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Interaction of human serum albumin and its clinically relevant modification with oligoribonucleotides

Yuliya V. Gerasimova [a,b], Irina A. Erchenko [b], Makhmut M. Shakirov [c], Tatyana S. Godovikova [a,b,*]

[a] Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of Russian Academy of Sciences, 8 Lavrentyev Ave., 630090 Novosibirsk, Russia
[b] Novosibirsk State University, 2 Pirogova St., 630090 Novosibirsk, Russia
[c] Novosibirsk Institute of Organic Chemistry, Siberian Branch of Russian Academy of Sciences, 9 Lavrentyev Ave., 630090 Novosibirsk, Russia

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ABSTRACT

Human serum albumin (HSA) was shown to mediate oligoribonucleotide cleavage. Nonenzymatic glycation of HSA decreased the ribonuclease-like activity of the protein. According to ^{31}P NMR data, both native and glycated albumins induced hydrolysis of RNA molecule through 2′,3′-cyclophosphate intermediates. A feasible mechanism of RNA hydrolysis by native albumin and its clinically relevant modification was discussed.

Human serum albumin (HSA) is the most abundant plasma protein in the human body with a plasma concentration of ~0.6 mM. HSA plays an important role in transport and metabolism of endogenous and exogenous compounds. Several enzymatic activities of HSA on different substrates or drugs have been studied and documented.[1] It has been reported recently that RNase-free bovine serum albumin induces RNA depolymerization.[2] However, no evidence of RNA-hydrolyzing activity for HSA has been demonstrated.

The albumin fraction in blood is known to be heterogeneous. For example, glycated albumin (gHSA) 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.[3] These adducts subsequently undergo irreversible rearrangements to form heterogeneous class of advanced glycation end-products. The level of gHSA in blood of healthy subjects is 6–15%.[4] The ratio of different albumin forms in blood plasma depends on a person's health. For instance, the level of the modified HSA is increased in blood of patients with various forms of cancer[5] and diabetes mellitus.[6] Moreover, the level of a definite modified albumin form in blood plasma depends on the disease stage and complications.

Earlier, we have reported on albumin affinity toward synthetic polyribonucleotides.[7] Using capillary electrophoresis it has been shown that gHSA binds poly(A) more efficiently than the unmodified HSA ($K_a = 1.4 \times 10^5$ M^{-1} and 0.8×10^5 M^{-1}, respectively). These data are in accordance with our previous results on the protein–polynucleotide binding obtained using poly(A)-immobilized affinity columns.[8] Glycation of HSA is mainly lysine-directed,[3] which might affect RNA cleavage. In this report, the modification-associated inhibitory effect on albumin-mediated RNA cleavage has been characterized. To explain the influence of glycation on albumin functionality, 'triester-like' mechanism for the albumin-mediated RNA hydrolysis has been suggested.

A sample of nonenzymatic glycated albumin has been prepared from RNase-free HSA in vitro under the conditions described earlier.[9] The fluorescence characteristics of the obtained protein are similar to those of in vivo glycated HSA.[10] According to MALDI-TOF MS, the obtained gHSA contained about 15 U of glucose per molecule. As it has been reported earlier,[11] up to 15 glucose molecules are condensed on serum albumin in blood of patients with badly controlled diabetes mellitus. Thus, the structure of gHSA obtained in the present study is similar to that of the in vivo protein state under the pathological condition.

To monitor the reaction of native and glycated albumin with oligoribonucleotides, the methods of denaturing PAGE analysis, anion-exchange chromatography and ^{31}P NMR spectroscopy were employed using pAGGAUCUAUAAAUGAC (ON16), $p(U)_6$ or $p(A)_6$ as substrates. For the analysis of protein-mediated cleavage the substrates were incubated with RNase-free and fatty acids-free HSA or gHSA at 37 °C for up to 35 h in a solution containing 50 mM Tris–HCl, pH 7.0, 0.2 M KCl, 0.5 mM EDTA.

We initially examined the sites of hydrolysis of 5′-^{32}P-labeled ON16 in the presence of HSA and gHSA by comparison of the obtained cleavage patterns with the electrophoretic ladders generated by imidazole hydrolysis,[12] or RNase T1 digestion.[13] The

* Corresponding author.
E-mail address: godov@niboch.nsc.ru (T.S. Godovikova).

