

# Effect of enzymatic desialylation of human serum amyloid P component on surface exposure of laser photo CIDNP (chemically induced dynamic nuclear polarization) – reactive histidine, tryptophan and tyrosine residues

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**Abstract** 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 created 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 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 tryptophan/histidine-characteristic signal is decreased by 0.1 ppm. One tryptophan/histidine signal disappeared and a new one was formed in the CIDNP difference spectrum of desialylated SAP, while the other signals were unaffected. The tyrosine 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.

**Key words:** Chemically induced dynamic nuclear polarization (CIDNP); Glycoprotein; Human serum amyloid P component (SAP); Sialic acid

## 1. Introduction

Human serum amyloid P component (SAP) belongs to the family of pentraxins, defined by their characteristic pentameric organization of identical subunits [1–4]. In comparison to the structure of the human acute phase reactant C-reactive protein with its five promoters, native SAP is arranged as a planar, non-covalently linked face-to-face dimer of two disc-shaped pentamers, constituting a decameric complex [2–7]. Further aggregation can be mediated by increasing the  $\text{Ca}^{2+}$  concentration [5–8]. 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 [9–12]. The protein part of human SAP and its tissue form shown no disease-associated alterations, which is also true for their glycosylation pattern [12]. SAP contains one invariant complex di-antennary glycan  $\text{Asn}^{32}$ , located on a  $\beta$ -strand under the single  $\alpha$ -helix on the promoter surface opposite of the  $\text{Ca}^{2+}$ - and ligand-binding site [12–15]. This lack of microheterogeneity is unusual and may thus be structurally and/or functionally important.

Enzymic 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 hepacytes via asialoglycoprotein receptor-mediated endocytosis for lysosomal degradation [15]. 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 to opposite locations of the carbohydrate chain and the ligand-binding site [13,15–17]. To answer the question, whether removal of the two sialic acid moieties from the single N-linked saccharide chain per promoter 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 [18,19].

## 2. 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 ( $3.3 \times 21$  cm gel bed dimensions for each 800 ml sample), as described [20,21], 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 units 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 dialyzed first against 20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM  $\text{CaCl}_2$ , subsequently against water containing 1 mM  $\text{CaCl}_2$  and finally against water, followed by lyophilization. Gel electrophoretic analysis and protein determination were carried out as described [22]. 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 [13]. Gel filtration of

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sialidase- and mock-treated SAP was performed with Superose 12 HR 10/30 (Pharmacia, Freiburg, FRG) using 20 mM PBS, pH 7.2, as eluent at a flow rate of 0.25 ml/min and detection at 220 nm. The sialidase was analyzed under the same conditions as control. Analytical procedures using the CIDNP approach were performed as outlined [18,19]. In detail,  $^1\text{H}$  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. Typical operation conditions were as follows: 1 s presaturation pulse, 0.6 s light pulse (5 W), 5  $\mu\text{s}$  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 1 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, the CIDNP difference spectrum was established, containing only lines of polarized residues. Surface accessibilities of the target residues were calculated from the X-ray structure of SAP (Brookhaven crystallographic data base) on a Silicon Graphics Personal Iris. The computer calculations were carried out with the help of the Connolly program in InsightII following an established method [23]. The assessment of the routine 'surface' implemented in InsightII pinpoints the accessible exterior part of the relevant portions of the molecule by smoothening the van der Waals surface with a test sphere that displays the average radius of the solvent water (1.5 Å). The dot density was generally set to a value of 1.

### 3. Results and discussion

In gel electrophoretic analysis, the sialidase-treated glycoprotein differs significantly from SAP that underwent identical processing except for the presence of the enzyme, as shown in Fig. 1. No residual protein at the position of SAP was seen in silver-stained gel lanes of sialidase-treated glycoprotein. It is similarly notable that the trace amount of enzyme is not visible in the stained gels. To determine whether desialylation may

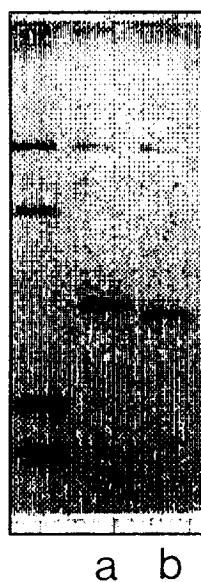


Fig. 1. Visualization of SAP (a) and its desialylated form (b) by silver staining after reducing SDS gel electrophoresis on a 10% running gel, using bovine serum albumin (66 kDa), ovalbumin (45 kDa),  $\beta$ -lactoglobulin (18.4 kDa),  $\kappa$ -lactalbumin (14.2 kDa) as markers for molecular mass designation.

