Investigating interactions of the pentraxins serum amyloid P component and C-reactive protein by mass spectrometry

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The oligomeric state of human SAP (serum amyloid P component) in the absence and presence of known ligands has been investigated using nanoelectrospray ionization MS. At pH 8.0, in the absence of Ca^{2+}, SAP has been shown to consist of pentameric and decameric forms. In the presence of physiological levels of Ca^{2+}, SAP was observed to exist primarily as a pentamer, reflecting its *in vivo* state. dAMP was shown not only to promote decamerization, but also to lead to decamer stacking involving up to 30 monomers. A mechanism for this finding is proposed. CRP (C-reactive protein), a pentraxin closely related to SAP, exists as a pentamer in the presence or absence of Ca^{2+}. Pentamers of CRP and SAP were shown to form mixed decamers in Ca^{2+}-free buffer; however, in the presence of Ca^{2+}, this interaction was not observed. Furthermore, no exchange of monomeric subunits was observed between the SAP and CRP oligomers, suggesting a remarkable stability of the individual pentameric complexes.

Key words: amyloid P component, C-reactive protein (CRP), mass spectrometry, nanoelectrospray ionization MS (nanoESI MS), pentraxin.

INTRODUCTION

As part of the systemic reaction to inflammation, significant changes in the liver, termed the acute-phase response, result in a radical alteration of the biosynthetic profiles of particular plasma proteins [1]. These proteins are termed APRs (acute-phase reactants), and in mammals include serum amyloid A protein, CRP (C-reactive protein) and SAP (serum amyloid P component). CRP is the major APR in humans [2], whereas SAP, which is the major APR in mice, shows only a slight increase in its plasma level [3]. SAP is, however, universally associated with amyloid deposits [4].

Both CRP and SAP are principal members of the pentraxin family of proteins, which are characterized by a planar disc arrangement of five, non-covalently associated, identical subunits [5]. The physiological functions of these pentraxins involve Ca^{2+}-dependent ligand binding. SAP has been shown to bind chromatin and DNA in apoptotic and necrotic cells, inhibiting the formation of antibodies against chromatin fragments, thus preventing antinuclear autoimmunity [6]. SAP also plays a dual role in bacterial infection, exhibiting a host-defence function against pathogens to which it does not bind; however, when SAP binds to bacteria, a strong anti-opsonic effect results in enhanced virulence of the infectious agent [7]. Serum CRP levels, which reflect the extent of systemic inflammation, have been found to have utility in the prediction of coronary events not only in patients with established coronary disease, but also for the prediction of future events in apparently healthy individuals [8].

Unlike CRP, each SAP monomer is glycosylated with a single N-linked biantennary oligosaccharide at Asn^{32} [9]. The two faces of the SAP pentamer, defined as A and B, are characterized by five α-helices and five double Ca^{2+}-binding sites respectively [5]. Oligomerization properties of SAP are highly dependent upon solution conditions. Until recently, the oligomeric assembly of SAP under physiological conditions was unknown, and was the subject of some controversy. It has been reported, however, that in whole serum, SAP is strictly pentameric [10,11], whereas dAMP, a model ligand for DNA and chromatin binding [12,13], stabilizes a B:B pentamer face interaction to form a stable decamer [13]. In the absence of Ca^{2+}, this interaction is reportedly through the A faces [5], whereas in the presence of Ca^{2+}, but not ligand, an A:B face-to-face interaction has been proposed, leading to stacking and precipitation [13–15].

SAP is so named because of its universal association with the deposits that characterize systemic amyloidosis, Alzheimer's disease and the transmissible spongiform encephalopathies, where it is bound to amyloid fibrils [9]. There is little evidence to suggest that SAP is involved in the formation of amyloid fibrils; however, it does bind in a Ca^{2+}-dependent manner, presumably via specific structural motifs on the fibrils [16]. Since SAP itself is highly resistant to proteolysis, this binding to amyloid fibrils is proposed to protect them from degradation and contribute to persistence of amyloid deposits [16].

Contrasting studies of the role of SAP in fibrillogenesis *in vitro* have shown both inhibition [17] and promotion [18] of Alzheimer's Aβ (β-amyloid peptide) aggregation, and binding to both immobilized and soluble Aβ [19]; however, the nature of these interactions is unclear. Although purified SAP has been shown to bind immobilized CRP in ELISA-based studies, the reverse interaction was not observed, nor was there any stable binding interaction in solution [20]. Earlier rigorous studies, utilizing whole human serum, found that immobilized CRP selectively bound lipoproteins [21], whereas fibronectin and C4-binding protein were selectively bound by immobilized SAP [22].

