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Interaction of cholesterol-conjugated alkylating oligonucleotide derivatives with cellular biopolymers

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Interactions of oligonucleotide derivatives with mammalian cells and cellular biopolymers have been investigated. The derivatives were oligonucleotides bearing an alkylating 2-chloroethylamino group at the 3'-end and a cholesterol residue at the 5'-terminal phosphate. These compounds are readily taken up by cells and react with cellular DNA, RNA and some proteins which may play a role in delivery of the compounds into cells.

Oligonucleotide derivative; Cellular uptake; Affinity modification

1. INTRODUCTION

It has been shown that conjugation of oligonucleotides to cholesterol dramatically improves their ability to enter animal cells and inhibit virus production [1–3]. Since conjugation of some other lipophilic groups [3,4] has proved to be much less efficient, the role of the cholesterol residue can not be explained simply by anchoring of the residue to the cell membrane due to its lipophilic nature and increase of the uptake of the compounds by the conventional endocytosis mechanism. It seems that efficient transportation of the cholesterol-conjugated compounds is provided by some specific mechanism, probably the mechanism for the natural delivering of cholesterol to cells. We investigated interaction of the cholesterol-conjugated alkylating oligonucleotide derivatives with cellular biopolymers, taking advantage of the possibility to accomplish affinity modification of nucleic acids and proteins with this reagent. It was found that the derivatives alkylate cellular DNA and RNA which proves that the compounds are capable of entering cell nuclei as well as the cytosol. A few proteins capable of interacting with the derivatives were detected that may play a role in delivering the compounds into cells.

2. MATERIALS AND METHODS

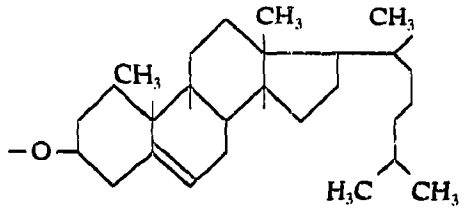
Oligonucleotides were synthesized by the phosphotriester method in solution [5,6]. The oligonucleotides were enzymatically labelled with ^{32}P, in some reagents the group ClRCH< was labelled with ^{14}C. Coupling of the alkylating and cholesterol groups to oligonucleotides was accomplished as described earlier [7,8].

Ascites carcinoma Krebs 2 (KAC) cells and a cell line COS-1 were treated with labelled oligonucleotide derivatives (in 0,5 μM) in DMEM serum free medium at 37°C, were washed and the oligonucleotide derivatives uptake was determined as described earlier [9,10]. Nuclear DNA, cytoplasmic RNA and poly(A)$^+$-mRNA were isolated as described elsewhere [1,11]. Subcellular fractions of plasma membranes, endoplasmic reticulum, cytosol, and lysosomes were isolated and characterized by distribution of marker enzymes as described in [12]. The labelled proteins of the cells and cellular fractions were analyzed by SDS electrophoresis in 9–15% gradient polyacrylamide gels according to Laemmli [13]. Gels were dried and autoradiographed.

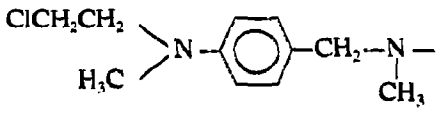
3. RESULTS AND DISCUSSION

To identify cellular biopolymers interacting with cholesterol-conjugated oligonucleotide derivatives, cells were treated with radiolabelled reagents:
5'-[^{32}P]-ClRp(Tp)$_{10}$ChS, ClR(pT)$_{10}$, ClR(pT)$_{16}$
[^{14}C]-ChS(pT)$_9$rUCHRCl, where

ChS =



ClR =



ClRCH< =

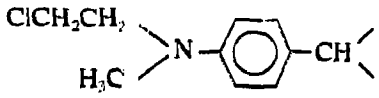


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Table I

Incorporation of the 5' ^{32}P-labelled alkylating oligonucleotide derivative RClp(Tp)$_{10}$Chs in different Krebs 2 ascite tumor cell fractions

Cell fraction	Radioactivity in fractions	
	cpm×10^{-3}	%
Plasma membrane	126	7.1
Nuclei	558	31.3
Cytosol	450	25.2
Lysosomes	6	0.35
Others	642	36.0

These alkylating derivatives can form covalent crosslinks with both nucleic acids and proteins [10]. Stability of the compounds in KAC cells and in culture medium was tested by gel electrophoresis [14]. According to the electrophoretic mobility data, the compounds were intact for at least 4 h and possessed the modifying groups.

