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Comparison between intact and desialylated human serum amyloid P component by laser photo CIDNP (chemically induced dynamic nuclear polarization) technique: an indication for a conformational impact of sialic acid

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The human pentraxin serum amyloid P component (SAP) exhibits no microheterogeneity in its complex di-antennary glycan. To elucidate whether the removal of sialic acids from this glycoprotein might affect the accessibility of certain amino acid residues of the protein we employed the laser photo CIDNP approach as a sensitive tool. The CIDNP effect is generated by the interaction of a photoexcited dye with reactive amino acids and results in enhanced absorption- or emission-signals which can be observed for the three aromatic amino acids histidine, tryptophan, and tyrosine if they are accessible to the dye. Therefore, this technique can be applied to explore surface exposure of these amino acid residues. The respective spectra of SAP and enzymatically desialylated SAP were determined. Six tryptophan/histidine signals and one tyrosine signal are present in the aromatic part of the CIDNP difference spectrum of SAP. The corresponding spectrum of desialylated SAP shows remarkable alterations. The chemical shift of one Trp/His-characteristic signal is decreased by 0.1 ppm. One Trp/His-signal disappeared and a new one was formed in the CIDNP difference spectrum of desialylated SAP, while the other signals were unaffected. The Tyr signal has a clearly enhanced intensity in desialylated SAP. Therefore, the removal of sialic acid moieties from the single N-glycan of each monomer apparently affects surface presentation of distinct CIDNP-reactive amino acids of SAP [1]. A conformational change of the protein part of SAP in relation with a different orientation of the desialylated oligosaccharide chain in comparison to the complete one is a possible explanation of our CIDNP results.

***Keywords*: human serum amyloid P component (SAP), chemically induced dynamic nuclear polarization (CIDNP), glycoprotein, sialic acid, protein conformation**

Introduction

Human serum amyloid P component (SAP) belongs to the family of pentraxins, defined by their characteristic pentameric organization of identical subunits [2–5]. In comparison to the structure of the human acute phase reactant C-reactive protein with its five protomers, native SAP is arranged as a planar, non-covalently linked face-to-face dimer of two disc-shaped pentamers, constituting a decameric complex [3–8]. Further aggregation can be mediated by increasing the Ca^{2+}-concentration [6–9]. This cation is also essential for the binding activities of this lectin-like molecule to glycosaminoglycans, the cyclic 4,6-pyruvate acetal of galactose, mannose-6-phosphate and the 3-sulfated derivatives of galactose, *N*-acetylgalactosamine and glucuronic acid, which are thought to influence the deposition of SAP to amyloid fibrils and bacterial surfaces [10–13]. The protein part of human SAP and its tissue form show no disease-associated alterations, which is also true for their glycosylation pattern. SAP contains one invariant complex di-antennary glycan at Asn32, located on a β-strand under the single α-helix on the protomer surface opposite of the Ca^{2+}- and ligand-binding site [13–16]. This lack of microheterogeneity is unusual and may thus be

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structurally and/or functionally important. Enzymatic removal of sialic acids provides a means to address the potential role(s) of this monosaccharide. With respect to catabolism, the desialylated form will be rapidly transported into hepatocytes via asialoglycoprotein receptor-mediated endocytosis for lysosomal degradation [16]. Structurally, it retains its decameric state of aggregation and its full capacity to bind *in vitro* to amyloid fibrils and to DNA, whereas its interaction with agarose is reduced by 7% despite opposite locations of the carbohydrate chain and the ligand-binding site [14, 16–18]. To answer the question, whether removal of the two sialic acid moieties from the single N-linked saccharide chain per protomer may cause a change in surface exposure of aromatic amino acids, a sensitive assay system is required. Technically, monitoring of signals, derived from corresponding histidine, tryptophan, and tyrosine residues with laser photo CIDNP (chemically induced nuclear polarization) studies affords such a system [19, 20].

