Glycosylation alterations of cells in late phase apoptosis from colon carcinomas

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Comparisons of carbohydrate profiles between control and apoptotic colon carcinoma cells were performed by flow cytometry using a set of lectins and anti-carbohydrate antibodies. The six cell lines analyzed presented distinct carbohydrate profiles before induction of apoptosis. PHA-L and MAA binding decreased after induction of apoptosis by UV-treatment. In contrast an increase of PNA binding was observed after induction of apoptosis, except on SW-48 cells for which a decrease occurred. A decrease of SNA binding was observed after induction of apoptosis from strongly positive control cell lines, whereas it increased on weakly positive ones. All the blood group related antigens A, H, Lewis a, Lewis x, Lewis b, and Lewis y, had their expression strongly diminished on apoptotic cells. These changes occurred irrespective of the mode of apoptosis induction since similar results were obtained after UV, TNFα, or anti-Fas treatment. Fucosyltransferases activities were also decreased after apoptosis induction, except for α1,3fucosyltransferase in anti-Fas treated HT-29 cells, where it was strongly augmented. This could be attributed to the IFNγ pretreatment required to induce Fas expression on these cells. Fucosidase activity decreased after induction of apoptosis suggesting that it was not responsible for the loss of fucosylated structures. In the rat PRO cell line, H blood group antigens are mainly carried by a high molecular weight variant of CD44. It could be shown that the loss of H antigen after induction of apoptosis correlated with a loss of the carrier glycoprotein.

Key words: apoptosis/colon carcinoma/glycosylation/fucosyltransferases/fucosidase

Introduction

Apoptosis, or programmed cell death, is an active mode of cell death that is highly conserved and regulated. It occurs physiologically from development through adult life where it participates to the maintenance of the dynamic steady state in cell turnover of many tissues. It also occurs pathologically in conditions such as cancer, autoimmune diseases, neurodegenerative diseases, or infectious diseases. Apoptosis is characterized by cell shrinkage, nuclear condensation, protease activation, and finally DNA fragmentation (Granville et al., 1998; Raff, 1998). Alterations in cell surface molecules and most specifically of glycan structures are also observed. These include a loss of sialic acid residues and inversely an increase in accessibility of galactose, mannose, fucose, and N-acetylgalactosamine residues (Morris et al., 1984; Akamatsu et al., 1996; Falasca et al., 1996; Russel et al., 1998). Once apoptotic cells or bodies have been generated, the terminal phase of the process is their rapid elimination by neighboring cells or by cells of the macrophage type. This elimination of apoptotic cells by phagocytes is of utmost importance since it prevents the release of unwanted molecules and the initiation of an inflammatory response (Ren and Savill, 1998). The engulfment of apoptotic bodies by phagocytes requires specific recognition systems, the best characterized being the exposure of phosphatidyserine at the surface of the apoptotic cell and its recognition by a specific receptor located at the surface of the phagocyte (Fadok et al., 1998). It is similarly believed that some changes in glycan exposure could be key steps in the process of removal of apoptotic cells (Duvall et al., 1985; Dini et al., 1995; Falasca et al., 1996). Indeed, an unmasking of galactose residues occurs after the loss of sialic acids and phagocytosis of rat apoptotic hepatocytes by neighboring hepatocytes carrying the asialoglycoprotein receptor could be inhibited by galactose, desialylated glycoproteins as well as by anti-asialoglycoprotein receptor antibodies (Dini et al., 1992). Similarly, participation of the mannose/fucose receptor to the phagocytosis of apoptotic bodies by neighbor cells has been suggested (Hall et al., 1994).

The alterations in glycosylation of apoptotic cells have previously been mainly studied on hepatocytes or on subsets of leukocytes, but since a large heterogeneity in cell surface glycosylation exists between cell types, it was of interest to compare the changes that would occur on cells originating from colorectal carcinomas. Such cells can express distinct carbohydrate structures which have their expression developmentally regulated and are tumor associated (Hakomori, 1996). These include increased α2,6sialylation, β1,6GlcNAc branching at the trimannosyl core of N-linked oligosaccharides and increased expression of ABH and Lewis related antigens (Kim and Varki, 1997). In the present study, we thus report our investigation of some changes in glycosylation that occur at the cell surface of colon carcinoma cell lines. This was performed using a set of lectins and of monoclonal antibodies of well defined carbohydrate specificity toward the above-mentioned developmental and tumor associated glycan structures.
Fig. 1. DNA fragmentation in HT-29 cells after anti-Fas treatment. Cells were treated with IFNγ and anti-Fas as described in the materials and methods section. Floating treated cells or adherent untreated cells were incubated for 2 h with proteinase K. The DNA was extracted with phenol-chloroform and precipitated. After RNase A treatment, the DNA fragments were resolved by electrophoresis on 1.8% agarose gel and visualized under UV light by ethidium bromide staining. Lane 1, size markers; lane 2, DNA from adherent untreated cells; lane 3, DNA from floating IFNγ + anti-Fas treated cells collected 24 h after treatment; lane 4, DNA from floating IFNγ + anti-Fas treated cells collected 48 h after treatment.