MS has long been used for peptide sequencing and protein mass measurement. More recently, however, when coupled with nanoelectrospray ionization (nanoESI), the analysis and mass measurement of non-covalent protein complexes, such as the SAP and CRP pentamers, has become feasible [23]. Under appropriate conditions of sample desolvation and instrument pressures, it has

Abbreviations used: Aβ, β-amyloid peptide; APR, acute-phase reactant; CRP, C-reactive protein; EB, electrospray buffer; ESI, electrospray ionization; SAP, serum amyloid P component; SEC, size-exclusion chromatography.

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been possible to preserve native structure and higher-order non-covalent interactions for protein assemblies with masses >1 MDa [24,25].

In the present study, we have used the technique of nanoESI MS to study the effects of solute conditions and presence of ligands upon the higher-order structure of SAP, as well as the interaction in solution of SAP with the related pentraxin, CRP.

MATERIALS AND METHODS

Freeze-dried SAP, purified from human serum, and recombinant human CRP, purified from *Escherichia coli*, were purchased from Calbiochem. A stock solution of 2.4 mg/ml (approx. 20 μM pentamer) was stored in 20 μl aliquots at -80 °C. Buffer salts were removed by applying 20 μl of stock solution to a Micro-BioSpin chromatography column (Bio-Rad Laboratories) previously equilibrated in an electrospray buffer (EB), 200 mM ammonium acetate, pH 8.0. The process was repeated to achieve satisfactory buffer exchange.

MS

NanoESI MS measurements were performed on an LCT mass spectrometer (Micromass). Typically, 2 μl of solution was electrosprayed from gold-coated glass capillaries prepared in-house. In order to preserve non-covalent interactions, the following instrument parameters were used: capillary voltage, 1.5 kV; cone gas, 100 l/h; sample cone, 140 V; extractor cone, 8 V; ion-transfer stage pressure, 8.0×10^{-3} mbar; ToF analyser pressure, 2.0×10^{-6} mbar. The pressure in the ion-transfer stage was decreased from 8.0×10^{-3} to 5.0×10^{-3} mbar to dissociate monomers from the pentamers and to remove excess solvent from the complex. All spectra were calibrated externally using a solution of CsI, and were processed with MassLynx software (Micromass).

SEC (size-exclusion chromatography)

A 10 μM sample (45 μl) of SAP in 10 mM Tris, 100 mM NaCl, pH 8.0, was loaded on to a Superdex 200HR10/30 gel filtration column connected to an Äkta Prime chromatography system (Amersham Biosciences) and was eluted at 0.4 ml $\cdot$ min^{-1} with 200 mM ammonium acetate. The column and buffers were kept at 4 °C throughout and the eluant was monitored at a 280 nm wavelength. To assess the effect of Ca^{2+} upon SAP oligomerization, an identical sample was eluted in 200 mM ammonium acetate buffer containing 1 mM calcium acetate. The column was calibrated using the high-molecular-mass gel-filtration calibration kit (Amersham Biosciences).

Ligand binding and CRP interaction

For the Ca^{2+}-binding experiment, SAP was diluted to 10 μM with an equivalent volume of 1 mM calcium acetate in EB and the complex was analysed directly after 30 min at room temperature (26 °C). Monomeric SAP was dissociated from the pentamer by increasing the sample cone voltage to 180 V. The SAP complex with Ca^{2+} and dAMP was formed by addition of calcium acetate to SAP, followed, after thorough mixing, by dAMP to final concentrations of 5 mM salt/ligand and 10 μM SAP. The mixture was incubated at pH 8.0 for 18 h at room temperature. Excess ligand was removed before analysis with a single Micro-BioSpin buffer-exchange step.

To study the interactions between SAP and CRP, mixtures were incubated for 60 min at a ratio of 5:4, in the presence or absence of 500 μM calcium acetate. Samples were taken directly from the incubation mixture for analysis by MS.