The methyl signal of the sialic acic moiety of SAP is resolved. Although the SAP-glycoprotein does not show a resolved spectrum, it was possible to assign this signal due to its disappearance after addition of sialidase.

Materials and methods

SAP was purified from serum of healthy donors, kindly provided by the local blood bank. Mannose-Sepharose 4B chromatography was used in order to deplete the serum of mannose-binding protein, and subsequent affinity chromatography on Sepharose 4B was performed (3.3×21 cm gel bed dimensions for each 800 ml sample), as described [21, 22] followed by dialysis against 20 mM sodium acetate buffer, pH 5.0, containing 5 mM EDTA. Individual preparations were divided and either incubated with 0.5 U sialidase from *Clostridium perfringens* (type V, from Sigma, Munich, FRG) in 250 mM sodium acetate buffer (pH 5.0), containing 5 mM EDTA, for 17 h at 37 °C or treated identically without addition of enzyme (mock-treated SAP). Then, the pH-value was adjusted to 7.4, and the samples were dialysed first against 20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM $CaCl_2$, subsequently against water containing 1 mM $CaCl_2$ and finally against water, followed by lyophilization. Gel electrophoretic analysis and protein determination were carried out as described [23]. Desialylated SAP is known to have an increased mobility in gel electrophoresis under denaturing conditions so that the removal of sialic acid residues can readily be assessed. Gel filtration of sialidase- and mock-treated SAP was performed with Superose 12 HR10/30 (Pharmacia, Freiburg, FRG) using 20 mM PBS, pH 7.2, as eluent at a flow rate of 0.25 ml min^{-1} and detection at 220 nm. The sialidase was analysed under the same conditions as control.

Analytical procedures using the CIDNP approach were performed as outlined. In detail, CIDNP experiments were carried out at 360 MHz on a Bruker AM-360 NMR spectrometer. As the source of light, a continuous-wave argon ion laser (Spectra Physics, Mountain View, USA) operating in the multiline mode with principal wavelengths of 488.0 and 514.5 nm was used. The light beam from the argon ion laser was directed to the sample by an optical fibre and chopped by a mechanical shutter controlled by the spectrometer. The generation of the CIDNP effect is explained in Figure 1. Typical operation conditions were as follows: 1 s presaturation pulse, 0.6 s light pulse (5 W), 5 μs RF pulse (90° flip angle), 1 s acquisition time, 5 s delay. The irradiation during the CIDNP experiment is short enough to prevent serious sample heating due to light absorption. For the used samples 16 or 32 light scans gave an adequate signal-to-noise ratio. CIDNP was generated by using flavin I mononucleotide. By alternately recording one light and four dark free induction decays, Fourier transformation and subtraction of the resulting light spectrum from the dark spectrum, CIDNP difference spectrum was established, containing only lines of polarized residues, as shown in Figure 2. Surface accessibilities of the targeted residues were calculated [24] from the X-ray structure of SAP (Brookhaven crystallographic data base) on a Silicon Graphics Personal Iris.

Results and discussion

No structural data about the carbohydrate chains of SAP are given in the X-ray derived model. However, the absence or presence of only sialic acid in the carbohydrate side chains leads to an altered surface accessibility of distinct amino acid residues in the protein part of SAP, *eg* for Tyr (Figure 3).

