Vol. 87, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 27, 1979 Pages 989-992

2-DEOXY-2-FLUORO-L-FUCOSE AND ITS EFFECT ON L-[1-^{14}C] FUCOSE UTILIZATION IN MAMMALIAN CELLS

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Received February 23,1979

Summary: 2-Deoxy-2-fluoro-L-fucose has been obtained as a chromatographically pure compound from 3,4-di-O-acetyl-L-fucal and its structure established by NMR measurements. Mouse fibroblasts (TAL/N P and TAL/N B) in culture incorporate L-[1-^{14}C] fucose into glycoproteins of trypsinates and of cell components. The labelling is competitively and progressively diminished when 2-deoxy-2-fluoro-L-fucose or L-fucose is added to the medium in the range 0.1mM to 10mM. Evidence suggests that the fluorosugar is incorporated into glycoprotein fractions.

A growing body of evidence indicates that N-fluoroacetyl-D-glucosamine (1,2) and fluorohexoses (3,4) are taken up by cells and become incorporated into hyaluronate and glycoprotein components. The fluoroanalogues appear to compete with the non-fluorinated sugars and to be incorporated apparently by the same enzymic steps (5).

So far little evidence has been obtained of any marked toxicity when fluoroanalogues are presented in low concentrations (less than 5mM). The structural effects of the incorporation of such a modified sugar on the antigenic determinant groups involving oligosaccharides remains to be explored. In a number of such groups, especially of the ABO system, L-fucose plays a critical role where it is present in terminal positions of carbohydrate side-chains.

The present work considers evidence for the effect of 2-deoxy-2-L-fucose on L-fucose labelling of glycoproteins synthesized by two mammalian systems, in vitro.

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0006-291X/79/080989-04$01.00/0

Experimental:

2-Deoxy-2-Fluoro-L-Fucose. The sugar was obtained by addition of CF_3OF to di-O-acetyl-L-fucal, chromatographic separation of the products on a silica column and deacetylation of the corresponding peak (6). The free sugar $[\alpha]_{546}^{22}$ - 91.2 (c 0.35, H_2O) had $R_{Glucose}$ 6.8 in ethyl acetate/pyridine/water, 8:2:1 v/v. (cf. L-fucose $R_{Glucose}$ 2.3)

TAL/N P and TAL/N B Fibroblast Cell Cultures. Cells obtained from Dr.P.T. Mora, National Cancer Institute, Md. U.S.A. were grown in Dulbecco's modification of Eagle's medium (DMEM) with added glutamine, sodium bicarbonate antibiotics and 10% foetal calf serum. Cultures were maintained in 100 ml medical flats containing 10 ml of the complete medium, in an atmosphere of 95:5 air/CO_2. Medium was changed on the second day and cells sub-cultured on the third when cells were usually in late logarithmic growth. Cells were harvested from the vessels by being washed with 0.25% or 0.01% trypsin for 5 min at room temperature.

Cell Labelling experiments. Radioactivity was measured in the trypsinates, in cells and in cells precipitated with trichloroacetic acid and collected on a millipore filter disc by the method used previously (7,8).

Results and Discussion

TAL/N P cells (2 x 10^5 per petri dish) grown for 5 days to near confluence were exposed for a further 20 h to additional medium (2.5 ml) containing 0.5 μCi/ml of L-[1-^{14}C]fucose. Labelling of the whole cells (TCA precipitated and washed) under these conditions was 12,000 dpm per 0.1mg of cell protein.

In other cultures maintained under the same conditions, simultaneously and with comparable cell numbers, cold carrier L-fucose added in the range 0.01M to 10mM, progressively diminished the ^{14}C-labelling of whole cells. Similar diminution in ^{14}C-labelling was brought out in corresponding experiments with 2-deoxy-2-fluoro-2-L-fucose as cold carrier in the same concentration range. (Table) At 5.0mM, the percentage of the control ^{14}C incorporation in the trypsinate was 40% (fucose) and 38% (fluorofucose) and in cells, values were 17% (fucose) and 14% (fluorofucose). In the trypsinate about 40% of the radioactivity was in low-molecular weight material. Above 10mM cytotoxic effects were observable by phase contrast spectroscopy.

Further experiments were carried out with TAL/N B (an early passage of the 'spontaneously' transformed cell line) in the logarithmic growth

Table Labelling of confluent TAL/N P cells by L-[1-^{14}C] fucose maintained for 20 hr in presence of L-fucose and 2-deoxy-2-fluoro-L-fucose.

L-Fucose						
Final concentration in medium (mM)	0	0.25	0.5	1.0	5.0	10
Labelling (dpmx10^{-2} in cells)	128.0	32.3	7.4	0.45	0.14	0.13
2-Deoxy-2-Fluoro-L-Fucose						
Final concentration in medium (mM)	0	0.25	0.5	1.0	5.0	10
Labelling (dmpx10^{-2} in cells)	84.0	55.0	22.4	1.14	0.14	0.13

Values are average of two experiments

phase and using the milder type of trypsinisation. Both the trypsinate and the trysinized cells readily incorporated L-[1-^{14}C]fucose. As in the preceeding experiment, addition of the 2-fluorosugar competitively diminished the degree of all labelling though the reduction (50% of control at 5mM fluorosugar) was less marked than in the previous experiment.

Double-labelling experiments of TAL/N P in which L-[1-^{14}C]fucose (0.5 μCi/ml) was accompanied by D-[1-^{3}H]glucosamine or L-[4,5-^{3}H]leucine or [6-^{3}H]thymidine or [5-^{3}H]uridine (each at 1 μCi/ml) were carried out under the preceeding conditions. The tritiated metabolites were without significant effect on labelling of cell components by ^{14}C-fucose, nor did they influence the pattern of labelling in dilution experiments with non-radioactive fucose or fluorofucose (in the concentration range 0.1 to 5mM).

With isotopically labelled 2-deoxy-2-fluoro L-fucose it may be possible to determine accurately the amount of the modified sugar entering macromolecular structures and its chemical location. The present preliminary results however indicate that the fluoroanalogue appears to be able to enter the cell to compete with the parent sugar in glycoprotein biosynthesis.

Similar results are observed in other systems for 2-deoxy-2-fluoro-D-mannose and -glucose (3,4) and for 6-deoxy-6-fluoro-D-glucose and related sugars (9,10).

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