RESULTS

The mass of monomeric human SAP purified from plasma has previously been determined by ESI-MS as $25\,462.7 \pm 0.3$ Da [9]. Our analysis of SAP under denaturing conditions was in agreement with this mass (results not shown), confirming the integrity of the SAP from this commercial source. Subsequent analyses were performed under conditions selected to preserve non-covalent interactions during the MS process. Figure 1 shows the mass spectrum of SAP in the absence of Ca^{2+} at pH 8.0. SAP exhibits two distinct charge state distributions under these conditions. The series centred on *m*/*z* 5100 and *m*/*z* 7500 correspond to the 23+ to 26+ charge states of the pentamer and the 31+ to 36+ charge states of the decamer respectively. SEC, carried out under identical solution conditions, demonstrated that SAP is exclusively in a decameric form. The fact that a proportion of pentamer is observed in the mass spectrum under these conditions is evidence for some destabilization of interactions in the gas phase of the MS.

The associated masses were calculated as $127\,479 \pm 9$ Da (pentamer) and $255\,626 \pm 42$ Da (decamer). The extra mass observed beyond that calculated from multiples of the mass of a monomer of SAP arises from residual solvation of the oligomers by water molecules and buffer salts, which is often observed for non-covalent interaction of macromolecular complexes in the gas phase [26]. At lower pH values (pH 7.0 and pH 5.5) and identical MS conditions, the ratios of pentamer to decamer were successively greater (results not shown), indicating that an alkaline pH enhances the stability of the decamer.

The Ca^{2+}-binding properties of SAP at pH 5.5 are established from X-ray analysis of the crystal structure of the pentamer [5]. Ca^{2+}, however, at physiological pH and in the absence of other ligands, has been proposed to cause rapid precipitation of SAP [15]. After incubation with calcium acetate at pH 8.0, the SAP–Ca^{2+} complex was analysed directly by ESI-MS (Figure 2). The spectrum indicated a pentamer with no appreciable amount of decameric species, in accord with our SEC data. Precipitation of SAP was not observed in the ammonium acetate buffer used in the present study, thus it was possible to observe the stoichiometry of Ca^{2+} binding to SAP at pH 8.0.

Individual monomers of SAP were dissociated from the pentameric complex by reducing the pressure in the ion transfer stage of the instrument. A section of the lower *m*/*z* region of the resultant spectrum (Figure 2, inset) depicts the 10+ and 9+ charge states of monomeric SAP dissociated from the pentamer in the gas phase. For each of these ions, two slightly higher *m*/*z* species were observed, corresponding in mass to the addition of one and two Ca^{2+} ions to the monomer. The fact that binding of three or more Ca^{2+} ions to SAP was not observed implies that binding is specific for two Ca^{2+} ions. Furthermore, the similar signal intensities arising from the binding of one and two Ca^{2+} ions, rather than a major population of doubly bound species, may be due to the decreased affinity observed for the second Ca^{2+}-binding site [5].

The observation of Ca^{2+} binding to SAP by MS at pH 8.0 provided an opportunity to look at the effect of dAMP, a model ligand for the Ca^{2+}-dependent binding of SAP to DNA [12], upon higher-order-complex formation. The mononucleotide dAMP has been shown to prevent Ca^{2+}-induced precipitation of SAP by

