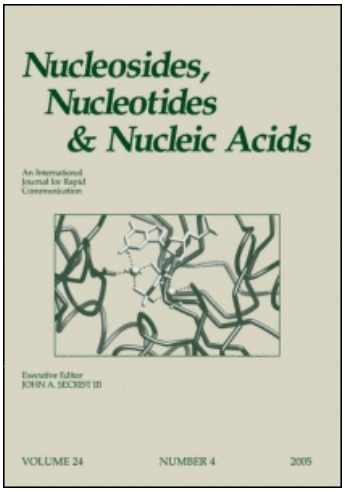

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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IS RESPONSIBLE FOR INTRANUCLEAR LOCALIZATION OF SOME OLIGONUCLEOTIDES

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ABSTRACT

Nuclear accumulation of ODNs has been associated with their binding to a series of nuclear proteins. These interactions could be responsible for the sequence-independent effects of ODNs as well as for their sequence-specific interactions and their intracellular distribution. Investigation of interaction of ODNs with these proteins may shed light on the mechanisms of cellular uptake and nuclear accumulation of oligonucleotides.

We have found, that ODN 5′-pTACAGTAAATATCTAGGAATG ($p(N)_{21}$) is efficiently taken up and enter into the nucleus of endothelial cells, human monocytes and HeLa cells whereas different other tested ODNs were found mainly in cytosole. Gel shift experiments have shown that $p(N)_{21}$ binds to 38 kDa protein in nuclear extract of HeLa cells. Using alkylating $[^{32}P]ClRp(N)_{21}$ conjugate Kd of $p(N)_{21}$ complex with 38 kDa protein was estimated to be 0,5 μM.

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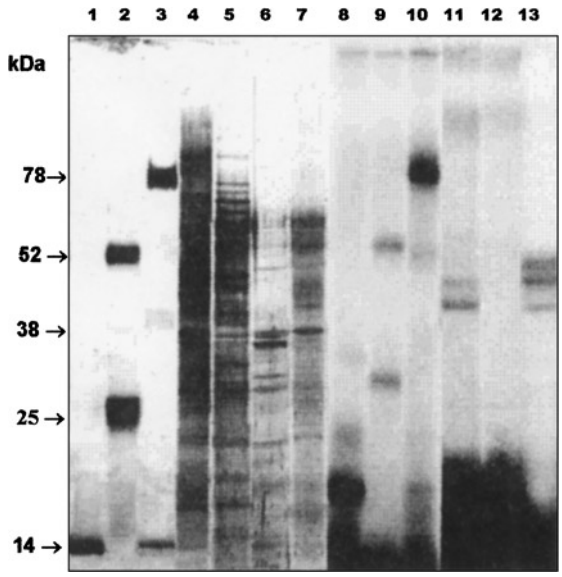

Figure 1. Isolation of TAAAT specific ODN-binding protein from nuclear extracts of HeLa cells. 1–7 - colloidal silver staining of nitrocellulose blot; 8–13 - autoradiography of the gel; 1, 2, 3 and 8, 9, 10 - molecular mass marker proteins (lysozyme; IgG; lactoferrin), modified with $[^{32}P]ClRp(N)_{16}$., 4, 11 - nuclear extracts modified with $[^{32}P]ClRp(N)_{16}$; 5, 12 - pellets AS 60% modified with $[^{32}P]ClRp(N)_{16}$; 6, 13 - pellets AS 60–80% modified with $[^{32}P]ClRp(N)_{16}$; 7 - affinity purified 37 kDa protein.

TAAAT sequence was identified as the major binding motif for the 38 kDa nuclear protein. To isolate the 38 kDa ODN-binding protein, HeLa cell nuclear extract was fractionated by stepwise addition of ammonium sulfate (AS) (Fig. 1). The precipitates were dialyzed against PBS and incubated with 1 μM $[^{32}P]ClRpCAGTAAATATCTAGGA$ ($[^{32}P]ClRp(N)_{16}$). It was found that 38 kDa ODN-binding protein was precipitated with 80% AS. Proteins pelleted by stepwise addition of AS at 60–80% saturation were applied to affinity chromatography. Two protein bands with molecular masses of about 38 kDa were revealed in the 60–80% pellet; the upper band was found to interact with the affinity sorbent. The affinity purified protein was separated from minor contaminants by 10–20% SDS PAGE.

