillation spectrometry, was related to the content of protein as determined by the Lowry method.

The conditions for assay are given in Table I.

The activity of galactosyltransferase was detectable only when Mn²⁺ and Triton X-100 were added to assay mixture. Maximal activity is obtained with 2 mM Mn²⁺, when incubated at pH 7, the optimum pH.

The Km, with respect to UDP-galactose was determined by varying the UDP-gal³H concentration in the incubation mixture. The data gave a Km of 1.8 x 10⁻⁵ M.

In order to evaluate the effect of Triton X-100, the variation in transferase activity was determined as a function of detergent and protein concentrations. As can be seen from Fig. 1, the efficiency of the action of Triton X-100 appeared to be dependent not only on the concentration of detergent but also on the detergent/protein ratio. Maximal activity is obtained with a Triton X-100 concentration of 2.5 ml/l for 1 mg protein.

Table I. The Assay System for the Galactosyltransferase from chick embryo cells. (The complete assay system contained in 280 µl : 10 µl Mn²⁺ 55 mM, 20 µl 1.5% Triton X-100, 200 µl of microsomes (0.5 mg protein) in 0.05 M Tris buffer pH 7 and 50 µl UDP-galactose-1³H (0.01 µmole, approximately 2.2 x 10⁶ cpm).)

<table>
<thead>
<tr>
<th>System</th>
<th>cpm incorporated into 1 mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>complete</td>
<td>2,200</td>
</tr>
<tr>
<td>Triton</td>
<td>60</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>150</td>
</tr>
<tr>
<td>Mn²⁺ + Mg²⁺</td>
<td>120</td>
</tr>
<tr>
<td>Triton + desoxycholate</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of Triton X-100 on galactosyltransferase from chick embryo cells. Protein concentration in incubation mixture: ●—● 0.4 mg; x—x 1 mg.

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12 R. L'ORANGE u. F. SCHNEWEISS, Biophysik 7, 40 [1970].
Similar results were obtained with the respiratory chain enzymes of a heart muscle preparation\(^1\) or with mannosyltransferase from *Aspergillus niger*\(^2\). Recently, the kinetic properties of three glycosyltransferases found in the synaptosome rich fraction obtained from embryonic chick brain were described\(^3\). All the three enzymes showed an absolute requirement for detergent, but optimal detergent/protein ratio was not determined.

\(^1\) D. SOLTYSIAK and Z. KANIUGA, European J. Biochem. 14, 70 [1970].

\(^2\) R. LETOUBLON, M. RICHARD, P. LOUBOT, and R. GOT, European J. Biochem. 18, 194 [1971].


Cross-Bridges Between Intramacronuclear Microtubules and Inner Nuclear Membrane

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(Z. Naturforsch. 26 b, 626—627 [1971]; received March 19, 1971)

During macronuclear division of ciliates, intranuclear microtubules are observed which seemingly play a functional role in the division mechanism\(^4\)\(^5\)\(^6\). Such microtubules can often be seen in a close association with the inner nuclear membrane. The question is if (and if so, how) these intramacronuclear microtubules are truly structurally connected with the membrane. Such a linkage has been previously suggested as being effected by a basal insertion of the tubules at the inner surface of the nuclear envelope\(^7\)\(^8\). As a consequence of the section thickness, which is usually more than the microtubular width, this type of basal anchoring is hard to demonstrate clearly. In the present study it is shown that such intramacronuclear microtubules in *Tetrahymena pyriformis* can be connected to the inner nuclear membrane by characteristic lateral "cross-bridges" (Figs. 1—5) with a mean width of 50 Å. Sometimes C-shaped side-arm profiles were also found (Figs. 2 and 3). In sections showing the microtubules lengthwise, i.e. paralleling the inner nuclear membrane, the bridges often appeared not to be arranged perpendicularly but rather construct a lower angle with the membrane (c.f. Figs. 4 and 5). The lengths of the cross-bridges varied from 80 to 250 Å. The microtubules of the present study were found to be rather wide with diameters up to 300 Å. The mode of connection described in this note fits into the concept that cross-bridging to microtubules is a general capacity of biomembranes\(^8\) and leads to the impression that certain proteins of the nuclear membrane, the microtubules (and possibly also of the chromosomes) behave as an integrated self-assembly system during nuclear divisions in which the nuclear envelope remains intact. The particular situation of the *Tetra*

Fig. 1—5. Microtubulus within the macronucleus of *Tetrahymena pyriformis* GL; N, nucleoplasma; C, cytoplasma; R, ribosomes. Microtubules can be cross-bridged with each other (Fig. 1, arrow in upper left) as well as with the inner nuclear membrane (Fig. 1 and 2). Sometimes pair of cross-links (C-shaped) are recognized beside the single bridges (Fig. 3, arrows). In longitudinal section the microtubules (denoted by black triangles in Fig. 4) show the side-arms as being perpendicularly oriented (Fig. 4) or as arranged under a lower angle (Fig. 5). Fig. 1: 100,000:1; Fig. 2, 72,000:1; Fig. 3, 210,000:1; Fig. 4, 140,000:1; Fig. 5, 110,000:1.


\(^5\) S. TAMURA et al., Exp. Cell Res. 55, 351 [1969].


\(^7\) F. WUNDERLICH and V. SPEITH, Protoplasma 70, 139 [1970].

\(^8\) W. W. FRANKE, Cyto biologie, in press.