COMPETITION BETWEEN SERUM AMYLOID PROTEIN AND APOPROTEIN E FOR BINDING WITH HUMAN SERUM ALBUMIN

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UDC 616.153.96:577.112.6]-074

KEY WORDS: serum amyloid protein; apoprotein E; human serum albumin; protein–protein interactions

Serum amyloid protein (SAP) is a component of amyloid fibrils – the characteristic structures of secondary amyloidosis [6]. These structures also contain protein AA, whose precursor, protein SAA, is associated with high-density lipoproteins of subclass 3 (HDL3) [4]. SAP binds with the amyloid fibril through the involvement of a Ca^{2+}-bridge, as a result of affinity for certain polyanions, such as sulfated glycosaminoglycans [5]. Since protein SAA is a component of HDL_3, whose amphipathic apoproteins (apoA, C, E) are in dynamic equilibrium between lipid-associated and water-soluble forms, an increase in the content of SAA on the surface of a lipoprotein particle, such as is observed, for example, in inflammation [4], may lead to displacement of the apoproteins, one of which, namely apoE, as we showed in a previous study [1], interacts with human serum albumin (HSA). In that case interaction between SAP and HSA, which has not yet been studied, may perhaps be modulated by a change in content of the water-soluble form apoE, which in turn, may lead to a change in clearance of SAP, with the appropriate pathophysiological consequences. The aim of the present investigation was an experimental assessment of interrelations within the HSA/SAP/apoE complex and the possible mechanism of such interaction.

EXPERIMENTAL METHOD

Isolation and purification of SAP, including adsorption on sepharose 4B, immunoaffinity chromatography on a column with immobilized rabbit immunoglobulins G against human serum proteins, and gel-chromatography on Sephacryl S-300 were carried out as described in [3]. Isolation of apoE and labeling of the apoprotein with fluorescein isothiocyanate (apoE/F) were carried out as described in [2]. To assess binding of SAP with sepharose 4B, SAP in different concentrations were incubated for 14 h at 4°C the matrix in 0.01 M Tris-HCl, pH 8.0, 0.14 M NaCl, 2 mM $CaCl_2$, 0.01% Na azide (Ca^{2+}-buffer) or in 0.01 M Tris-HCl, pH 8.0, 0.14 M NaCl, 10 mM EDTA, 0.01% Na azide (EDTA-buffer). The sepharose was separated by low-speed centrifugation, and the SAP concentration in the supernatant was determined fluorometrically (excitation at 281 nm, recording at 340 nm). The percentage of binding was calculated by the equation: bound SAP (%) = [(I (EDTA) – I (Ca^{2+}))/I (EDTA)] × 100, where I (EDTA) and I (Ca^{2+}) denote the intensity of fluorescence of SAP in the supernatants after incubation with sepharose 4B in EDTA- and Ca^{2+}-buffer solutions respectively. The gel-chromatographic study of dissociation of SAP was carried out by chromatography of 25 μl of the protein solution in different concentrations on a serially connected Polyol Si 100 HPLC precolumn (10 μ, 4.6 × 75 mm) and Polyol Si 300 HPLC column (5 μ, 7.1 × 500 mm) (both from "Serva," Germany), with elution at the rate of 0.2 ml/min with 50 mM phosphate buffer, pH 7.0, 0.15 M NaCl, 1 mM EDTA (buffer A). The effect of apoE/F on binding of SAP with HSA with completely reduced S–S bonds was studied during gel-chromatographic fractionation of a mixture of 2.21 μM SAP and 24.5 μM HSA, containing 0.45 μM apoE/F or in the absence of the apoprotein, after preliminary treatment of the mixture with dithiothreitol in a concentration of 20 mM. Fractionation of 100 μl of the mixture was carried out on the same Polyol Si 300 column

Research Center for Preventive Medicine, Ministry of Health of Russian Federation, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 12, pp. 598-600, December, 1992. Original article submitted March 30, 1992.

