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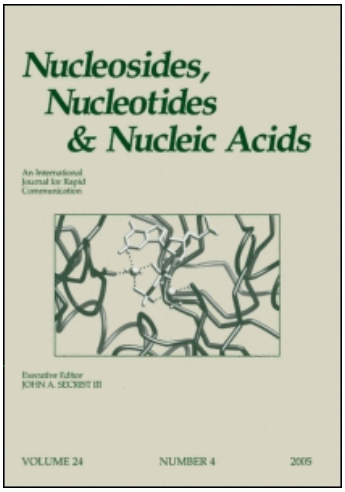

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INTERACTION OF OLIGONUCLEOTIDES WITH CELLULAR PROTEINS

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INTERACTION OF OLIGONUCLEOTIDES WITH CELLULAR PROTEINS

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ABSTRACT

Oligonucleotides (ODNs) conjugated to 4-[(N-2-chloroethyl-N-methyl) amino] benzylamine were used to investigate ODN-binding proteins in cells of different origin. The data obtained demonstrate that 68, 46, 38 and 28 kDa ODN-binding proteins are universal for tested cell lines.

Receptor mediated endocytosis was shown to be the main process mediating penetration of oligonucleotides into cells (1). Cellular proteins of different molecular masses were shown to participate in absorption of ODNs at the surface of different cells (2–4). Originality or universality of ODN-binding cellular proteins and reasons of diversity of ODN-binding proteins remains to be questionable.

Interaction of different oligodeoxynucleotides (ODNs) with cellular proteins was investigated using ^{32}P-labeled alkylating oligonucleotide conjugates (ClR-ODNs) as affinity labels (5). Cells of different origin (Cos-7, A431, HeLa, K562, KB, NIH/3T3, MCF-7, HEp-2, human lung primary epithelial cells, pig primary kidney cells) were used. Intact cells, treated cells and cellular fractions were incubated with ClR-ODNs in different conditions and ODN-binding proteins were analyzed with SDS-PAGE followed with autoradiography. Two different oligonucleotides $p(T)_{16}$ and pCAGTAAATATCTAGGA ($p(N)_{16}$) were used for synthesis of affinity reagents.

Incubation of intact cells with ClR-ODNs in PBS led to modification of membrane-cytosolic proteins with molecular masses 68, 38 and 28 kDa (Fig. 1).

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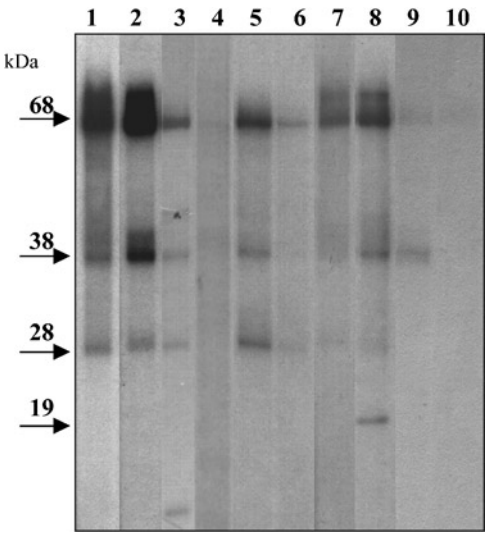

Figure 1. Affinity modification of the MC proteins with [^{32}P]ClRp(N)$_{16}$ in PBS. The culture medium was discarded, cells were washed three times with PBS and then incubated with 1 μM [^{32}P]ClRp(N)$_{16}$ in PBS during 1 h at 37°C, 5% CO_2, washed three times with PBS, scraped from the plate and pelleted by centrifugation at 3000 rpm. Cells were resuspended in 25 μl of lysis buffer with 1 mM PMSF, 10 μg/ml aprotinine and 150 μg/ml leupeptin containing 1% NP-40 and separated into the membrane-cytosolic (MC) and nuclear fractions by centrifugation at 14000 rpm. 10–20% SDS PAGE, followed by autoradiography. 1 - Cos-7 cells. 2 - A431 cells. 3 - HeLa cells. 4 - K562 cells. 5 - KB cells. 6 - 3T3 cells. 7 - MCF-7 cells. 8 - pig primary kidney cells. 9 - HEp-2 cells. 10 - human lung primary epithelial cells.