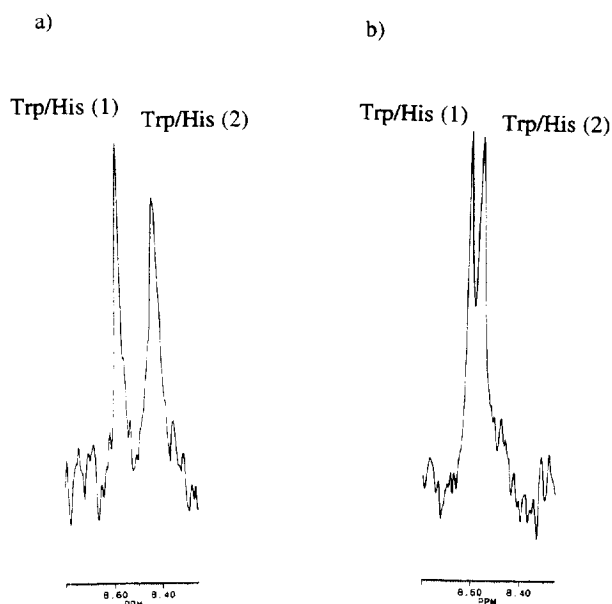


Fig. 2. Part of a CIDNP difference spectrum of mock-treated (a) and sialidase-treated SAP (b) which shows the chemical shift differences of two Trp/His signals.

affect the state of aggregation of the pentraxin, HPLC gel filtration analysis was performed, which revealed no changes between mock-treated and sialidase-treated SAP (not shown). This results confirms a previous note that desialylation has no impact on decamer formation [15]. Subsequently, aliquots of individual preparations were subjected to CIDNP measurements.

Six Trp/His signals occur in positive direction of the CIDNP difference spectrum obtained from SAP with a complete oligosaccharide chain, as shown in Fig. 2a and 3a. A comparative monitoring with desialylated SAP is illustrated in Fig. 2b and 3b. The intensities and chemical shift values of the Trp/His signals 1, 3, 5, and 6 are not altered. Thus, the total surface presentation of the respective side chains is apparently not affected by the enzyme treatment. However, Trp/His signal 2 is shifted 0.1 ppm downfield (Fig. 2b). Such an effect will be caused when the surface accessibility of the corresponding residue is unchanged, whereas the chemical vicinity is altered. Trp/His signal 4 has disappeared and a new Trp/His signal, referred to as Trp/His 7, occurs in the CIDNP difference spectrum of asialo SAP (Fig. 3b). Furthermore, the intensity of the Tyr signal, which is present in negative direction in the CIDNP difference spectrum, is enhanced, when the oligosaccharide chain of each promoter 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 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 to exclude an impact on the state of aggregation as explanation for this response. Although the available X-ray structure of SAP does not provide precise spatial information for the oligosaccharide chain