The KAC (5×10^6 cells/ml) cells were incubated with the reagents for 2 h at 37°C and the cellular uptake of the compound and incorporation of the label in different cellular biopolymers were determined. Under the conditions used, approximately 1.3% of ClR(pT)$_{10}$ and 25% of CRlp(Tp)$_{10}$ChS or Chs(pT)$_9$rUCHRCl were taken up by the cells. Incorporation of the label occurred in both cellular RNA and DNA, which proves that the compounds can reach these nucleic acids in cytoplasm and nucleus. Under the above incubation conditions, the extent of modification of total RNA, poly(A)$^+$-mRNA and DNA with Chs(pT)$_9$rUCHRCl equaled 4.5×10^{-5}, 5.0×10^{-5} and 3.5×10^{-5} mol of reagents per mol of nucleotides, respectively.

The data on labelling of biopolymers by ClRp(Tp)$_{10}$ChS in different cellular fractions are summarized in Table I. It can be seen that the major part of the labelled biopolymers is located in the cell nucleus and in cytosol. A substantial amount of the label is associated with plasma membrane fractions, and only a small part of the labelled material is associated with lysosomes which suggests either fast degradation and release of the compounds from lysosomes or processing of the compounds in the cells, which does not involve lysosomes.

Analysis of the radiolabelled proteins in whole cells and in different cellular fractions (Figs. 1 and 2) reveals a number of labelled peptides. The labelled peptide patterns are characteristic for each of the fractions investigated.

Fig. 3 shows results of the analysis of the COS-1 cells proteins labelled by ClR(pT)$_{16}$ and ClRp(Tp)$_{10}$ChS. It can be seen, that both derivatives react with the 80-kDa protein, identified earlier as putative nucleic acids binding receptor [10] and that the cholesterol-conjugated

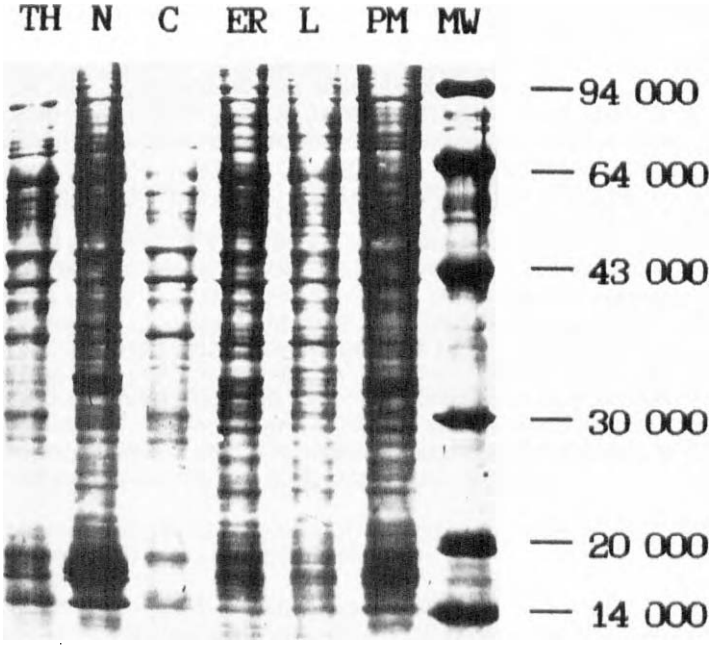

Fig. 1. SDS gradient (5–9%) PAGE of isolated cell fractions, silver staining. TH, total homogenate; ER, endoplasmic reticulum; PM, plasma membrane; N, nuclei; C, cytosol; L, lysosomes; MW, molecular weight markers.

