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Affinity separation of polyribonucleotide-binding human blood proteins

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Abstract—Using affinity columns with immobilized poly(A), poly(G), poly(U), poly(C), and poly(A)·poly(U) and poly(G)·poly(C) duplexes several polyribonucleotide-binding blood plasma proteins have been captured. Albumin and keratins K1 and K2e have been detected to bind polypurine tracts. The in vitro glycated albumin binds poly(A) and poly(G) more efficiently than the unmodified protein. The major polypyrimidine-binding blood plasma protein (28 kDa) can catalyze the hydrolysis of poly(U).

Several reports have documented the detection of extracellular RNA molecules in human serum or plasma.[1] These ribonucleic acids have been shown to be protected from degradation by plasma ribonucleases.[2] One of the possible mechanisms of this protection is that extracellular RNA molecules are bound to proteins. However, little is known about the structure of such nucleic acid–protein complexes.

The stability of mRNA in cytoplasma is regulated by binding of specific intracellular proteins to poly(A) tracts on its 3′-terminus.[3] The poly(A) tracts are the first mRNA segments to be degraded in the absence of poly(A)-binding proteins. This fact demonstrates the importance of poly(A) in protecting mRNAs from indiscriminate destruction by cellular nucleases. Moreover, it proves that the formation of poly(A)–protein complexes is required to maintain mRNA stability in cells.

The basal and regulated stability of a particular mRNA may be also determined by cooperative interactions between several *cis*-elements spread throughout the mRNA and their binding by *trans*-acting RNA-binding proteins.[3] Among the *cis*-elements identified to date are AU-rich elements, many of which contain numerous copies of the pentamer AUUUA or the nonamer UUAUUUA(U/A)(U/A), as well as one or more U-rich stretches. Other well-characterized elements are poly(C)- and UC-rich regions of mRNAs. It may be suggested that a similar protection mechanism exists for RNA molecules drained into blood, and the extracellular RNAs are protected from nuclease degradation in blood through binding of certain proteins to their A-, U- or C-rich sequences. In this respect it was interesting to investigate blood plasma proteins which are able to bind polyribonucleotides.

In order to capture blood plasma proteins that are able to specifically bind polyribonucleotides, we have prepared affinity resins carrying synthetic homopolymers of adenylic, uridylic, cytidylic, and guanylic acids, as well as poly(A)·poly(U) and poly(G)·poly(C) duplexes. Blood plasma samples from healthy donors (blood type AB(IV)Rh(+)) were incubated with immobilized polyribonucleotides and their duplexes and subsequently washed by increasing concentration of NaCl solution. The highly bound proteins were eluted with 1 M NaCl, and the eluate was then analyzed by 10% SDS–PAGE with subsequent silver staining.[4]

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The data obtained have shown that the sets of the captured proteins (Fig. 1) bound to purine- and pyrimidine-containing polynucleotides are different, thus indicating the different specificity of the polynucleotide-binding proteins. Five major bands corresponding to the proteins with molecular weights of ~73, 67, 54, 39, and 28 kDa are visualized using the affinity columns containing poly(A) and poly(A)·poly(U) duplex (lanes 1 and 3, respectively). The same proteins are captured with poly(G)- and poly(G)·poly(C)-containing affinity columns (lanes 4 and 6, respectively). It is interesting to note that the captured proteins bind single-stranded poly(A)-tracts more effectively than poly(A)·poly(U) duplex, whereas the affinity of the proteins to both poly(G) and poly(G)·poly(C) duplex is practically the same. In the case of the immobilized polyuridylic and polycytidylic acids only one major protein band with molecular weight of 28 kDa is visualized (lanes 2 and 5, respectively). The data obtained indicate that a large number of both low- and high-molecular-weight blood proteins are capable of binding to polypurine tracts. At the same time, the proteins that possess the affinity toward polypyrimidines predominantly are of low molecular weight.

RNases of different specificity with molecular weight of ~30 kDa have been reported to exist in human blood.[5] In order to test the hypothesis that the 28 kDa protein is able to catalyze polyribonucleotide hydrolysis, the corresponding protein fraction was incubated with polyuridylic acid at 37 °C, and the reaction mixture was analyzed by ^{31}P NMR spectroscopy. The complex signal in the region of ~20 ppm, which can be attributed to polynucleotide 2′,3′-cyclophosphodiester, along with the signal of internucleotide phosphates ($\delta = -0.1$ ppm) were observed in NMR spectrum of sodium salt of polyurydilic acid after 2 h of incubation.[6] At the same time, the only signal in the spectrum of the control poly(U) sample, which had been incubated in the absence of the protein, was that of the phosphorus atom of internucleotide bond ($\delta = -0.2$ ppm). When poly(A) was used as a substrate, no changes in the ^{31}P NMR spectrum of poly(A) were observed. The data obtained allowed us to suggest that the polynucleotide-binding protein with molecular weight of 28 kDa possesses RNase activity with specificity to poly(U)-tracts.

