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Short Communication

Demonstration of Endogenous Sialyltransferase on the Surface of Ehrlich Tumour Ascites Cells

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The plasma membrane of several tumour cells is capable of forming ATP under certain experimental conditions. Part of this ATP synthesis occurs on the outer surface of the intact tumour cell (1, 2, 3, 4, 16). Furthermore, the newly formed ATP can be converted to other nucleoside-triphosphates on the tumour cell surface (5). Among the nucleoside-triphosphates formed on the tumour cell surface is CTP, which is a necessary metabolite for the formation of sialoproteins and sialolipids of the cell. This focused our interest on the sialic acid metabolism on the surface of Ehrlich tumour cells.

Earlier investigations have mostly been concerned with sialyltransferase in subcellular fractions or the total homogenate of cells and tissue (8, 10, 15). However, recently, some studies on intact cells have given strong evidence for the presence of sialyltransferase activity on the cell surface (6, 9). The first report of such an ecto-sialyltransferase present in normal and virus-transformed fibroblasts was given by Bosman (7).

In the present work it is clearly shown for the first time that endogenous sialyltransferase activity exists on the surface of intact Ehrlich ascites cells. Several control experiments were performed to emphasize that the reaction studied was due to the existence of cellsurface-bound sialyltransferase and endogenous glycoprotein or glycolipid acceptors.

The Ehrlich cells were obtained by a slight modification of a method previously described (16). 0.85×10^8 cells were incubated in a Krebs-Ringer-bicarbonate (KRB) buffer which was equilibrated with 6% CO_2 and 94% O_2. The cells were incubated in a final concentration of 10 μM CMP-*N*-acetyl-neuraminic acid (CMP–NANA). Unlabelled CMP–NANA was prepared and purified on a Dowex 1-X2 column using a $H_2O \rightarrow 1$ M NH_4 HCO_3 gradient according to Kean et al. (12, 13). CMP-^{14}C-NANA was purchased from NEN Chemicals (Mass. USA). Incubation was for 30 min at 37°C unless otherwise stated. The incubation was terminated by dilution severalfold with ice-cold KRB buffer followed by centrifugation. The centrifuge tubes were rinsed with cold KRB and the cell pellet was washed twice in 3% ice-cold sulfosalicylic acid containing unlabelled sialic acid. The cell pellet was then digested in Protosol and counted in Aquasol. (NEN Chemicals). The incorporation of sialic acid from the CMP-^{14}C-NANA precursor into the cell pellet was within 20–40 pmoles per 30 min and 1×10^8 cells.

Figure 1 illustrates a Lineweaver-Burk plot where apparent K_m and V_{max} values can be obtained. In this experiment the apparent K_m was 7.0 μM and the apparent V_{max} 36 pmoles per 30 min and 1×10^8 cells. The time dependency of the incorporation is given in Fig. 2. The cells were incubated with 10 μM CMP-^{14}C-NANA. In order to maintain the viability of the cells during the long incubation period, glucose was added to a final concentration of 100 mg % (w/v) in all experiments. To diminish the variability between similar experiments it was necessary to use cells from one single animal.

A number of control experiments were necessary to exclude the possibility of unspecific adsorption of radioactive metabolites. One type of experiments involved incubation with 10 μM ^{14}C-NANA showing only insignificant labelling of the cell pellet, indicating the necessity of CMP–NANA as a precursor metabolite. Another type of control was the incubation with CMP-^{14}C-NANA as has been de-

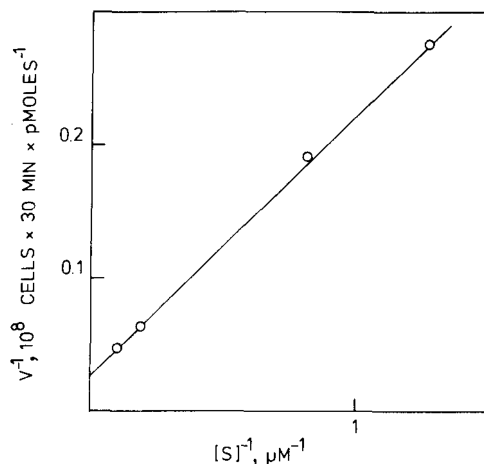

Fig. 1. A Lineweaver-Burk plot of the surface-bound sialyltransferase of Ehrlich cells. 1×10^{8} cells were incubated for 30 min at 37° with different concentrations of CMP-^{14}C-NANA. For further details, see text.