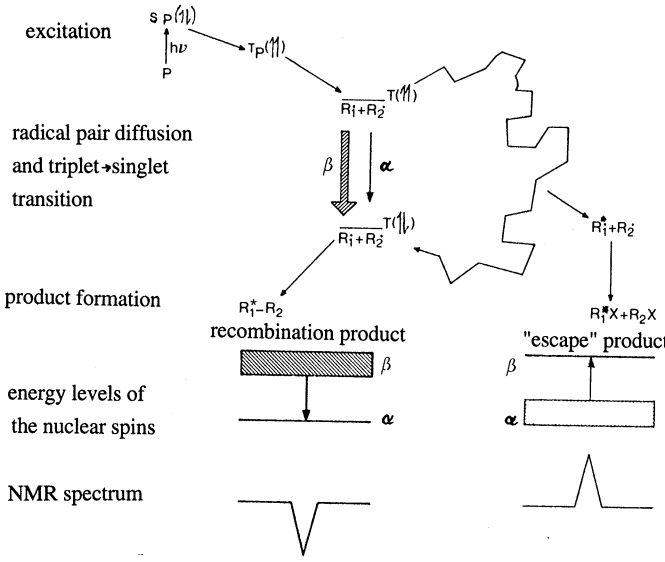

Figure 1. Radical pair mechanism of CIDNP. The scheme visualizes the generation of CIDNP.

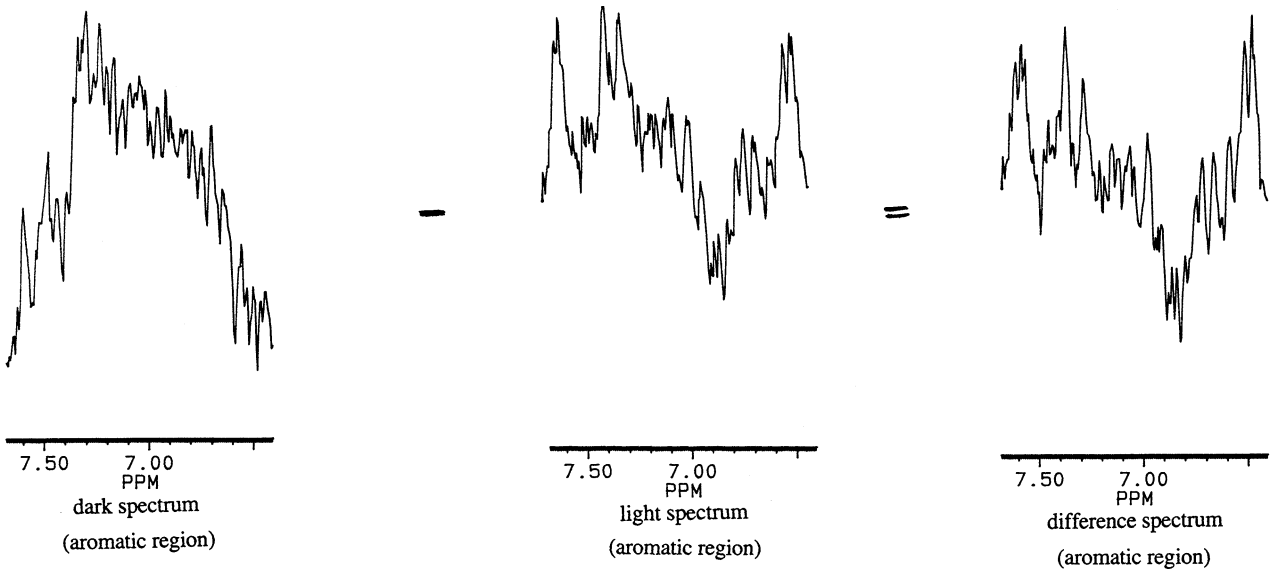

Figure 2. Overview of the generation of a CIDNP difference spectrum of SAP by subtraction of the light spectrum from the dark spectrum.