Substitution of PBS for culture medium with or without serum resulted in increase of modification of 68 and 28 kDa (but not 38 kDa) proteins, labelling of an additional 46 kDa protein and modification of small minor proteins (Fig. 2). It was found that Ca^{2+} is the main component increasing affinity modification of cellular ODN-binding proteins in culture medium. Incubation of cells with ClR-ODN in PBS supplemented with 2 mM Ca^{2+} led to modification of the same proteins, as under incubation in culture medium. Fetal calf serum, Mg^{2+} or ATP do not influence the modification of proteins with ClR-ODNs. It was shown that the 68 kDa protein is exposed at cell surface, because it was degraded by a mild trypsin treatment of cells. 38 kDa protein is localized mainly in cytosolic fraction, 46 and 28 kDa proteins are present mainly in membrane fraction and to a lesser extent in nuclear fraction, 68 kDa protein was found both in membrane and nuclear fractions (Fig. 3). In contrast to living cells, incubation of cytosolic, membrabe-cytosolic or nuclear fractions with the affinity reagents resulted in labelling of a great number of proteins. The 68, 46, 38 and 28 kDa proteins were found as minor components among other labelled proteins.

Treatments of cells disturbing the integrity of cellular membrane (scraping, treatment with trypsin or non-enzymatic cell dissociation solution, saponin

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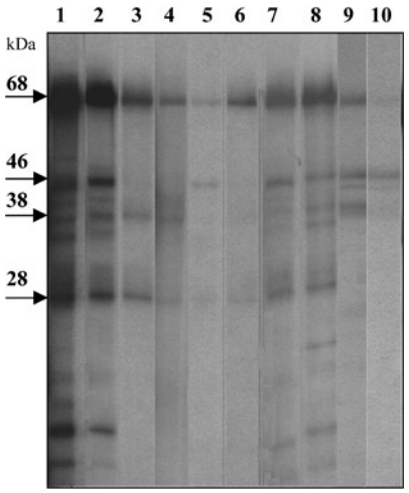

Figure 2. Affinity modification of the MC proteins with [^{32}P]CIRp(N)$_{16}$ in IM medium. The culture medium was discarded, cells were washed three times with PBS and then incubated with 1 μM [^{32}P]CIRp(N)$_{16}$ in IM medium during 1 h at 37°C, 5% CO_2, washed three times with PBS, scraped from the plate and pelleted by centrifugation at 3000 rpm. Cells were resuspended in 25 μl of lysis buffer with 1 mM PMSF, 10 μg/ml aprotinine and 150 μg/ml leupeptin containing 1% NP-40 and separated into the membrane-cytosolic (MC) and nuclear fractions by centrifugation at 14000 rpm. 10–20% SDS PAGE, followed by autoradiography. 1 - Cos-7 cells. 2 - A431 cells. 3 - HeLa cells. 4 - K562 cells. 5 - KB cells. 6 - 3T3 cells. 7 - MCF-7 cells. 8 - pig primary kidney cells. 9 - HEp-2 cells. 10 - human lung primary epithelial cells.

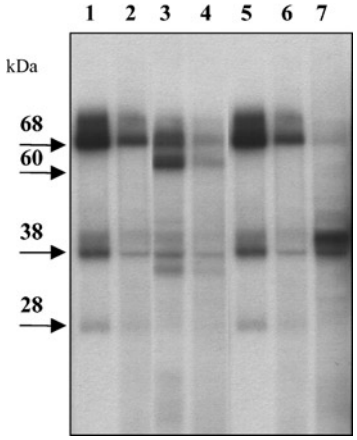

Figure 3. A431 cells modified with [^{32}P]CIRp(N)$_{16}$ in PBS were treated with 0,25% trypsin (5 min 37°C) or saponin (40 μg/ml, 20 min at room temperature), pelleted at 3000 rpm, and supernatants were collected. Cellular pellets were washed three times with excess of PBS and cellular fractions were prepared as described. The proteins of the supernatant of treated with saponin cells (corresponding to "cytosole" fraction), MC and nuclear fraction were analyzed by 10–20% SDS PAGE, followed by autoradiography. 1,2 - MC and NF of A431 cells (control), 3,4 - MC and NF of A431 cells treated with trypsin. 5,6,7 - MC, nuclear and cytosolic fractions of A431 cells treated with saponin.

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treatment) were shown to decrease affinity modification of ODN-binding cellular proteins dramatically. Permeabilization of cell membrane with saponin or streptolysin O abolishes the labelling of all the proteins except for the 38 kDa protein. The 38 kDa protein was identified as glyceraldehyde-3-phosphate dehydrogenase.

Identical proteins were modified with conjugates of ClR group with $p(T)_{16}$ and $p(N)_{16}$ oligonucleotides, but extent of modification of ODN-binding proteins was higher with oligonucleotide $p(N)_{16}$. The data obtained propose some sequence specifity of ODN-binding proteins. 50x molar excess of ODN abolishes modification of ODN-binding proteins with ClR-ODNs, suggesting specificity of affinity modification. Such active transport inhibitors as NaN_3, chloroquine and monensin do not prevent binding of ClR-ODN with proteins in PBS or culture medium.

The data obtained demonstrate that ODN-binding proteins are universal for tested cell lines. Revealing of ODN-binding proteins depends from integrity of cellular membrane, incubating conditions, sequence of oligonucleotide and method of preparation of cellular fractions.

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