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Serum immunoglobulins interact with oligonucleotides

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Abstract

The interaction of reactive derivatives of oligonucleotides bearing a 4-[(*N*-2-chloroethyl-*N*-methyl)amino]benzylamin residue at the 5'-terminal phosphate with serum blood proteins has been investigated. It was found that the compounds react with serum albumin and immunoglobulins M and G, the reactivity increasing in the order: albumin < IgG < IgM. The reactions with immunoglobulins were inhibited in the presence of different oligonucleotides, DNA and heparin, suggesting the oligonucleotide binding to some cationic region of the proteins. Myoglobin inhibited the interaction of oligonucleotide derivatives with myoglobin-specific monoclonal antibodies which indicates that the derivatives interact with the proteins within or near the antigen binding site.

Key words: Oligodeoxynucleotide; Immunoglobulin; Affinity modification

1. Introduction

The ability of oligonucleotide derivatives targeted to specific genetic messages to suppress the multiplication of viruses in cell cultures and to affect expression of specific genes (for reviews see [1]) suggests that compounds of this type may become efficient therapeutics. A few animal tests have already demonstrated antiviral activity of oligonucleotides at the organism level and recently human tests have been started [2]. However the fate of the compounds in the organism is poorly investigated and little is known about their interactions with biomolecules in the organism. Oligonucleotides imitate natural nucleic acids and may be expected to interact not only with the target nucleic acids but also with a number of proteins of nucleic acids metabolism, polymerases of nucleic acids, ribosomes and perhaps with many other known and unknown factors capable of binding to nucleic acids. These interactions may influence distribution and the fate of oligonucleotides in the organism.

Earlier we have investigated the distribution of oligonucleotide derivatives in mice. It was found that they enter all organs showing less efficient accumulation in the brain and persist in the blood in an undegraded state for 20–60 min. [3]. Also it was found that the life time of oligonucleotides in the organism can be prolonged by chemical modification of terminal residues [4] and that conjugation of oligonucleotides to steroids promotes their binding to blood cells [5], thus changing the distribution and increasing their life time. In this report we describe experiments in which we have found that the oligonucleotides interact with certain blood proteins, namely, serum albumin and the immunoglobulins M and G.

2. Materials and methods

Oligodeoxynucleotides and the radiolabeled reactive derivatives of oligonucleotides were synthesized as described earlier [6–8]. The compounds were 90–95% pure as shown by electrophoresis the specific radioactivity being 50 Ci/mmol. Heparin and bovine serum albumin were from Sigma. Total fractions of the human immunoglobulins G and mouse immunoglobulin M were purchased from Biosan (Novosibirsk). The human myoglobin-specific mouse antibodies 4D4 and 8D6 were characterized elsewhere [9]. Human blood serum was prepared by standard procedures and stored at −20°C.

Affinity modification of the proteins was performed in physiological salt solution (0.14 M NaCl, 0.01 M NaH_2PO_4, pH 7.5) in the presence of various concentrations of the alkylating oligonucleotide derivatives. In the competition experiments, the reaction samples were supplemented with competitors, which were introduced before the reactive compounds. Incubation was for 40 min at 37°C. The reaction was stopped by the addition of the equal volume of 0.06 M Tris-HCl, pH 6.8, containing 2% SDS, 2% 2-mercaptoethanol and 20% glycerol followed by incubation for 5 min at 100°C. The labeled proteins were analyzed by SDS electrophoresis in gradient polyacrylamide gels (7–25%), according to Laemmli [10]. the gels were autoradiographed and the films scanned to quantitate the data.

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Abbreviations: pT_{16} oligodeoxyribothymidilate 16-mer; dsDNA, double-stranded DNA; $CIRCH_2NH$-, 4-[(*N*-2-chloroethyl-*N*-methyl)-amino]benzylamine residue.

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3. Results and discussion

Affinity modification is an easy method to investigate macromolecular interactions of biologically active compounds. Oligonucleotides are large molecules allowing conjugation of reactive groups which does not interfere with their binding to the complementary nucleic acids and proteins [1]. In this study we have used a reactive alkylating aromatic 2-chloroethylamino group, which can interact with both nucleic acids and proteins. The group was conjugated to the terminal phosphate which is the least important position for interactions of oligonucleotides.