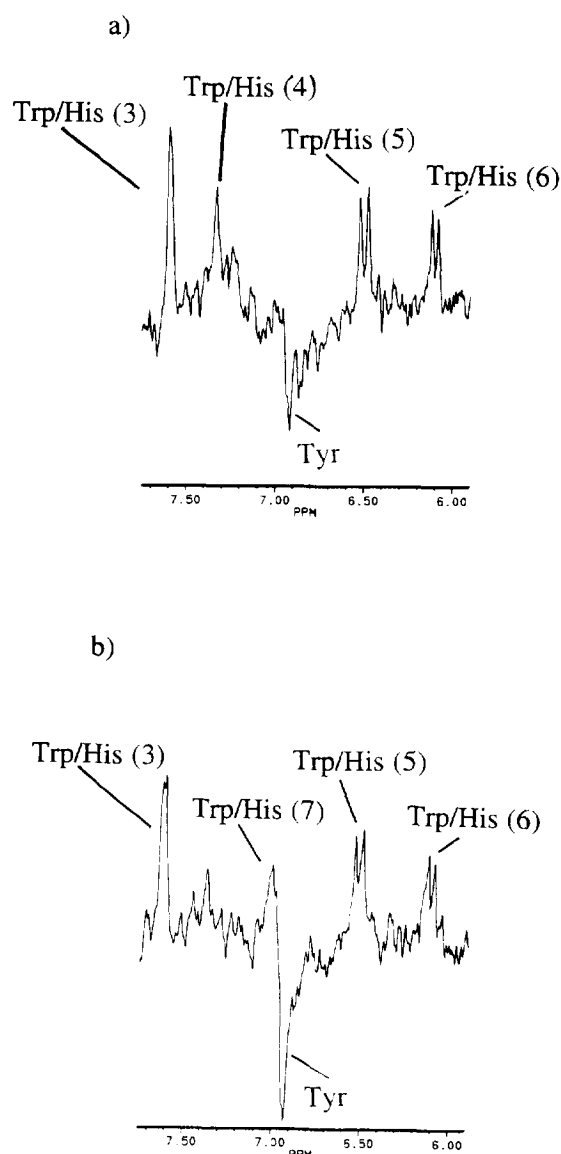


Fig. 3. Spectrum of mock-treated (a) and sialidase-treated SAP (b), which shows the strong decrease of Trp/His signal 4, the appearance of Trp/His signal 7 and the increase in intensity of the Tyr signal.

Table 1  
Surface accessibilities [ $\text{\AA}^2$ ] of CIDNP-reactive histidine (His) and tryptophan (Trp) residues of the five SAP subunits

	A	B	C	D	E
His 1	121.7	89.3	104.8	141.7	142.5
His 19	20.3	20.5	18.0	133.8	144.3
His 78	129.1	53.4	62.5	59.0	129.7
His 93	135.6	24.3	23.6	138.9	151.2
Trp 98	182.8	0	0	52.3	190.7
Trp 108	184.4	34.7	33.6	154.9	201.0
Trp 160	198.8	18.2	27.6	208.2	193.9
Trp 185	6.1	4.9	0	205.6	199.2
Trp 203	28.1	32.4	33.2	197.6	186.1

(dot density: 1, sphere radius: 1.5  $\text{\AA}$ ); SAP (subunit A, B, C, D, E).

Table 2

Surface accessibilities [ $\text{\AA}^2$ ] of CIDNP-reactive tyrosine (Tyr) residues of the five SAP subunits

	A	B	C	D	E
Tyr 40	22.5	20.9	21.2	177.1	177.3
Tyr 47	35.1	25.0	30.7	172.3	163.4
Tyr 52	136.3	3.7	0	174.0	164.8
Tyr 64	75.1	25.8	32.2	189.1	175.3
Tyr 71	152.5	10.9	9.0	81.0	181.8
Tyr 74	169.6	35.7	29.3	118.9	178.2
Tyr 123	171.0	39.0	46.1	88.8	174.7
Tyr 140	120.1	83.9	69.9	180.3	167.6
Tyr 158	175.9	37.2	34.0	177.1	166.2
Tyr 173	168.0	63.5	73.5	162.2	170.4
Tyr 190	49.2	47.9	52.1	184.2	160.9
Tyr 195	48.1	61.6	49.4	172.7	170.4

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 promoter. Since His or Trp residues with an accessibility area below 30  $\text{\AA}^2$  are considered to give rise to negligible CIDNP signals, involvement of such residues in generation of the detected desialylation-dependent changes is not very likely. His or Trp residues with an accessibility area above 100  $\text{\AA}^2$  for His and above 150  $\text{\AA}^2$  for Trp, however, should produce clearly visible CIDNP signals. Eleven His- and twelve Trp-molecules can display such a high level of accessibility that can be different for individual amino acids residues of different promoters (Table 1). Since only six signals from His or Trp residues are present in the CIDNP spectrum of SAP, a signal overlap may be responsible for this observation. A similar situation is seen in the case of Tyr, when the accessibilities of the 60 Tyr units (12 at each promoter) are determined on the basis of the X-ray crystallographic structure (Table 2). Remarkably, the determined accessibility values of all Tyr residues of promoters B and C are low. Of these Tyr units 18 have accessibility values below 50  $\text{\AA}^2$ , none of these residues has a value above 85  $\text{\AA}^2$ . Although an unequivocal correlation of each signal to individual amino acids is presently not possible, it is reasonable to conclude that at least the contribution of side chains from the promoters B and C to the CIDNP tyrosine signals will be small. To infer which His, Trp and Tyr residues participate in the measured responses to desialylation, extensive computer-assisted calculations of SAP in comparison to asialo-SAP are required and now in progress. In summary, these results may suggest that sialylation of the glycans is important to sustain at present not precisely defined aspects of the protein conformation and thus the biological activity.

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