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PAGE revealed that HSA promoted ON16 cleavage in a concentration-dependent manner (data not shown). Equivalent amounts of glycated albumin had smaller effect (Fig. 1A). For instance, after 2.5 h of incubation with native HSA (1×10^{-5} M) more than 70 % of ON16 (1×10^{-6} M) was cleaved, whereas gHSA induced only 27% cleavage under the same conditions (Fig. 1B). This result suggested that glycation of the protein might inhibit RNA cleavage. Both native and glycated albumin hydrolyzed ON16 in a sequence-independent manner. However, the major cleavage occurred within the U7-A8 and U9-A10 sequences (Fig. 1B). Similar cleavage specificity was demonstrated in the case of RNase-free bovine serum albumin.[2]

It is known that UpA sequence is a major cleavage site in the reaction catalyzed by RNase A and some other human blood RNases.[14] However, the HSA catalytic properties distinguish the protein from other known human RNases. For example, we have shown that albumin is only two times more active in the hydrolysis of $p(U)_6$ in comparison with $p(A)_6$, whereas known human RNases are almost inactive with oligo(A) substrate (hydrolysis rate ratio for $p(U)_{10}$ and $p(A)_{13}$ is 500–2000).[14b]

In order to shed light on the mechanism of HSA-mediated oligoribonucleotide cleavage, $p(U)_6$ was used as a substrate. Since low rate of the oligoribonucleotide cleavage was expected, the reaction was carried out under second-order conditions, that is, the initial concentrations of $p(U)_6$ and HSA or gHSA were the same (1×10^{-3} M). The reaction between $p(U)_6$ and native or glycated albumin was monitored over 35 h. No spontaneous oligonucleotide degradation was detected under the same conditions for the same time period.

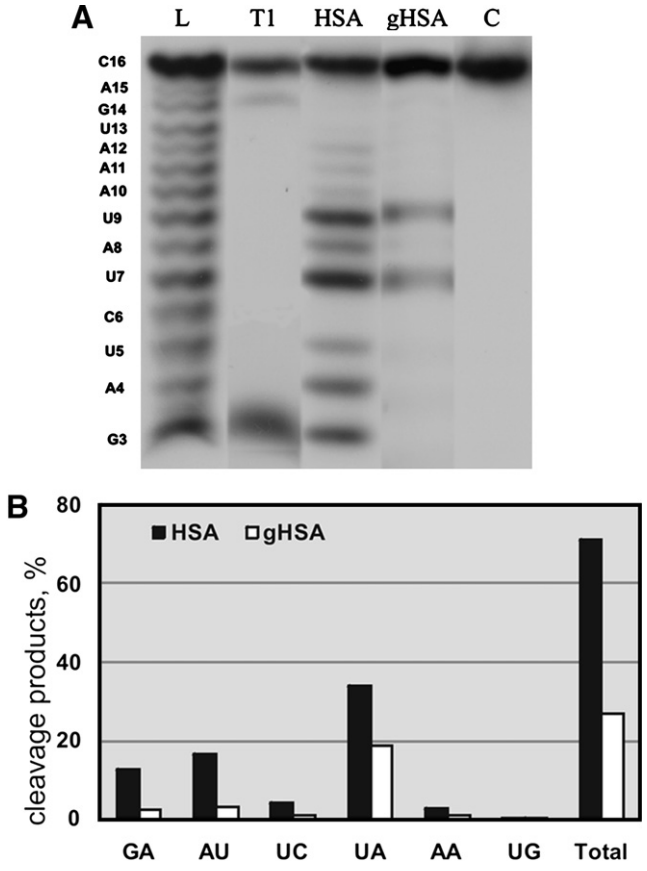

Figure 1. Cleavage of ON16 in the presence of native or glycated HSA. Panel A: autoradioaftograph of 20% PAAG/7 M urea after analyzing the cleavage products. Lane L represents the imidazole ladder; lane T1, RNase T1 digestion; lane C, incubation of ON16 without proteins. Panel B: efficiency and specificity of ON16 cleavage with HSA (black bars) and gHSA (white bars). Cleavage conditions: 50 mM Tris–HCl, pH 7.0, 0.2 M KCl, 0.5 mM EDTA, [α-^{32}P]ON16 (1×10^{-6} M), HSA or gHSA (1×10^{-5} M). The reaction mixtures were incubated at 37 °C for 2.5 h.