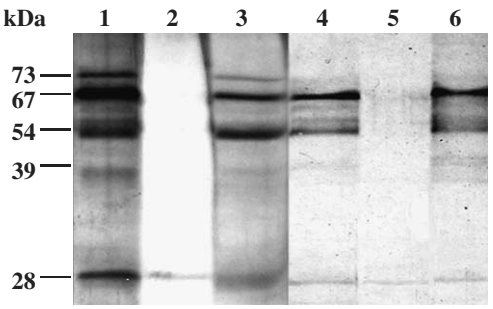

Figure 1. 10% SDS–PAGE of the AB(IV)Rh(+) blood plasma proteins that bind poly(A) (lane 1), poly(U) (lane 2), poly(G) (lane 4), poly(C) (lane 5), and duplexes poly(A)·poly(U) (lane 3) and poly(G)•poly(C) (lane 6).

As can be seen in Figure 1, the molecular weight of the major polypurine-binding blood plasma protein corresponds to that of human serum albumin (67 kDa). Using the method of affinity modification with ^{32}P-labeled reactive oligodeoxyribonucleotide derivatives, it has been demonstrated that albumin is the major oligonucleotide-binding protein of human blood plasma.[7] Moreover, serum albumin has been reported to take part in oligonucleotide cellular internalization.[8]

In order to identify some polynucleotide-binding blood proteins, immunoenzyme analysis using anti-albumin and anti-keratins rabbit polyclonal antibodies was carried out. The specific eluate from poly(A)-containing affinity column was separated by 10% SDS–PAGE, and the protein bands were transferred onto a nitrocellulose membrane and incubated with rabbit anti-albumin or anti-keratins polyclonal antibodies. The antigen–antibody complex was detected by goat anti-rabbit IgG, conjugated to horseradish peroxidase, and visualized using 4-chloro-1-naphthol in the presence of H_2O_2. The poly(A)-binding proteins with molecular mass of ~67 kDa were identified as keratin K1, keratin K2e, and serum albumin. These results have demonstrated that albumin and keratins are capable of binding to polynucleotides, particularly poly(A) tracts of RNAs, in human blood plasma. The roles and identity of other polyribonucleotide-binding proteins captured by polypurine-containing affinity columns have not been identified so far.

The results of the present study as well as the data reported by others indicate that human serum albumin binds to both DNAs and RNAs.[7,9] However, the albumin fraction in blood serum is known to be heterogenic,[10] and the data on the efficiency of nucleic acids' binding by different albumin forms are absent.

It is known that albumin fraction in blood plasma contains glycated albumin, which is formed in vivo by nonenzymatic reaction of D-glucose with the ε-amino group of exposed lysine side chain within the protein resulting in the formation of reversible Schiff's bases.[11] These adducts subsequently undergo irreversible rearrangements to form heterogeneous class of advanced glycation endproducts, which have been implicated in the pathogenesis of diabetes.[12] In order to test the ability of glycoalbumin to bind polynucleotide sequences and to compare its affinity to that of the unmodified protein, human serum albumin was glycated in vitro with 300 and 500 mM glucose solutions within 14 days.[13] The molecular masses of the obtained glycated proteins were determined using MALDI-TOF mass spectrometry. The increase in molecular mass of 2169 and 3357 Da in the case of 300 and 500 mM glucose-containing reaction mixtures corresponds to the condensation of about 13 and 21 glucose units per protein molecule, respectively. As it has been reported earlier,[14] up to 15 glucose molecules are condensed on serum albumin in blood of patients with badly controlled diabetes mellitus. Thus, the glycated albumins obtained in the present study imitate the in vivo protein state under the pathological condition.

It has been demonstrated that the level of glycoalbumin in blood of healthy subjects is 6–15%.[15] To estimate the level of glycoalbumin in blood plasma (type AB(IV)Rh(+)) used in this study, the plasma proteins were separated by anion-exchange chromatography. As can be seen in Figure 2, the retention times of the blood plasma proteins eluted in fractions 1 and 2 (a) are similar to that of native (b) and in vitro glycated (c) albumins. Electrophoretic mobility of the protein 2 corresponds to that of glycated albumin (data not shown). Moreover, the appearance of glucose-derived fluorescence[13] (excitation 365 nm, emission 440 nm) indicates that the protein fraction 2 (Fig. 2a) contains the glycated protein. The level of the modified albumin in the blood plasma was estimated to be about 23%. The data obtained allow us to suggest that glycoalbumin is a natural constituent of blood plasma (type AB(IV)Rh(+)) from healthy subjects, and its level in blood plasma is rather high.