Results

Induction of apoptosis

Three different treatments known to induce apoptosis were tested on six colon carcinoma cell lines: UV, TNFα and anti-Fas treatments. In order to confirm that these treatments induced apoptosis in the cell lines used, DNA fragmentation was monitored. As shown in Figure 1, anti-Fas treatment of HT-29 cells induced DNA fragmentation characteristic of apoptosis in detached cells recovered in the supernatants 24 or 48 h after addition of the anti-Fas antibody. Seventy-two hours after induction, only the low molecular weight fragments were visible, the unfragmented high molecular weight DNA being no longer visible (not shown). However in the latter case, floating cells incorporated trypan blue and thus were not used for the study of cell surface glycans. No such degradation was evidenced in the untreated cells. Staining of nuclei with Hoescht 33258 confirmed that floating treated cells presented nucleolar condensation typical of apoptosis, no such sign being visible in control untreated cells (data not shown). Upon induction of apoptosis, epithelial cells are known to detach from their substrate. As already reported by others (Günthert et al., 1996), only detached HT-29 cells showed DNA fragmentation and nucleolar condensation. Adherent treated cells did not present these signs of apoptosis. Similar observations were made with the two other inducers of apoptosis, UV or TNFα treatments, on HT-29 and other cell lines (data not shown). Twenty-four hours after induction of apoptosis, from 10 to 20% cells were floating. After 48 h, this proportion doubled. Therefore, comparisons between floating and adherent untreated cells could be performed to compare glycosylation patterns between control and apoptotic cells.

Cytofluorimetric analysis of apoptotic cells

The binding of various lectins to the colon carcinoma cell lines was determined by flow cytometry. As depicted in Figure 2A, large differences were visible among the cell lines. For example, cell lines such as PRO and SW-48 were strongly stained by PNA, whereas the others were only weakly stained. The same 2 cell lines were also strongly stained by PHA-L, but MAA staining was strong only on HT-29 and SW-48. After UV treatment (Figure 2B), the binding of PHA-L and of MAA was largely decreased from all cell lines that were significantly labeled by these two lectins before treatment. Concerning the
Fig. 4. Inhibition of the binding of SNA and PNA lectins to apoptotic cells by free ligands. (A) UV-treated floating PRO cells were stained with FITC-labeled SNA alone or in the presence of 420 μM 6’sialyl-lactose and analyzed by flow cytometry. Fluorescence intensities are given in arbitrary units. (B) anti-Fas-treated HT-29 cells were stained with FITC-labeled PNA alone or in the presence of 0.2 M galactose. Adherent untreated cells were also stained with the lectins (control) showing the low binding of SNA to standard PRO cells and of PNA to standard HT-29 cells.

The fate of fucosylated structures was next analyzed using the UEA-1 lectin. It showed an increased binding to all cell lines after induction of apoptosis. However, while the binding to spontaneously positive cells such as PRO was readily inhibited by 0.2 M fucose, the binding to apoptotic cells could not be inhibited at all, indicating that it was nonspecific (data not shown). A panel of antibodies specific for some of the fucosylated antigens was therefore used. A control irrelevant antibody showed only a small background on both untreated and treated cells. As shown on Figure 5A, each specific antibody presented a unique binding pattern on the six cell lines. Only two of them SW-707 and HT-29 were blood group A positive; three cell lines expressed H type 3/4 antigens (PRO, SW-1116, and SW-48). Similarly, the Lewis type antigens were unevenly distributed among cell lines. After UV induction of apoptosis, the binding of the antibodies to all cell lines largely decreased (Figures 3, 5B). Different inducers of apoptosis were used: TNFα on PRO and SW-48 and anti-Fas on HT-29. The same results were obtained in each case. The expression of fucosylated antigens and of α2,3 linked sialic acids decreased after apoptosis induced by each of the three treatments (Figure 6). In the case of HT-29, induction of apoptosis by TNFα and anti-Fas required an IFNγ pretreatment. This pretreatment itself did not induce any detectable apoptosis. However, it strongly enhanced the binding of the anti-Lewis y and Lewis x antibodies on adherent cells.
Changes in fucosyltransferases activities upon induction of apoptosis