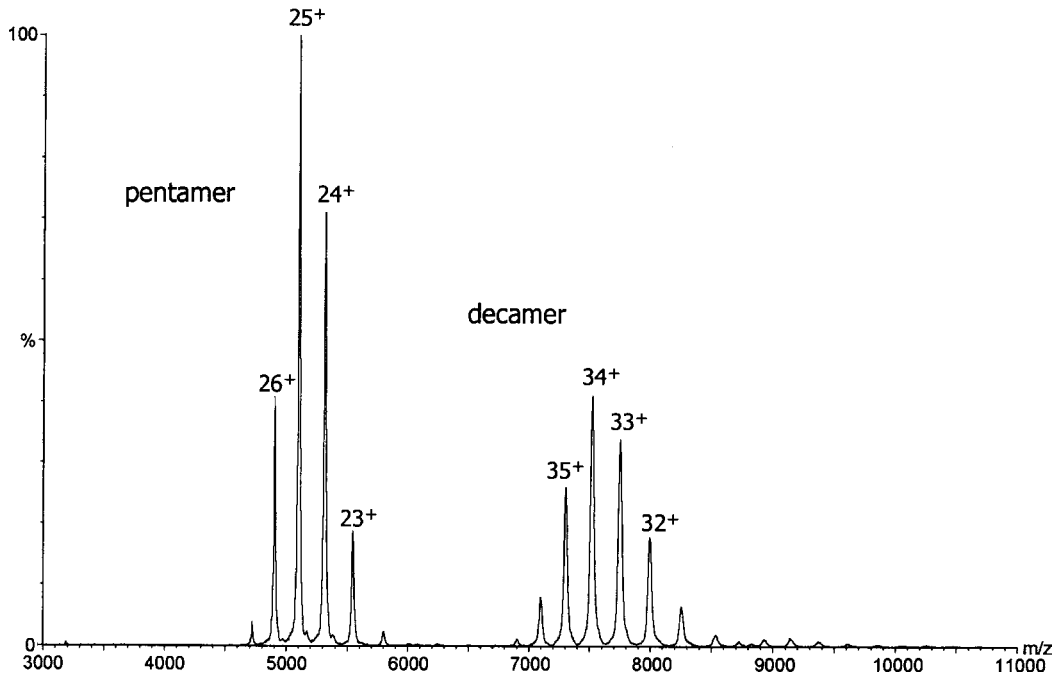

Figure 1 NanoESI mass spectrum of 10 μM SAP in 200 mM ammonium acetate, pH 8.0

Charge-state series centred on *m/z* 5100 can be assigned to the SAP pentamer, whereas the series around *m/z* 7500 arises from decameric SAP.

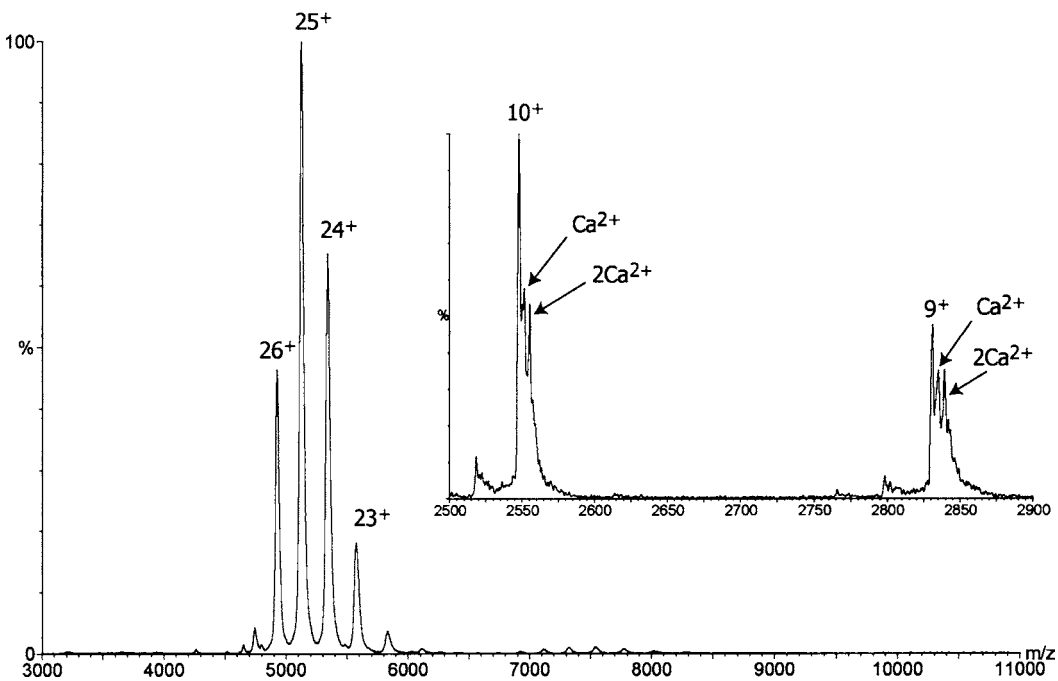

Figure 2 Ca^{2+} binding to the SAP-pentamer complex and individual monomers

A 10 μM solution of SAP in 200 mM ammonium acetate was incubated with 500 μM calcium acetate for 30 min at pH 8.0. The spectrum obtained by direct analysis of the mixture indicated that the amount of decameric SAP was greatly decreased in the presence of Ca^{2+}. Each SAP monomer has two Ca^{2+}-binding sites. Two consecutive ions in the series of charge states assigned to the monomer show the sequential addition of Ca^{2+} to the Ca^{2+}-binding site (inset). In contrast with earlier reports, SAP did not precipitate rapidly in the absence of ligand at pH 8.0.