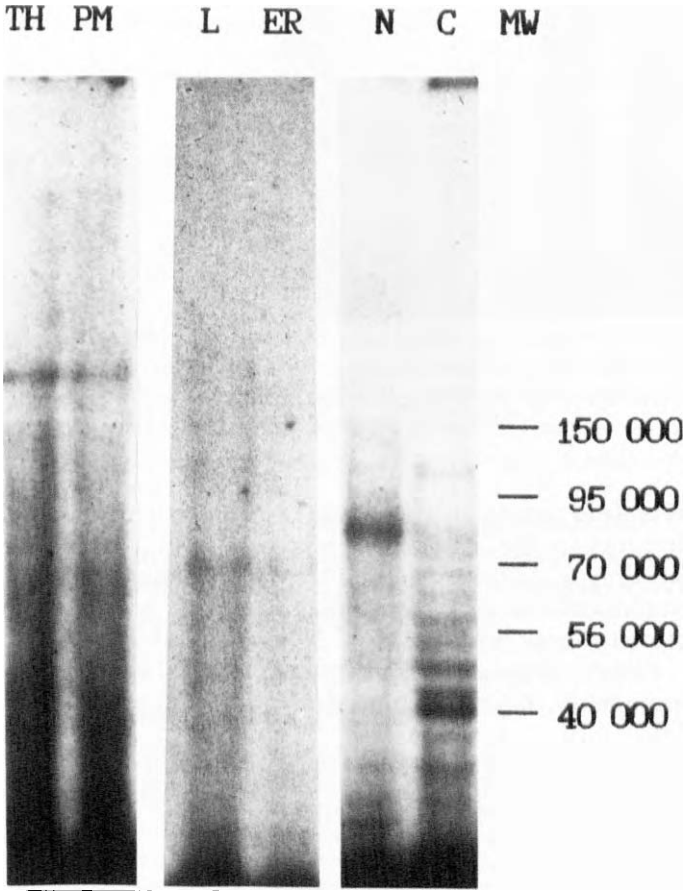

Fig. 2. Distribution of radioactivity among fractions of ascite carcinoma cells alkylated by [^{32}P]-ClRp(Tp)$_{10}$Chs SDS-PAGE. Cells ($5 \cdot 10^6$ per ml) were incubated with the oligonucleotide derivatives (0.5 μM) for 2 h at 37°C in DMEM, washed and fractionated.

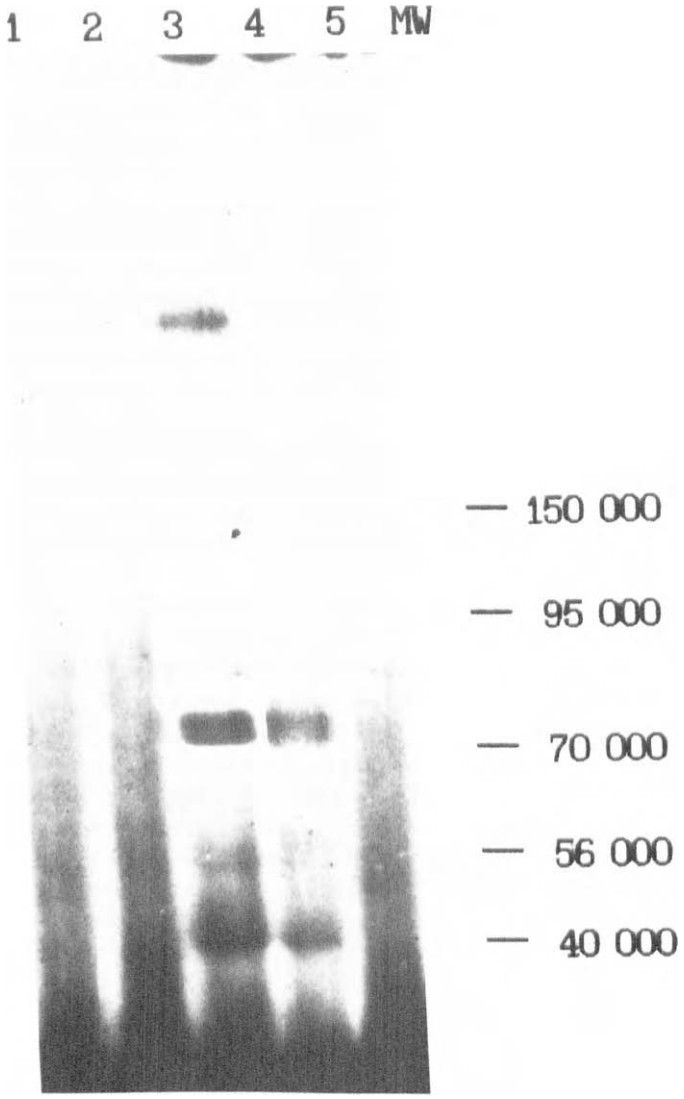

Fig. 3. Proteins of COS-1 cells alkylated by $ClR(pT)_{16}$ and $ClR(pT)_{10}ChS$. 1, $ClRp(Tp)_{10}ChS$ + excess DNA; 2, $ClRp(Tp)_{10}ChS$ + excess oligonucleotide; 3, $ClRp(Tp)_{10}ChS$; 4, $ClR(pT)_{16}$; 5, $ClR(pT)_{16}$ + excess oligonucleotide.

derivative labels also a 250-kDa protein which is not detected in the experiment with $ClR(pT)_{16}$. That the labelling is specific is proved by the fact, that an excess oligonucleotide or DNA in the cell culture prevents the proteins from labelling (Fig. 3, lanes 1 and 2).

Results of these experiments suggest, that cholesterol-conjugated oligonucleotide derivatives interact with a protein which apparently do not bind the parent oligonucleotides. A number of proteins were shown to interact with cholesterol. Thus a 14-kDa protein that binds cholesterol and is probably involved in cholesterol absorbtion in the brush border membrane was described [15]. Recently spontaneous association of cholesterol conjugated oligonucleotides with LDL was reported [16]. The protein detected in our experiments has a different molecular mass and it seems to be a different protein which may be either directly or indirectly involved in the efficient transportation of the compounds into cells.

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