The analysis of $p(U)_6$ incubated in the presence of HSA by anion-exchange chromatography suggested that the protein enhanced the cleavage of the hexanucleotide internucleotide bonds (Fig. 2A). As shown in Fig. 2A (profile b), the retention times of the products were smaller as compared with that of the initial hexaribonucleotide.

Initial ^{31}P NMR spectra showed disappearance of the intact $p(U)_6$ peaks (the resonance typical of internucleotide phosphate groups at 0.19–0.35 ppm) with the concomitant appearance of two groups of resonance peaks (the ~20.55 and ~20.66 ppm peaks in Fig. 2B, spectrum a). These signals can be assigned to the oligonucleotides of different length caring 2′,3′-cyclophosphate group.[15] For longer reaction times (up to 35 h) the uridine 2′,3′-cyclophosphate peak (the 20.91 ppm peak in Fig. 2B, spectrum b) was accumulated at the expense of other peaks (Fig. 2B, spectrum c). This cyclic intermediate is hydrolyzed by a water molecule to a 3′- (or 2′)-phosphate (the 4.29 and 4.68 ppm peaks in Fig. 2B, spectrum c).

The ^{31}P NMR spectra analysis of $p(U)_6$ incubated in the presence of gHSA demonstrated the presence of two peaks for terminal 5′-phosphate (2.63 and 3.52 ppm) (Fig. 2C) instead of one broader signal (3.52 ppm) for $p(U)_6$ after incubation with native HSA (Fig. 2A, spectrum a). At the same time, the chemical shifts of internucleotide phosphates remained practically the same. The chemical shifts values of phosphate monoester group in aqueous solutions are known to depend on pH in ionization region of these groups and change in ~4 ppm interval.[15] The chemical shifts of phosphate esters are also sensitive to medium effects. In case of $p(U)_6$/gHSA reaction mixture, the observed difference in chemical shifts might be provided by conformational peculiarities of the oligonucleotide inside gHSA binding sites. Unfortunately, albumin binding sites for nucleic acids have not been revealed so far.

The ^{31}P NMR data indicated that cleavage of the $p(U)_6$ in the presence of gHSA resulted in the formation of products containing 2′,3′-cyclophosphate group (the 20.45 ppm peak in Fig. 2C).[15] The efficiency of $p(U)_6$ cleavage in the presence of gHSA was significantly lower than in the presence of HSA (compare Fig. 2B and C). This result suggests that the unmodified lysine residues are important for cleavage of the RNA molecule. It should be mentioned that Lys41 in the active site of RNase A participates both in phosphoryl group binding and in the catalysis.[16]

The obtained data allow us to suggest that the albumin-catalyzed RNA cleavage can follow a modified version of 'triester-like' mechanism (Fig. 3). The mechanism depends on the protonation of one of the nonbridging phosphoryl oxygens, rendering the transition state more like a phosphotriester, which is 10^3 to 10^5 times more reactive than the corresponding phosphodiester.[17] In the Breslow mechanism,[18] the proton comes from the catalytic acid. In an internal proton transfer mechanism,[19] the proton comes from the 2′-OH group. Either way, the resulted intermediate is a monoanionic cyclic pentaoxyphosphorane. After the formation of the transition state structure, the catalytic proton is abstracted from the nonbridging oxygen by the catalytic base, leading to the expulsion of the leaving group by the formation of the 2′,3′-cyclic phosphate diester. In the first step the attacking hydroxyl is OH group on C-2 of ribose; in the second step it is the OH group of a water molecule that cleaves the cyclic phosphate to generate the C-2 or C-3 OH groups.