bridging the two Ca^{2+} ions of each monomer and stabilizing a B:B face decamer via ligand-base stacking [13]. MS analysis of an SAP–Ca^{2+}–dAMP complex (Figure 3) revealed no significant increase in the ratio of decamer to pentamer compared with SAP in the absence of Ca^{2+} (Figure 1). Integration of the areas underneath the charge-state peaks yielded decamer to pentamer ratios of 1.095:1 and 1.097:1 for Figures 1 and 3 respectively. Significantly, two new series of charge states centred on *m/z* 9900 and *m/z* 11 900 were observed in the presence of Ca^{2+} and dAMP, corresponding to masses of 515 kDa and 761 kDa respectively. These masses

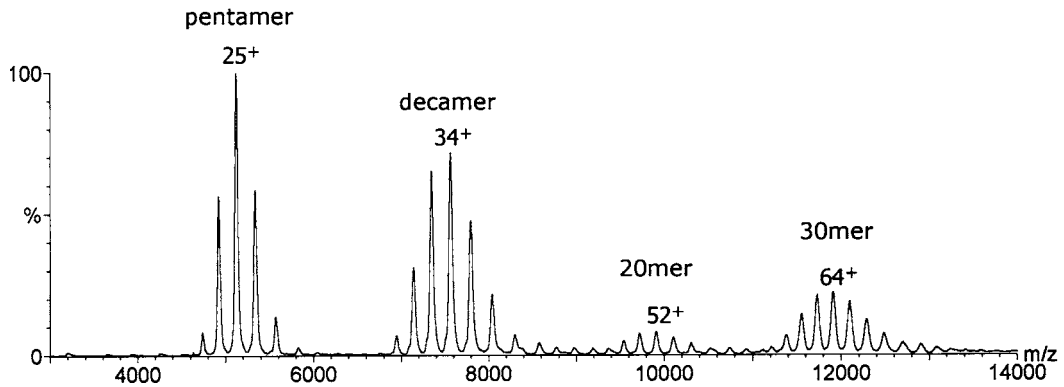

Figure 3 Spectrum of SAP after 18 h of incubation in the presence of 5 mM calcium acetate and 5 mM dAMP

The formation of a significant population of a species with a mass of approx. 761 kDa, corresponding to three SAP decamers (30 monomers), was observed. A small amount of 20mer was present around *m/z* 9900, and the ratio of decamer to pentamer was higher than for SAP in a Ca^{2+}-free environment.

correspond to oligomers of twenty (20mer) and thirty (30mer) subunits. The absence of a 15mer or 25mer, despite the relative abundance of pentamer, suggested that these high-molecular-mass species were a result of the association of decamers, rather than the alternative scenario whereby decamers interact with pentamers. These higher-order assemblies indicate that the presence of dAMP effected an aggregation of SAP, and this phenomenon could account for the observed lack of increase in the ratio of decamer to pentamer, as much of the decamer population is involved in the formation of 30mer.

Oligomerization and interaction of the related pentraxins SAP and CRP

SAP and CRP have high sequence homology, and both exist as pentamers *in vivo*. It was therefore of interest to see if any interaction occurs between these proteins.

The lower two spectra (Figures 4a and 4b), acquired for SAP and CRP before mixing, in the absence of Ca^{2+}, show the oligomers formed by the respective pentraxins. SAP forms a significant proportion of decamer, whereas CRP is almost exclusively pentameric. CRP has a lower monomeric mass than SAP, which is reflected in the proportionally lower *m/z* values observed for the corresponding charge states of the pentamer. Upon mixing SAP and CRP in the absence of Ca^{2+} (Figure 4c), the CRP signal was no longer observed and a new higher *m/z* series was seen in the same region as the SAP decamer. This species has a molecular mass equivalent to the sum of one SAP pentamer and one CRP pentamer, suggesting that, in the absence of Ca^{2+}, CRP binds preferentially to SAP to form a mixed decamer. In the presence of Ca^{2+}, formation of this mixed decamer was greatly retarded, and a mixture of homopentamers was detected (Figure 4d). These results suggest that, although these proteins share significant tertiary and quaternary homology, their interaction in the *in vivo* situation, where Ca^{2+} is present, is likely to be transient or minimal.