There exist various fucosyltransferases which can participate to the synthesis of the antigens revealed by the panel of antibodies that was tested as described above (Costache et al., 1997). We previously showed that PRO cells contain a significant α1,2fucosyltransferase activity, in accordance with their expression of H antigens. After UV and TNFα treatments, PRO apoptotic cells presented a largely decreased enzymatic activity (Figure 7). HT-29 present a strong α1,3fucosyltransferase activity detected using the H type 2 trisaccharide as acceptor. This enzyme activity was largely diminished after
induction of apoptosis by UV irradiation. Inversely, after induction by anti-Fas, the activity was increased over 2.5-fold. Yet, this increase was not due to the apoptotic process itself, since it was already visible in cell extracts from IFNγ-treated cells, which do not show any sign of apoptosis (Figure 7). The synthesis of Lewis x and Lewis y antigens requires an α1,3fucosyltransferase. Thus, the increased expression of these antigens after IFNγ treatment can be explained by an induction of the enzyme activity. Nevertheless, this does not correlate with the antigenic expression which decreases from apoptotic cells, irrespective of the inducer (data not shown). Synthesis of the Lewis a and Lewis b antigens requires an α1,4fucosyltransferase which was assayed using the biotinylated H type I trisaccharide as acceptor. This enzyme activity was unchanged after IFNγ treatment, but was decreased in cell extracts from apoptotic cells irrespective of the inducer (data not shown).

Changes in fucosidase activity upon induction of apoptosis

Since the loss of fucosylated antigens observed after induction of apoptosis could not always be correlated to a decrease of the corresponding fucosyltransferase activity, we tested whether there was an increase of fucosidase activity that could be responsible for the degradation of the antigens. After UV treatment, both HT-29 and SW-1116 showed a diminished fucosidase activity (Figure 8). The other cell lines presented a lower fucosidase activity that was unchanged after UV treatment (data not shown). Therefore, the changes in this enzymatic activity could not explain the drop of fucosylated antigens.

Loss of the carrier protein from apoptotic cells

Various proteases are known to be involved in the process of apoptosis. Such enzymes could potentially release the glycoproteins that carry some of the glycans lost after induction of apoptosis. We had shown earlier by immunoprecipitation experiments, that most of the H antigen present at the surface of PRO cells was born by a high molecular weight variant of CD44 carrying the product of exon v6 (Labarrière et al., 1994; Hallouin et al., 1999). We therefore tested the expression of the CD44v6 peptide on PRO cells, using a specific antibody, together with that of the H antigen, before and after induction of apoptosis. After both UV and TNFα treatments, the expression of H antigen strongly decreased (Figure 9). A parallel decrease was noted for the CD44v6 epitope, indicating that the loss of the fucosylated antigen was due to a loss of the carrier protein itself.

Discussion

In this study, the previously suggested loss of cell surface sialic acid residues on apoptotic cells was confirmed on some cell
Untreated UV treated TNF treated

\% activity relative to control

Control UV treated IFN treated IFN + anti-Fas treated

\% activity relative to control

Fig. 7. Fucosyltransferases activities after induction of apoptosis. 
\(\alpha_2\) fucosyltransferase (\(\alpha_2\)FT) activity was determined in adherent untreated and floating UV-treated or TNF treated PRO cells using Gal\(\beta_3\)GalNAc-biotin as acceptor. Enzyme activity in PRO control cells was 227 pmol/h/mg protein. \(\alpha_3\) fucosyltransferase (\(\alpha_3\)FT) activity was determined, using biotinylated H type 2 trisaccharide as acceptor, in adherent untreated and IFN-treated HT29 cells, as well as in floating UV-treated, IFN++ anti-Fas-treated cells. Enzyme activity in HT29 control cells was 4.75 nmol/h/mg protein. The percentages of activity relative to those of untreated control cells are given and represent the means of two independent experiments.