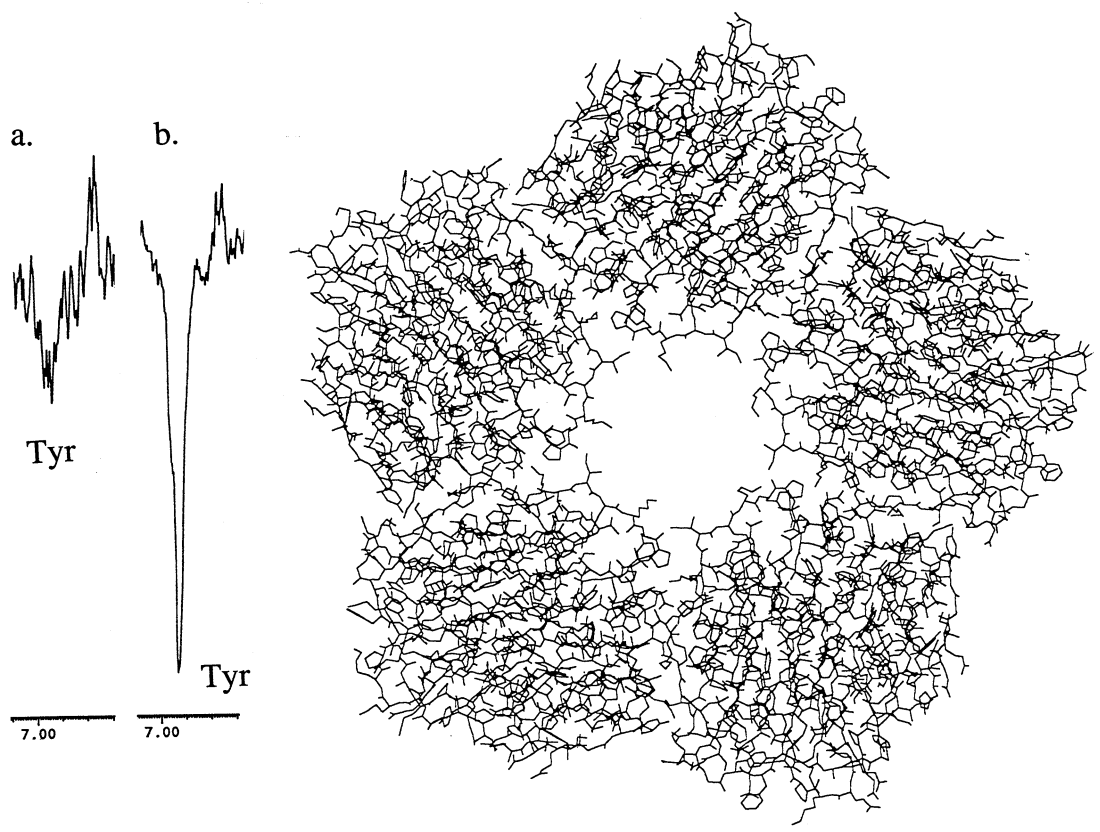

Figure 3. Tyr-peak from a CIDNP difference spectrum of mock-treated (a) and sialidase-treated SAP (b) supplementing the X-ray data [15].

The intensity of the Tyr signal, which is present in a negative direction in the CIDNP difference spectrum, is enhanced when the oligosaccharide chain of each protomer of SAP is desialylated. The change of intensity of the Tyr signal in the CIDNP difference spectrum of asialo-SAP indicates that the surface presentation of such a residue is increased. However, it cannot be determined whether this increase concerns a single Tyr signal or overlapping signals from