DISCUSSION

In the present study, MS has been used to examine some of the solution properties of SAP and CRP and the effect of Ca^{2+} and ligand binding upon higher-order assembly. At pH 8.0, in the absence of Ca^{2+}, SAP has been shown by X-ray and neutron scattering to be decameric [27]. Commercial SAP is purified into a Tris buffer containing EDTA at pH 8.0 [28], thus upon removal of these salts by buffer exchange, a Ca^{2+}-free solution of SAP can be prepared. Under the conditions used in the present study, SAP was found to be decameric by SEC, but dissociated to form a proportion of pentamer when analysed by MS. This result is probably due to weakened interactions as a result of the transition from the solution to gas phase.

The ability of ESI-MS to provide a clear and rapid distinction between the pentameric and decameric forms of SAP represents a valuable tool for screening the effects of ligand binding upon quaternary structure, thus the reported Ca^{2+}-induced precipitation of SAP was also investigated. At pH 8.0, in the presence of Ca^{2+}, no precipitation of SAP was observed; however, the proportion of decamer was greatly diminished, resulting in a predominantly pentameric population. Acetate has been proposed to occupy co-ordination sites involved in Ca^{2+} ligation by SAP [5]. In this case, acetate, rather than acidic residues of SAP, would be expected to be acting as ligands binding to Ca^{2+}, thus preventing any A:B face interactions and subsequent aggregation. This result supports previous studies that propose SAP to be an uncomplexed pentamer in whole serum, where, although the concentration of Ca^{2+} is approx. 1.5 mM [29], a multitude of anions that are present exert a similar effect to that observed here with acetate buffer.

The stability of the Ca^{2+}-bound SAP pentamers in ammonium acetate was exploited to examine the ability of dAMP binding to promote decamer formation. In the presence of Ca^{2+} and dAMP, SAP was present as pentamer, decamer, 20mer and 30mer at approx. proportions of 32:35:4:17, based on integration of the peak areas. This result confirmed that dAMP promotes decamerization, since it was the major species observed, and led to the novel observation of multimers of 20 and 30 subunits. These oligomers may form via A:A face binding of dAMP-ligated decamers, which would explain the absence of 15mer and 25mer in the spectrum (Figure 3). A model of the observed states of SAP oligomerization is presented in Figure 5.

SAP and the closely related pentraxin CRP display approx. 70% sequence homology [20], and co-exist in human plasma, which presents the question, do they interact in solution? In the present study, CRP, which exists as a pentamer in the presence or absence of Ca^{2+}, was shown to form mixed decamers, in which a pentamer of CRP is associated with pentameric SAP, in Ca^{2+}-free buffer. In the presence of Ca^{2+}, however, no interaction between the species was observed, indicating that they do not interact in solution. It might be anticipated, with such similar structure and sequence homology, that exchange of subunits between pentraxins