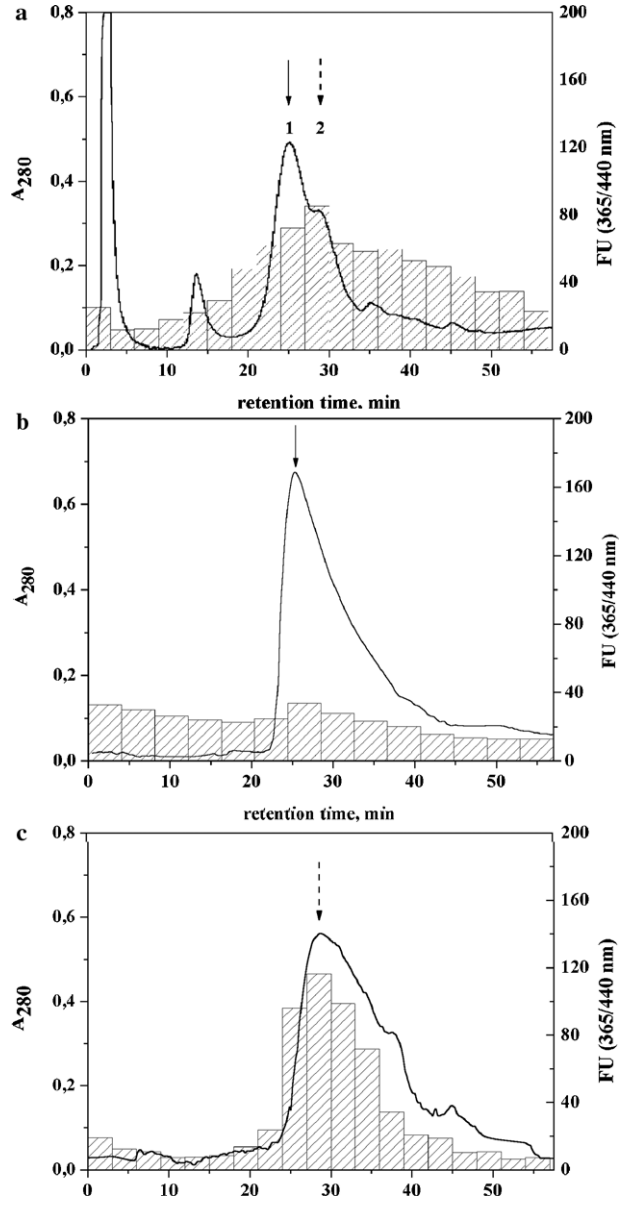

Figure 2. Anion-exchange chromatography of blood plasma (type IV(AB)Rh(+)) (a); unmodified human serum albumin (b) and albumin modified with 13 glucose units (c). The dashed bars represent the fluorescence intensity of the collected fractions. Arrows indicate the position of the peaks corresponding native (solid) and glycated (dashed) albumins.

To compare the affinity of native and glycated albumin toward polypurine tracts, the protein samples in equal amount were loaded onto poly(A)- and poly(G)-containing affinity columns, and eluted with increasing concentration of NaCl, as described above for blood protein separation. The protein fractions eluted with 1 M NaCl were analyzed by 10% SDS–PAGE with subsequent silver staining (Fig. 3). It can be seen that the protein bands of the modified albumins (lanes 2, 3, 5, 6) are more intensive than that of the unmodified protein (lanes 1 and 4). In order to estimate the efficiency of native and glycated albumin binding to polyadenylic and polyguanylic acids, the intensities of the gel bands, corresponding to the polynucleotide-bound albumins, were normalized on the intensities of the gel bands, corresponding to the proteins that had not been incubated with the affinity resin. It was demonstrated that the affinity of serum albumin toward poly(A) tracts depended on the degree of glycation and was about 2 and 3 times higher for the proteins modified with 13 and 21 glucose units than for the unmodified protein, respectively. In the case of poly(G), the modified albumins bound to polyribonucleotides about 2.5 and 6.5 times more effectively than the native protein.

It has been recently demonstrated that the ratio of different albumin forms in blood plasma depends on a person's health. The level of modified albumin in blood of patients with various forms of cancer[16] and diabetes mellitus[17] is increased. Moreover, the level of a definite modified albumin form in blood plasma depends on the disease stage and complications. Besides, the level of extracellular nucleic acids in blood plasma has been reported to increase in the case of autoimmune diseases and cancer.[18] Therefore, it is possible that the reception and transport of nucleic acids are directly connected with posttranslational modification of serum albumin. Moreover, the increased affinity of modified albumin toward poly(A) tracts, as well as the data on albumin cellular uptake, allow us to suggest that the protein can participate in regulation of cytoplasmic mRNA stability via its binding to mRNA 3′-poly(A) tail. Thus, a possible role of glycated serum albumin in regulation of gene expression, probably by inhibiting mRNA

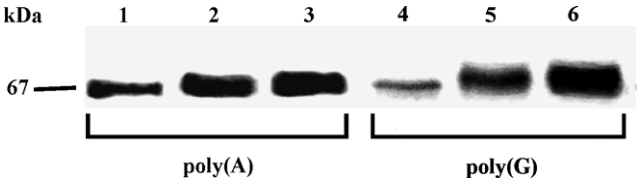

Figure 3. 10% SDS–PAGE of human serum albumin (lanes 1 and 4) and albumin modified with 13 (lanes 2 and 5) and 21 glucose molecules (lanes 3 and 6) after affinity chromatography using poly(A)- (lanes 1–3) and poly(G)-containing columns (lanes 4–6).

deadenylation resulting from albumin binding to mRNA poly(A) tail, cannot be excluded.

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