Since glycation, which is known to be targeted mainly on lysine ε-amino groups,[3] inhibits albumin RNA-hydrolyzing activity, a lysine residue might serve as a catalytic base in the case of HSA-med-

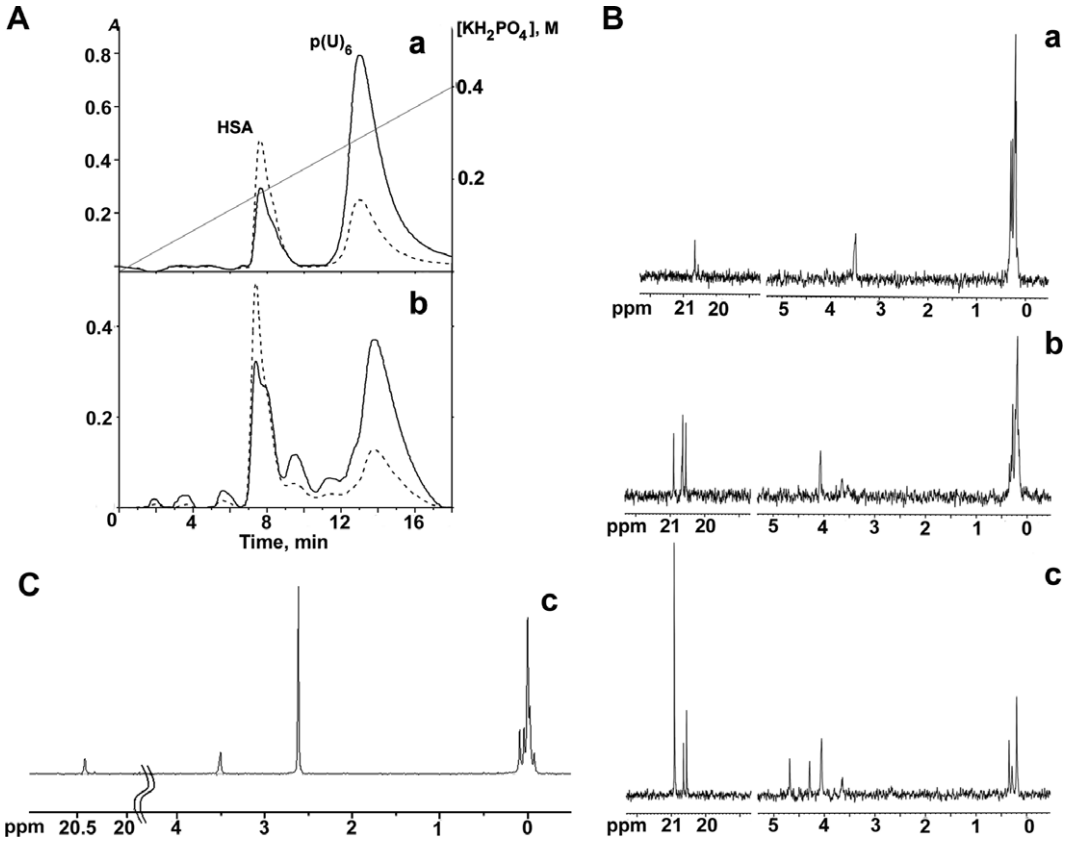

Figure 2. Cleavage of $p(U)_6$ in the presence of native or glycated HSA. Panel A: profiles of anion-exchange chromatography of the reaction mixture $p(U)_6$/HSA (solid line—A_{260}, broken line—A_{280}). Panels B and C: ^{31}P NMR spectra of the reaction mixtures $p(U)_6$/HSA (B) and $p(U)_6$/gHSA (C). Cleavage conditions: 50 mM Tris–HCl, pH 7.0, 0.2 M KCl, 0.5 mM EDTA, $p(U)_6$ (1×10^{-3} M), HSA or gHSA (1×10^{-3} M). The reaction mixtures were incubated at 37 °C for 1 h (a), 29 h (b), 35 h (c).