We incubated whole human blood serum with a ^{32}P-labeled alkylating derivative of $(pT)_{16}$, to label proteins capable of interacting with oligonucleotides. Results of the labeling experiments show (Fig. 1) that there are a few proteins in the serum which can interact with oligonucleotides. Among the labeled proteins we have found those corresponding to the immunoglobulins G and M according to their electrophoretic mobility. Experiments with the isolated pure immunoglobulins G and M have confirmed that the oligonucleotide derivatives react with these proteins under the conditions used (Fig. 2). The reactivity changed in the order: IgM > IgG > albumin. The oligonucleotide affinity to the proteins (dissociating constants) may be estimated [11] as 4 and 6 μM for IgM and IgG, and about 20 μM for albumin.

To investigate the specificity of interaction of the oligonucleotides with the proteins, we performed affinity modification of the proteins by an alkylating derivative of oligothymidylate in the presence of various competitors: oligonucleotides, heparin and dsDNA. The compounds competed efficiently with the reagent in the reaction with immunoglobulins. Since some of these compounds were not nucleophilic in nature, we suggested that they inhibited the modification process by interfering with the binding of the reagents to the proteins. Because heparin showed high competitive efficiency, it was assumed that oligonucleotides bind to some polyanionic binding site of low specificity which is capable of tethering nucleic acids and polyanions. In the case of albumin, the competition was poor, suggesting

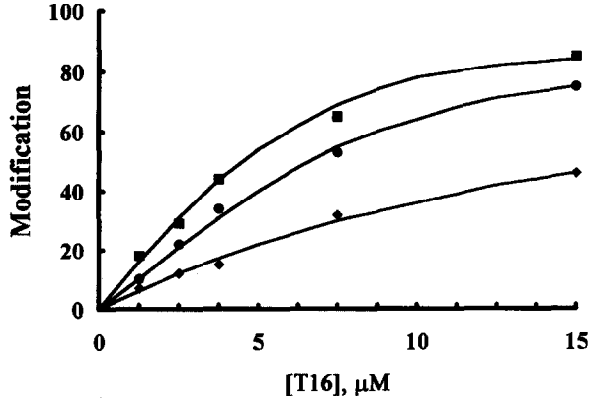

Fig. 2. Effect of the reagent concentration on the specific labeling of the proteins. Albumin (◆), IgG (●) and IgM (■) (all at 1 μM concentration) were incubated with ^{32}P-labeled $ClRCH_2NH(pT)_{16}$ in buffered (pH 7.5) physiological salt solution for 30 min at 37°C. [T16], μM – the alkylating reagent concentration in micromoles. Modification, specific ^{32}P label incorporation in the protein bands (arbitrary units).

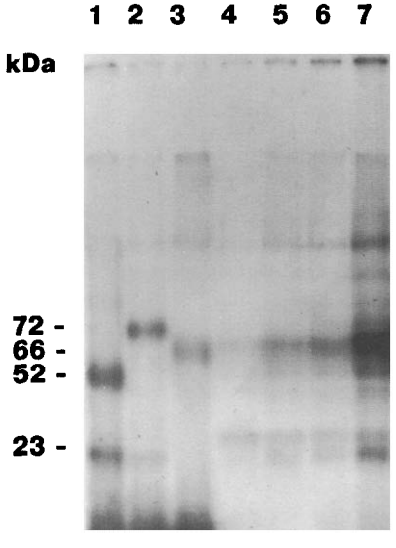

Fig. 1. Labeling of the human blood plasma proteins with the alkylating oligonucleotide derivative $ClRCH_2NH(pT)_{16}$. Autoradiogram of the SDS electrophoresis in the polyacrylamide gel. Proteins were incubated with 10 μM reactive derivative of oligonucleotide for 30 min at 37°C. Lanes 1–3, IgG, IgM and bovine serum albumin, respectively. Lanes 4–7, human serum incubated with 1.2, 2.5, 5.0 and 10 μM reagent.