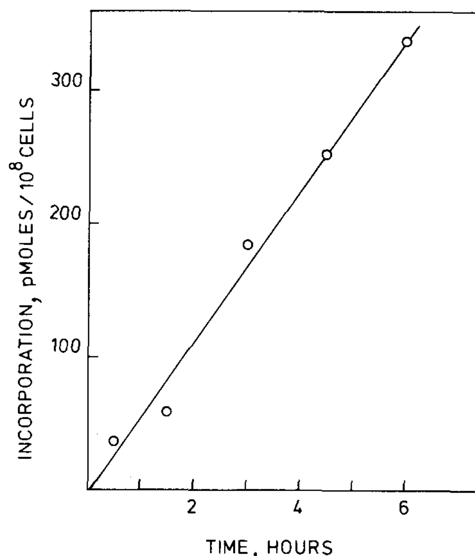

Fig. 2. Time dependency of CMP-^{14}C-NANA incorporation into 1×10^{8} intact Ehrlich cells. 70 to 80% of the incorporated substrate was hydrolysed by extracellularly added neuraminidase. For further details, see text.

scribed, now also together with 1 mM unlabelled NANA or 3 μM of unlabelled CMP. The presence of these metabolites does not at all influence the incorporation ability of the cells. The degree of spontaneous hydrolysis of the prepared CMP–NANA did not exceed 5% as judged by the thiobarbituric acid colour method (14). Furthermore, incubation with CMP-^{14}C-NANA at 0°C does not give any significant incorporation. Preincubation with 10 μM unlabelled CMP–NANA for 30 min at 37°C followed by the addition of radioactive CMP–NANA and the immediate termination of the reaction resulted in negligible amounts of radioactivity in the cell pellet. This excludes the possibility of adsorption to successively accumulating cell debris.

The following experiments were performed in order to demonstrate that sialyltransferase as well as the acceptor molecules, were endogenous and firmly bound to the surface of the Ehrlich cells. The cells were washed up to 10 times in the KRB buffer before incubation with CMP-^{14}C-NANA. Their incorporating ability still remained intact. When the cells after incubation with CMP-^{14}C-NANA were washed and suspended in 100-times their volume and finally incubated in the KRB buffer for 30 min at 37°C no label was lost. When the cells were grown for 2 days in F-10 medium[1] and then incubated with CMP-^{14}C-NANA as previously described they still displayed their ability to incorporate the labelled compound. At the end of the culturing period the Ehrlich cells were contaminated by 4‰ of granulocytes and lymphocytes. The granulocytes were predominating. No macrophages were present (K. Nilsson, personal communication). The incorporating ability thus seemed to be due to the Ehrlich cells.

Leakage of soluble or membrane-bound sialyltransferase from the cell interior was ruled out by the following experiments. Two cell pellets each containing 0.85×10^{8} cells prepared from the same mouse were suspended and incubated in the following way: The first one was suspended in 1.5 ml KRB buffer and incubated with 10 μM CMP-^{14}C-NANA for 30 min at 37°C. The second pellet was suspended in the same volume of the fourth cell-free washing from the preparation of Ehrlich cells and incubated with labelled CMP–NANA. A cell-free supernatant was obtained by centrifuging for 10 min at 1 500 *g*. The incorporation values were 34 and 35 pmoles per 10^{8} cells, respectively. It is clearly seen that there is no significant difference in the incorporating ability regardless of the composition of the medium. From these results it is reasonable to assume that the sialyltransferase is firmly

[1] F-10 is a synthetic medium containing 10% of calf serum.

bound to the intact cell surface and not derived from the cell interior.

The most convincing evidence for the real incorporation of the extracellular CMP-^{14}C-NANA into sialoproteins and sialolipids of the surface membrane came from experiments in which the cells after labelling with this substrate were washed and postincubated for 30 min at pH 6.5 with neuraminidase from *Clostridium perfringens* (Sigma). A modified Krebs-Ringer-phosphate buffer was used. It is known that neuraminidase specifically splits terminal glucosides of α-*D*-configurated *N*-acetylneuraminic acids (11). Such an enzymatic treatment of the intact cells reduced the label of the cell pellet to only 20–30%. A corresponding treatment with the buffer lacking this enzyme did not change the label.

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