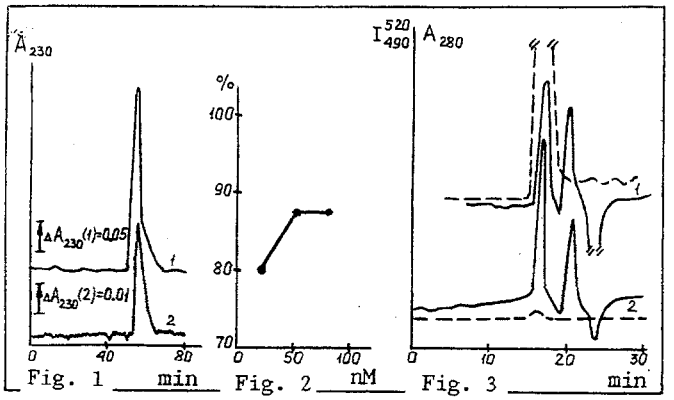

Fig. 1. Gel-chromatographic study of dissociation of SAP. SAP concentration 5.3 (1) and 0.53 μM (2). Protein detected by absorption at 230 nm (A_{230}).

Fig. 2. Binding of SAP with sepharose 4B depending on protein concentration.

Fig. 3. Gel-chromatographic study of displacement of serum amyloid protein by apoprotein E from complex with HSA with reduced S–S bonds. 1) Fractionation of SAP/HSA/apoE/F mixture, 2) fractionation of SAP/HSA mixture. Elution profile based on absorption at 280 nm (A_{280}) denoted by continuous line; that based on fluorescence of fluorescein – excitation at 490 nm, recording at 520 nm; I_{490}^{520} – broken line.

with elution at the rate of 0.5 ml/min with buffer A containing 14.7 μM HSA. The protein concentration was determined as in [7] in the presence of 5% SDS.

EXPERIMENTAL RESULTS

During assessment of the degree of purity of the SAP after its isolation, by gel-filtration on an HPLC column (Fig. 1) the protein came out as a single peak, and if the SAP concentration was reduced tenfold(from 5.3 to 0.53 μM) the peak was shifted toward the zone of high volumes and became heterogeneous. Since SAP is an oligomeric protein containing 10 identical subunits with mol. wt. of 23 kilodaltons, arranged in the form of two adjacent pentamers [8], the concentration-dependent behavior of SAP may indicate dissociation of the oligomeric structure of SAP in the course of gel-filtration. It can be tentatively suggested that the degree of Ca^{2+}-dependent binding of SAP in the oligomeric and dissociated states with sepharose 4B will differ. In fact, the efficiency of binding of protein with matrix decreased with a fall in the SAP concentration in the incubation medium from 81 nM to 27 nM (Fig. 2). Thus the existence of equilibrium between associated and dissociated forms of SAP was established by two independent approaches. The mechanism of oligomerization may include hydrophobic interactions between individual subunits of SAP. In this case, the provision of an additional surface for binding of the subunits in the composition of the oligomeric protein may induce its dissociation, followed by interaction with this surface. Such a mechanism was suggested by the present writer [1] in the case of interaction of a different oligomeric protein, namely apoprotein E, which exists in solution in the form of a tetramer, and of human serum albumin with completely reduced S–S bonds, forming aggregates. During gel-chromatography of the mixture of reduced HSA and SAP (the molar monomeric ratio is 11:1), two peaks are observed: high-molecular-weight, with shorter retention time on the column, and a second peak corresponding to removal of SAP (Fig. 3, profile 2). Addition of fluorescein-labeled apoprotein E to the incubation mixture (ratio HSA:SAP:apoE 11:1:0.2) led to complete binding of the apoprotein with the aggregated form of reduced HSA and displacement of SAP from its complex with reduced albumin (Fig. 3, profile 1): whereas in

the absence of apoE/F the ratio of the heights of the high-molecular-weight peak and the SAP peak was 1.74, in the presence of apoprotein this ratio fell to 1.15.

Consequently, the presence of a hydrophobic surface in the aggregated form of albumin leads to binding of the apoprotein and to competitive displacement of serum amyloid protein from its complex with the hydrophobic surface. It can be tentatively suggested that a similar mechanism also is realized in the case of lipoprotein – SAP interaction, where the hydrophobic surface is formed by the lipid phase. Thus in the present investigation competitive interrelations between SAP and apoE were found during binding with albumin, and a contribution of the oligomeric state of SAP to this relationship is suggested. Interactions of this kind in the SAP/HSA/apoE system, in the presence of any changes of equilibrium toward the water-soluble form of apoE, in particular, with an increase in the content of the water-soluble form of apoE (in particular, during an increase in concentration of protein SAA on the surface of the lipoprotein particle) may lead to a change in clearance of SAP due to a change in the ratio between free and albumin-associated forms of SAP. The contribution of the lipoprotein particle and of amphipathic apoproteins to the development of secondary amyloidosis may be realized in this way.

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