lines since the binding of the \(\alpha_2,3\) linked neuraminic acid specific lectin MAA to apoptotic HT-29 and SW-48 cells was much lower than on their untreated counterparts. Similarly a decrease in \(\alpha_2,6\) linked neuraminic acid could be monitored on apoptotic bodies from LS-174 T and SW-1116 using the SNA lectin. It has been previously suggested that the appearance of galactosyl residues at the surface of apoptotic cells would stem from a loss of sialic acid residues (Morris et al., 1984; Dini et al., 1992; Falasca et al., 1996). This was indeed the case for HT-29 and SW-1116. However, this phenomenon cannot be considered as universal since it was not observed in the case of SW-48. An increase in PNA reactive terminal \(\beta\) galactosides was nevertheless observed on apoptotic bodies from the five other cell lines. In the case of PRO and SW-707, it could not be accounted for by a decrease of sialic acid residues since these cells were hardly stained by SNA or MAA before induction of apoptosis. The use of SNA yielded interesting results since the two cell lines (SW-48 and PRO) that primarily expressed high levels of SNA binding sites prior induction of apoptosis (SW-1116 and LS-174T), no change being visible for the two cell lines that expressed intermediate levels of SNA binding sites (SW-707 and HT-29). The increase in SNA binding to apoptotic PRO cells could not be inhibited when apoptosis was induced in the presence of either benzyl-N-acetylglactosamine or deoxymannojirimycin which are inhibitors of O-linked and N-linked glycosylation processes, respectively (data not shown). This would indicate that the appearance of \(\alpha_2,6\) linked neuraminic acid residues detected by SNA after induction of apoptosis results from an unmasking of previously undetectable molecules and not from de novo synthesis. This unmasking could result from the loss of larger molecules such as polylactosamines synthesized from the \(\beta_1,6\) GlcNAc branch at the trimannosyl core of N-linked oligosaccharides, as assessed from the major decrease of PHA-L reactivity from all cell lines after induction of apoptosis. It could also result from a loss of glycoproteins such as high molecular weight variants of CD44, as we observed in the case of PRO cells and that others previously observed in the case of HT-29 (Günther et al., 1996). After the loss of large glycans the access of the SNA lectin to shorter structures, close to the membrane, could become possible.

Concerning the fucosylated developmentally and tumor associated antigens, their pattern of expression was quite variable from a cell line to the other. Yet, all of them were lost after induction of apoptosis irrespective of the mode of induction.

The loss of such structures from apoptotic cells could complement the loss of sialic acids and of \(\beta_1,6\) GlcNAc branching at the trimannosyl core of N-linked oligosaccharides to allow an unmasking of underlying galactose residues or of SNA binding sites. Two fucosyltransferase activities involved in the synthesis of these antigens were also strongly decreased when apoptosis was induced by UV irradiation or TNF\(\alpha\) alone. However, fucosidase activity was also diminished in apoptotic cells, making it unlikely that the loss of fucosylated structures merely resulted from a degradation by fucosidases. Instead, it could result from a degradation of polylactosamine structures on which they are often carried, or from the release of the entire carrier glycoprotein, as exemplified in the case of PRO cells that mainly carry H antigens on a high molecular weight.
Glycosylation in late phase apoptosis from colon carcinomas

**UV treatment**

**TNF treatment**

![Image of cytofluorimetric profiles](image)

**Fig. 9.** Changes in cytofluorimetric profiles of the H antigen and its carrier glycoprotein CD44 after induction of apoptosis by UV or TNFα in PRO cells. The logs of fluorescence intensities are plotted against cell numbers. Adherent untreated cells (filled histograms) or floating UV and TNFα-treated cells (unfilled histograms) were stained with the anti-H/Lewis b MAb LM-137, or with the anti-CD44v6 peptide 1.1ASML. Fluorescence intensities of cells incubated with the secondary FITC-labeled anti-mouse IgG are shown (controls).

variant of CD44 which expression dropped to the same extent as that of the H antigenic sites after induction of apoptosis.

In contradiction with the results presented above, earlier reports proposed that fucosylated structures increase rather than decrease on apoptotic cells. Russel et al. (1998) recently observed an increased binding of the lectin from *Tetragonolobus purpurea* (LTA) on apoptotic thymocytes and on the mouse mastocytoma cell line P815. It could be that the use of different cell types explains the discrepancy between the two studies. Alternatively, the increase of LTA binding to apoptotic cells could have been nonspecific since it was not shown that this increase corresponded to a higher fucosyltransferase activity or that it could be inhibited by fucose. We observed an increase in UEA-I binding to all tested cell lines that could not be inhibited by fucose (data not shown). Similarly an increase in MAA binding to some cell lines that could not be inhibited by 3’sialyllactose was observed after induction of apoptosis. In addition, the binding of an irrelevant antibody to apoptotic bodies, although quite low, was always higher than on native cells (see controls in Figure 3). It is therefore quite likely that the apoptotic cell surface is prone to bind proteins nonspecifically. The loss of glycan structures as observed in the present study could be in part responsible for this effect.