Purified protein was identified by Edman degradation procedure as human glyceraldehyde 3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12). Commercially obtained GAPDH modified with $[^{32}P]ClRp(N)_{16}$ showed the electrophoretic mobility (Fig. 2) and affinity to $cPLA2p(N)_{16}$ (dissociation constant 0.2 μM) similar to that of the 38 kDa ODN-binding protein of HeLa nuclear extract.

Confocal microscopy experiments have revealed that GAPDH and fluorescein labeled $p(N)_{21}$ co-localize in the nucleus.

Effect of ODN on a dehydrogenase function of GAPDH and influence of glyceraldehyde 3-phosphate (G3P), cofactors and polyanions on binding of GAPDH

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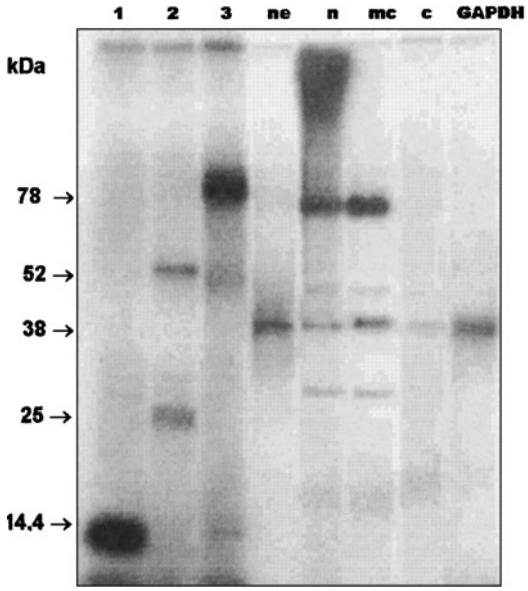

Figure 2. Affinity modification of proteins of living HeLa cells, nuclear extract of HeLa cells and GAPDH from human erythrocytes with [^{32}P]ClRp(N)$_{16}$. Hela cells, nuclear extract of Hela cells and GAPDH were incubated 1 hour at 37°C with 1 mM [32P]ClRp(N)$_{16}$. Cytosolic fraction was obtained as soluble extracellular proteins recelived after permeabilization of the cells with saponine. 10–20% SDS-PAGE, followed with autoradiography. 1,2,3 - molecular mass marker proteins (lysozyme; IgG; lactoferrin), modified with [^{32}P]ClRp(N)$_{16}$.

with ODNs were investigated. Binding of ODNs with GAPDH leads to some non-competitive inhibition of the dehydrogenase activity. Poly U, poly A, poly C, NAD^+ and Heparin efficiently inhibit binding of ODNs with GAPDH. ATP, GTP, DexS, NADH, poly IC, dsDNA and G3P inhibit binding to a lesser extent. Thus the dehydrogenase activity of the enzyme is not affected by p(N)$_{16}$, whereas NAD^+ prevents binding of oligonucleotide to the enzyme. The data obtained demonstrate that NAD^+ binding site and oligonucleotide binding region of GAPDH are not the same. Effects of NAD^+ on ODN binding to GAPDH are supposed to be related to conformational rearrangement of the enzyme caused by NAD^+ binding (2).

ODN-binding region of the GAPDH molecule was localized after hydroxylamine hydrolysis of the enzyme modified with [^{32}P]ClRp(N)$_{16}$. It was shown that the 4,9 kDa C-terminal peptide is responsible for binding of p(N)$_{16}$ (Fig. 3). It is known that NH_2- domain of GAPDH (1–151) is responsible for NAD^+ binding whereas COOH-domain (151–334) is responsible for binding of G3P (aminoacids 179, 231, 148, 195, 208, 209) (2). Our data demonstrate that ODN-binding site of GAPDH is localized within peptide 286–334 that is not involved in G3P or NAD^+ binding sites.