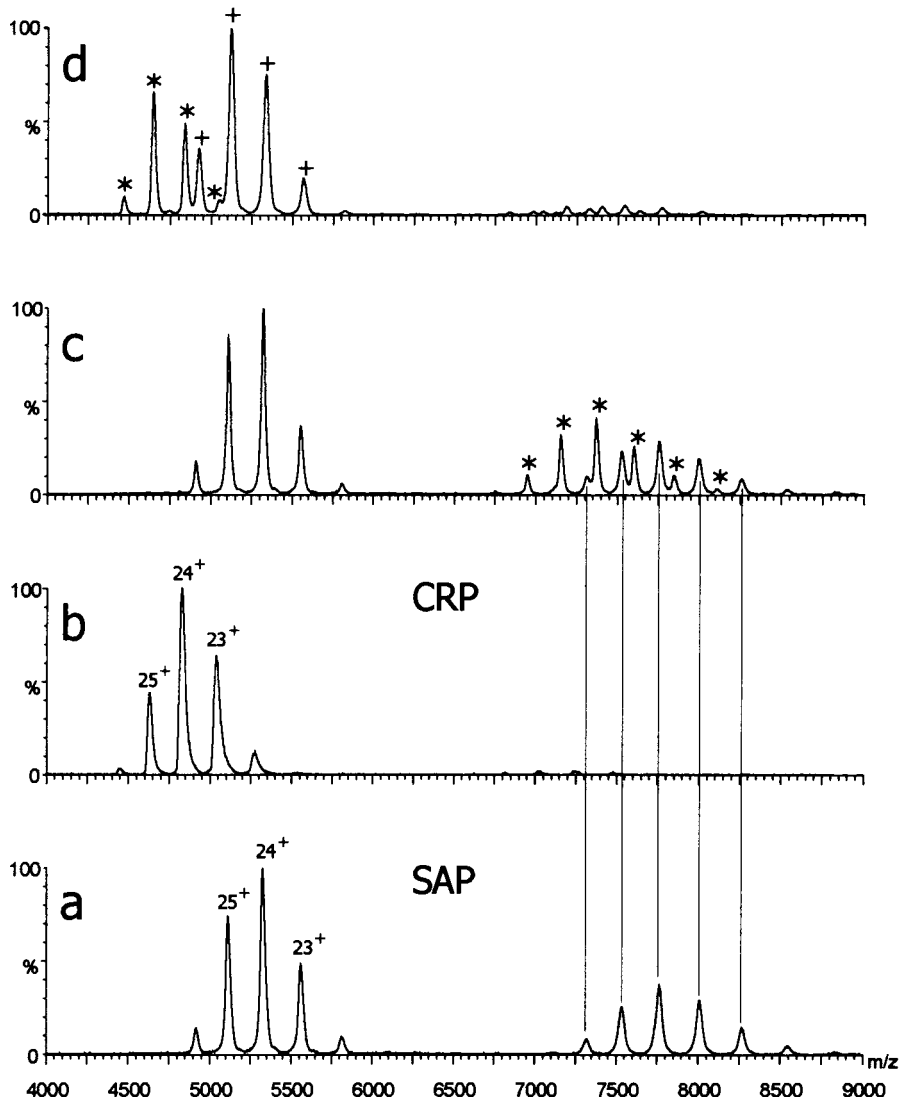

Figure 4 Interactions between SAP and CRP in the presence or absence of Ca^{2+}

(**a**), (**b**) Spectra of SAP and CRP acquired in the absence of Ca^{2+}. SAP forms a significant proportion of decamer, whereas CRP is almost exclusively pentameric. (**c**) SAP and CRP were mixed at a ratio of 5:4 in the absence of Ca^{2+}, and a new decameric species was observed (*). This decamer had a molecular mass equivalent to the sum of one SAP pentamer and one CRP pentamer. The majority of CRP was involved in formation of this decamer as evidenced by the absence of signal for the CRP pentamer. (**d**) In the presence of Ca^{2+}, formation of the mixed decamer was greatly retarded, and a mixture of homopentamers remained. +, SAP; *, CRP.

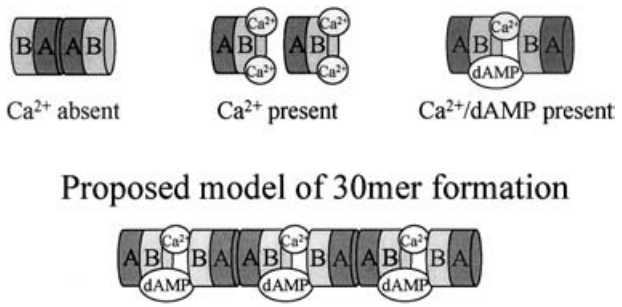

Figure 5 Possible model for higher-order SAP oligomerization

A model for higher-order SAP oligomerization, which combines the Ca^{2+}/dAMP-dependent decamer formation observed previously with the non-Ca^{2+}-dependent A:A face binding of decamers, proposed from the present study.

would occur. Such properties have been observed for small heat-shock proteins originating from the same class [30]. The fact that no subunit (monomer) exchange was observed demonstrates the very specific non-covalent interactions responsible for the 5-fold symmetry of the pentraxins and suggests quite distinct physiological roles.

In summary, higher-order quaternary structures of SAP were readily observed by nanoESI-MS, which permitted an analysis of some different buffer and ligand effects and it is proposed that SAP is stabilized in its pentameric form by carboxylate ions. The ability of MS to readily observe substrate binding and changes in SAP structure due to ligands makes it a potentially useful technique for the screening of pharmacological molecules in the treatment of amyloidosis [31].