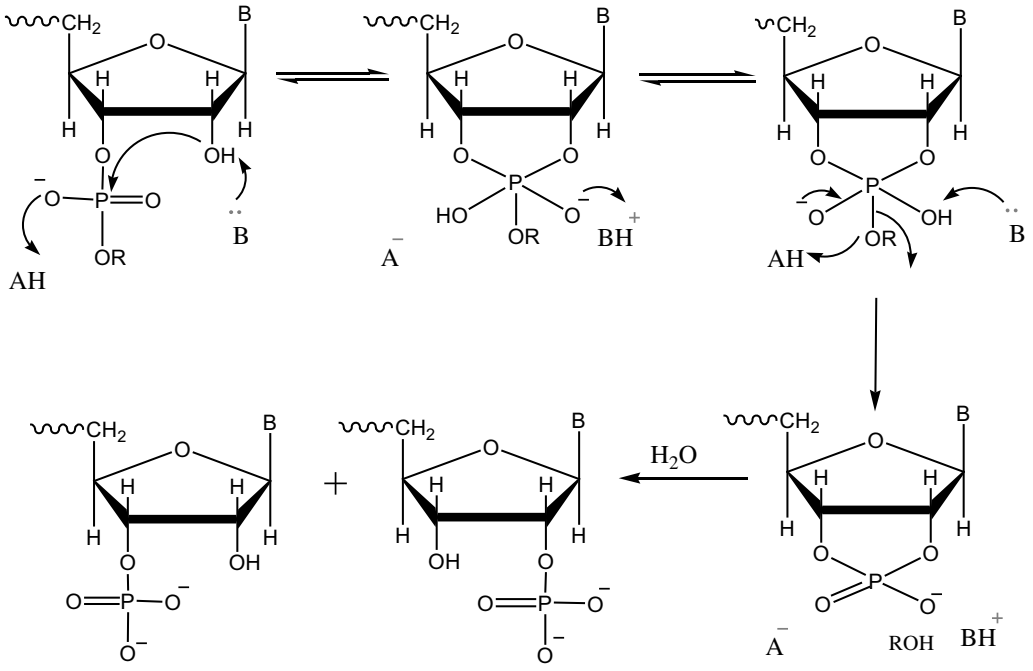

Figure 3. Proposed mechanism of RNA cleavage catalyzed by HSA.

iated RNA cleavage. The critical feature of this mechanism would require ε-amino group of the reactive lysine to be deprotonated in order to facilitate the attack toward poor acid such as 2′-OH group. Interestingly, the nonenzymatic reaction of HSA with glucose has revealed the presence of exceptionally nucleophilic lysine residues, and Lys-199 is one of these.[20] This chemical behavior is

consistent with an unusually low pK_a of ~8 for Lys-199, which implies of a significant amount of the neutral form at physiological pHs. The analysis of the solvent structure and dynamics indicates that the basic form of Lys-199 is likely connected to the acid form of Lys-195 through a network of H-bonding water molecules with a donor-network of H-bonding water molecules with a donor-acceptor character.[20] Furthermore, the X-ray structure of HSA[21] has revealed similar pairing of Lys541/Lys545 residues, which are also modified under the protein glycation.[22]

Based on the data of competition binding assay, as well as molecular dynamics simulation, two possible binding sites for nucleic acids were suggested: warfarin-specific binding site I and additional binding site located in domain IIIA in C-terminal region of the protein molecule.[23] Interestingly, the pairs Lys195/Lys199 and Lys541/Lys545 are located in the region of binding site I and the proposed C-terminal oligonucleotide-binding site, respectively. This allows us to speculate about possible catalytic role of Lys in RNA-hydrolyzing activity of HSA.

Although albumin displays a low reactivity toward RNA compared to RNase A and other known human RNases,[14] the large quantity of albumin in blood circulation and lymph may provide a substantial reservoir for sequestering and hydrolyzing extracellular nucleic acids. The level of extracellular nucleic acids in blood plasma has been reported to increase in the case of different diseases, thus indicating the possible role of extracellular nucleic acids in the development of pathological processes.[24] This study shows that one of albumin modification results in significant loss of its RNA-hydrolyzing activity. Since albumin may play a critical role in ligand binding due to its abundance in serum, these findings have implications in disease states characterized by increased levels of extracellular nucleic acids, for instance autoimmune diseases and cancer.

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Supplementary data

The details of the experimental procedures can be found in Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.060.

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