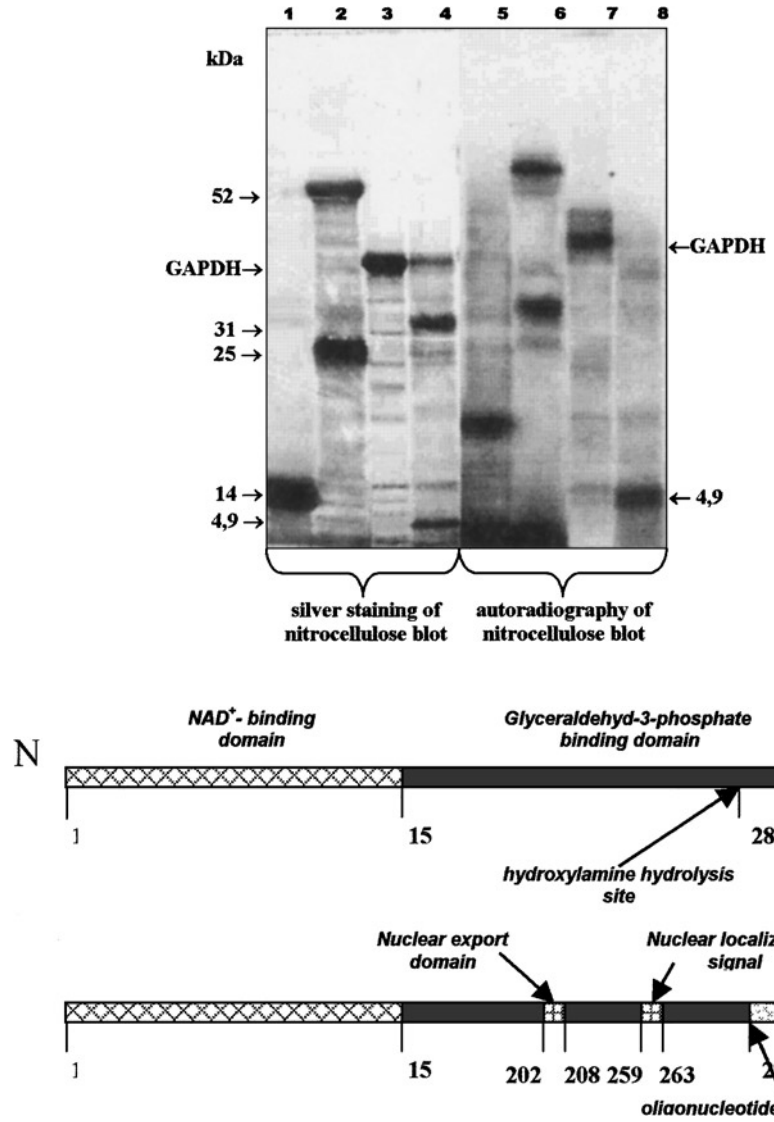

Figure 3. Identification of oligonucleotidebinding region of GAPDH. GAPDH from human erythrocytes affinity modified with [32P]ClRp(N)$_{16}$ was treated with hydroxylamine. Products of hydrolysis of GAPDH were analyzed by 15–20% SDS-PAGE, followed with autoradiography. 1,2,5,6 - molecular mass marker proteins (lysozyme (14 kDa); IgG (52; 25 trkDa)) modified with [32P]ClRp(N)$_{16}$; 3, 7 - GAPDH, modified with [32P]ClRp(N)$_{16}$; 4, 8 - product of hydroxylamine hydrolysis of GAPDH, modified with [32P]ClRp(N)$_{16}$.

It should be noted that GAPDH has nuclear localization (259–263) and nuclear export (202–208) sites that are outside of the ODN-binding sequence. These data enable us to suggest that binding of ODN with GAPDH do not interfere with nuclear shuttling of GAPDH, and this protein may be involved in intranuclear delivery of ODN.

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GAPDH was shown to bind ODNs in living cells (Fig. 2.). These data suggest that GAPDH may be responsible for both irregular nuclear distribution of the $p(N)_{21}$ and for transportation of ODNs into the nucleus.

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