Akamatsu and colleagues (1996) also reported that Fas-induced apoptosis in HT-29 was accompanied by an increase in the expression of the Lewis x antigen and of the FUT IV α1,3fucosyltransferase activity necessary for its synthesis. This is in clear contradiction with our results since using the same cell line, we observed a major decrease of the Lewis x antigen, as well as of the other fucosylated antigens that we tested. In our hands, this decrease occurred when cells were induced in apoptosis by UV irradiation, but also by TNFα or anti-Fas treatments. Since induction of apoptosis by TNFα or anti-Fas in HT-29 required a pretreatment with IFNγ, we tested the effect of IFNγ alone. It induced a strong increase in both Lewis x and Lewis y antigenic expressions as well as in α1,3fucosyltransferase activity on adherent cells. The enzymatic activity was still high after induction of apoptosis. Since Akamatsu and colleagues (1996) did not provide a control experiment with IFNγ treatment alone, it is quite possible that the increase in FUT IV transcription and enzyme activity that they reported was due to an induction by IFNγ and not to the apoptotic process. The reported corresponding increase in Lewis x antigen on apoptotic cells could be due to a contamination of the apoptotic cells by non apoptotic cells since in their analysis the authors mixed up floating and adherent cells. In our hands and those of others (Günther et al., 1996), HT-29 IFNγ + anti-Fas treated adherent cells show no sign of apoptosis. Yet, such cells could be at an early stage of apoptosis.

Hiraishi and colleagues (1993) showed earlier by histological methods that expression of the Lewis y antigen in normal and tumoral tissues correlated with apoptosis. This might also appear in contradiction with the results presented herein since we observed a loss of that antigen from apoptotic cell surfaces. However, we analyzed a rather late phase of the apoptotic process since DNA fragmentation was well advanced. The nick-end labeling technique used by Hiraishi and colleagues (1993) should detect earlier steps of DNA fragmentation. In addition, the expression of Lewis y antigen that they observed extended around the apoptotic areas to nonapoptotic cells. We recently showed an increase in resistance to apoptosis of rat colon carcinoma cells expressing α1,2fucosylated structures after transfection of cDNAs coding for either human or rat α1,2fucosyltransferases (Goupille et al., unpublished observations). Based on these results and those reported by Hiraishi et al. (1993), we suggest that before induction of apoptosis, an overexpression of such fucosylated antigens occurs that will
Materials and methods

Cell culture

PRO cells, obtained from Dr. F. Martin (Dijon, France) is a clone derived from a rat colon carcinoma cell line. Human colon carcinoma cell lines HT-29, LS-174 T, SW-48, SW-707, and SW-1116 were obtained from the American Type Culture Collection and cultured in RPMI 1640 (GIBCO, Cergy Pontoise, France) supplemented with 10% FCS and 2 mM glutamine. For induction of apoptosis by UV, cells at near confluency were subcultured to a UV light for 1 min and cultivated in complete medium for 24 h. After 24 h, cells were collected again for floating cells were collected. For induction of apoptosis by TNF α, cells at near confluency were treated with 5 ng/ml human recombinant TNF α (Boehringer, Mannheim, Germany) for 24 h and floating cells were collected. For induction of apoptosis by anti-Fas, HT-29 cells at about 60% confluence were treated with 1000 U/ml IFN γ (Boehringer Ingelheim, Gagny, France) in complete medium for 24 h. After removal of the medium, fresh medium containing 100 ng/ml of anti-Fas antibody clone CH-11 (BIOMOL Research Labs, Plymouth Meeting, PA) was added for 17 h and floating cells were collected.

DNA extraction and assessment of fragmentation

For analysis of DNA fragmentation, floating and adherent treated or untreated cells were treated with 2 µg/ml proteinase K (20 µg/ml). The DNA was extracted with phenol-chloroform and then precipitated overnight at -20°C following addition of ethanol. After incubation for 3 h at 37°C in Tris-EDTA containing 10 µg/ml RNase A, the DNA fragments were resolved by electrophoresis for 2 h at 40 V on 1.8 % agarose gel and visualized under UV light after ethidium bromide staining.