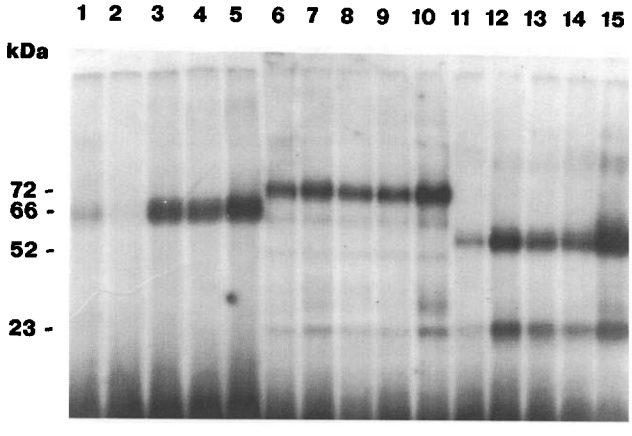

Fig. 3. Effect of competitors on the reaction of the blood serum proteins with alkylating derivative of oligothymidylate. Autoradiograph of the polyacrylamide gel. Lanes 1–5, alkylation of bovine serum albumin; lanes 6–10, alkylation of IgM; lanes 11–15, alkylation of IgG. Reaction mixtures analyzed in lanes 1, 6 and 11 contained 1.0 mg/ml dsDNA (0.5 μM). The reaction mixtures analyzed in lanes 2, 7 and 12 contained 50 mg/ml heparin. The reaction mixtures analyzed in lanes 3, 8 and 13 contained the 10 μM oligonucleotide pTGACCCTCTTCCCATT. The reaction mixtures analyzed in lanes 4, 9 and 14 contained 10 μM $(pT)_{16}$. Lanes 5, 10 and 15, the mixtures contained no competitors.

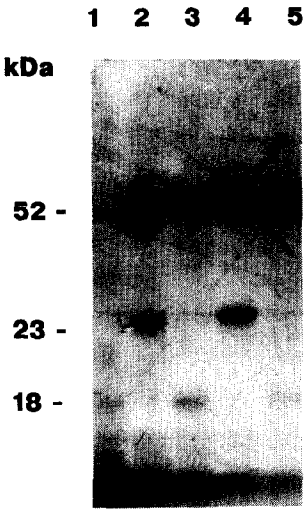

Fig. 4. Effect of the antigen (human myoglobin) on the reaction of the myoglobin-specific monoclonal mouse antibodies with the alkylating derivative $ClRCH_2NH(pT)_{16}$. Concentration of the derivative was 10 μM. Lane 1, human myoglobin (4 μM); lanes 2 and 4, antibodies of the clones 4D4 and 8D6 (1 μM); lanes 3 and 5, antibodies of the clones 4D4 and 8D6 (1 μM) in the presence of 4 μM human myoglobin.

labeling without strong complex formation. Analysis of the labeled immunoglobulins has revealed that the oligonucleotide derivatives modified both heavy and light chains of the proteins. The competitors blocked the reaction with both the immunoglobulin chains (Fig. 3).

Experiments with a myoglobin-specific mouse monoclonal antibody (subtype G1) have been performed to shed light on the localization of the oligonucleotide binding sites. The antibodies 3D4 and 8D6 showed high affinities to myoglobin: K_d (dissociating constants) 6 nM and 1 nM, respectively [9]. As shown in Fig. 4 the alkylating derivative of the oligonucleotide reacts with both monoclonal antibodies. Introduction of the myoglobin (4 μM) in the reaction mixture inhibits the reaction completely suggesting the oligonucleotide binding at or in close proximity to the antigen binding site; the bound antigen shields the site from the oligonucleotides. An alternative explanation may be that the interaction with the specific antigen causes conformational change of the site which is responsible for the oligonucleotide binding.

The described experiments show that oligonucleotides can interact with immunoglobulins. This interaction should be taken into account when considering the fate of oligonucleotides in the organism. Earlier we have found that the oligonucleotides bind with CD4 receptor [11] which is of the same protein family as immunoglobulins. The observed interactions pose the question if the interactions of nucleic acids with immunoglobulins and immunoglobulin-like receptors is of any biological significance.

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