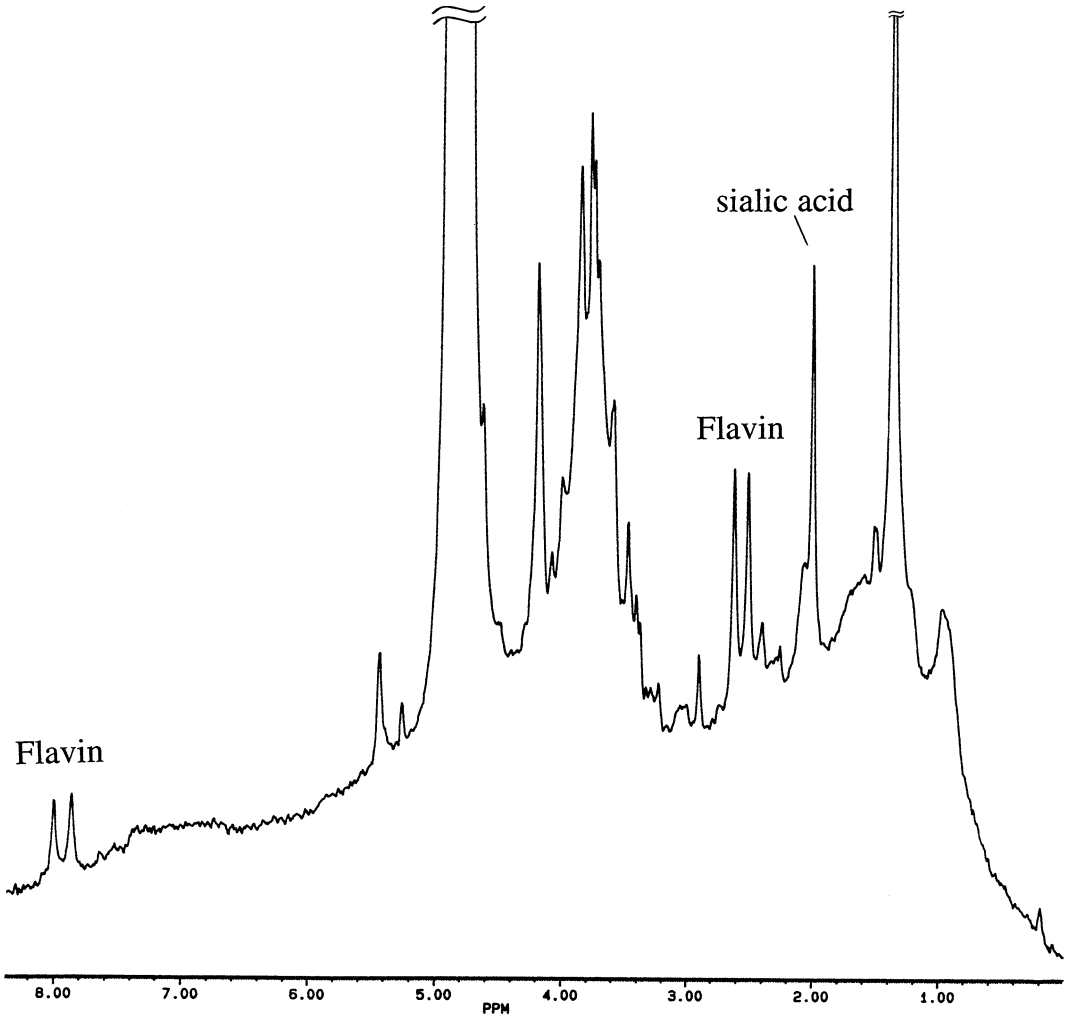

Figure 4. One-dimensional ^{1}H-spectrum of SAP in which the signal of the *N*-acetyl group of sialic acid is marked.

several Tyr units. These results reveal that desialylation causes a different surface accessibility of aromatic amino acid residues of the pentraxin. The gel filtration analysis allows the exclusion of an impact on the state of aggregation as an explanation for this response. Although the available X-ray structure of SAP does not provide precise spatial information for the oligosaccharide chain that would permit unambiguous correlation of detected signals to certain residues, the molecular model can be used for the calculation of the areas of accessibility of the four histidine- and the five tryptophan-residues of each SAP protomer [1]. The *N*-acetyl signal of sialic acid is clearly visible in the one-dimensional ^{1}H-NMR spectrum, as shown in Figure 4. The *N*-acetyl signal of sialic acid (which disappears after sialidase treatment) is marked. We have found no indication by signal line broadening for a direct contact between the protein part of SAP and the sialic acid residue of the oligosaccharide chain of SAP. The remarkable number of unaffected CIDNP signals of desialylated compound indicates that no overall conformational change of the protein part has occurred. In computational studies of sialic acid containing oligosaccharides in comparison to their asialo analogues we have found evidence for the conformational influence of sialic acid. Therefore, a conformational change in relation with an altered dynamic behaviour of the oligosaccharide chain may lead to a different orientation thereby affecting the surface accessibilities of several aromatic amino acids.

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