Lectins and antibodies

Digoxigenin-labeled lectins from Maackia amurensis (MAA), Sambucus nigra (SNA) and Arachis hypogaea (PNA) were obtained from Boehringer (Mannheim, Germany). FITC-labeled lectin from Phaseolus vulgaris (PHA-L) was obtained from Sigma (St. Louis, MO). MAb 3–3A, 19-OLE, and 7-LE were obtained from Dr. Bara (Paris, France). 3–3A recognizes all types of A determinants: types 1, 2, 3, 4, and difucosylated types 1 and 2 (Bara et al., 1988). 19-OLE is an anti-Lewis y and 7-LE is an anti-Lewis a (unpublished observations). MAb MBr1, obtained from Dr. Conaghi (Milan, Italy) is an anti-H types 3 and 4 specific antibody (Clausen et al., 1986). MAb 3E1 obtained from Dr. Blanchard (Nantes, France) is an anti-Lewis x specific antibody (unpublished observations). MAb LM137/276, obtained from Dr. Fraser (Glasgow, UK). This antibody reacts equally well with Lewis b and H antigens (Good et al., 1992). MAb 1.1ASML was obtained from Dr. Herrlich (Karlsruhe, Germany). It recognizes a peptide epitope lying within the sequence of exon vi of the rat CD44 molecule. MAb E4, obtained from Dr. Douillard (Nantes, France), recognizes the glycoprotein Tage 4 (Chadeneau et al., 1994) and was used as an irrelevant control antibody. FITC-labeled anti-mouse Ig and FITC-labeled anti-digoxigenin were purchased from Sigma and Boehringer, respectively.

Cytofluorimetric analyses

Adherent cells were recovered by scraping after two washings and an incubation in Versene solution for 10 min at 4°C. Floating cells obtained after treatments were washed three times in 0.1% gelatin in PBS. Treatments inducing apoptosis were set so that only a small percentage of floating cells were stained with trypan blue. Cells were then incubated with the lectins or antibodies at the appropriate concentrations in the same buffer for 30 min at 4°C. After washings, if required, cells were incubated with the FITC-labeled secondary reagents, anti-digoxigenin or anti-mouse Ig. After final washings, fluorescence analysis was performed on a FACSscan (Becton-Dickinson, San Jose, CA).

Assays for fucosyltransferases activities

Adherent cells were rinsed with ice-cold PBS, pH 7.2, then recovered by scraping. After washing again with ice-cold PBS, cells were solubilized in 50 mM potassium phosphate pH 6.0, containing 2 % (v/v) Triton X-100 on ice for 30 min. Floating cells obtained after induction of apoptosis were pelleted and solubilized in the same buffer as adherent cells. Following a centrifugation at 13,000 x g for 10 min, supernatants were collected and used as crude enzyme preparations. Protein concentrations were determined using bichinchonic acid obtained from Pierce (Rockford, IL).

The reaction mixtures contained 20 µM GDP-[3H]-Fucose (23 mCi/mmol, NEN Chemical Center, Dreieichenhain, Germany), 26 mM Ga1β1-3GalNAcα1-s-biotin (Syntheson, Munich, Germany) for the α1,2fucosyltransferase activity or Fucα1–2Galβ1-4GlcNAcβ1-s-biotin.
for the α1,3fucosyltransferase activity, 10 mM L-fucose, 7.7 mM MgCl₂, 1.9 mM ATP, and 50 μg protein extract in a final volume of 33 μl. After an incubation at 37°C for 3 h, the reaction mixtures were quenched with 5 ml of distilled water and applied to freshly conditioned C-18 Sep Pak cartridges (Waters-Milipore). The cartridges were washed with 20 ml water. The radiolabeled products were then eluted with 5 ml methanol and counted in 10 ml scintillation liquid (Ready Safe, Beckman, Palo Alto, CA). Background levels of radioactivity were obtained from controls without exogenous acceptor. Values obtained for the controls were then subtracted from those obtained for the assays.

**Assay for fucosidase activity**

Cell lysates prepared as for the fucosyltransferases assays containing 25 μg of proteins in 0.2 M sodium acetate buffer, pH 4.2 were incubated in 96 wells plates with 100 μl 0.2 mM sodium 4-methylumbelliferyl-α-L-fucose (Sigma) in the same buffer for 90 min at 37°C. The presence of free 4-methylumbellifereone was determined in a spectrofluorimeter (Fluorolite 1000, Dynatech, Guyancourt, France) using excitation light at 365 nm and fluorescence emission at 450 nm.

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