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REFERENCES

1 Kushner, I., Ganapathi, M. and Schultz, D. (1989) The acute phase response is mediated by heterogeneous mechanisms. Ann. N.Y. Acad. Sci. **557**, 19–29
2 Pepys, M. B. and Baltz, M. L. (1983) Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. **34**, 141–212
3 Pepys, M. B., Dash, A. C., Markham, R. E., Thomas, H. C., Williams, B. D. and Petrie, A. (1978) Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. Clin. Exp. Immunol. **32**, 119–124
4 Pepys, M. B. (1994) in Immunological Diseases (Frank, M. M., Austen, K. F., Klaman, H. N. and Unanue, E. R., eds.), pp. 637–655, Little Brown, Boston
5 Emsley, J., White, H. E., O'Hara, B. P., Oliva, G., Srinivasan, N., Tickle, I. J., Blundell, T. L., Pepys, M. B. and Wood, S. P. (1994) Structure of pentameric human serum amyloid P component. Nature (London) **367**, 338–345
6 Bickerstaff, M. C., Botto, M., Hutchinson, W. L., Herbert, J., Tennent, G. A., Bybee, A., Mitchell, D. A., Cook, H. T., Butler, P. J., Walport, M. J. and Pepys, M. B. (1999) Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nat. Med. **5**, 694–697
7 Noursadeghi, M., Bickerstaff, M. C., Gallimore, J. R., Herbert, J., Cohen, J. and Pepys, M. B. (2000) Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. Proc. Natl. Acad. Sci. U.S.A. **97**, 14584–14589
8 Rifai, N. and Ridker, P. M. (2001) High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin. Chem. **47**, 403–411
9 Pepys, M. B., Rademacher, T. W., Amatayakul-Chantler, S., Williams, P., Noble, G. E., Hutchinson, W. L., Hawkins, P. N., Nelson, S. R., Gallimore, J. R., Herbert, J. et al. (1994) Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. Proc. Natl. Acad. Sci. U.S.A. **91**, 5602–5606
10 Sorensen, I. J., Andersen, O., Nielsen, E. H. and Svehag, S. E. (1995) Native human serum amyloid P component is a single pentamer. Scand. J. Immunol. **41**, 263–267
11 Hutchinson, W. L., Hohenester, E. and Pepys, M. B. (2000) Human serum amyloid P component is a single uncomplexed pentamer in whole serum. Mol. Med. **6**, 482–493
12 Pepys, M. B. and Butler, P. J. (1987) Serum amyloid P component is the major calcium-dependent specific DNA binding protein of the serum. Biochem. Biophys. Res. Commun. **148**, 308–313
13 Hohenester, E., Hutchinson, W. L., Pepys, M. B. and Wood, S. P. (1997) Crystal structure of a decameric complex of human serum amyloid P component with bound dAMP. J. Mol. Biol. **269**, 570–578
14 Painter, R. H., De Escallon, I., Massey, A., Pinteric, L. and Stern, S. B. (1982) The structure and binding characteristics of serum amyloid protein (9.5 S α1-glycoprotein). Ann. N.Y. Acad. Sci. **389**, 199–215
15 Baltz, M. L., de Beer, F. C., Feinstein, A. and Pepys, M. B. (1982) Calcium-dependent aggregation of human serum amyloid P component. Biochim. Biophys. Acta **701**, 229–236
16 Tennent, G. A., Lovat, L. B. and Pepys, M. B. (1995) Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. Proc. Natl. Acad. Sci. U.S.A. **92**, 4299–4303
17 Janciauskiene, S., Garcia de Frutos, P., Carlemalm, E., Dahlback, B. and Eriksson, S. (1995) Inhibition of Alzheimer β-peptide fibril formation by serum amyloid P component. J. Biol. Chem. **270**, 26041–26044
18 Hamazaki, H. (1995) Amyloid P component promotes aggregation of Alzheimer's β-amyloid peptide. Biochem. Biophys. Res. Commun. **211**, 349–353
19 Hamazaki, H. (1995) Ca^{2+}-dependent binding of human serum amyloid P component to Alzheimer's β-amyloid peptide. J. Biol. Chem. **270**, 10392–10394
20 Swanson, S. J., Christner, R. B. and Mortensen, R. F. (1992) Human serum amyloid P-component (SAP) selectively binds to immobilized or bound forms of C-reactive protein (CRP). Biochim. Biophys. Acta **1160**, 309–316
21 de Beer, F. C., Soutar, A. K., Baltz, M. L., Trayner, I. M., Feinstein, A. and Pepys, M. B. (1982) Low density lipoprotein and very low density lipoprotein are selectively bound by aggregated C-reactive protein. J. Exp. Med. **156**, 230–242
22 de Beer, F. C., Baltz, M. L., Holford, S., Feinstein, A. and Pepys, M. B. (1981) Fibronectin and C4-binding protein are selectively bound by aggregated amyloid P component. J. Exp. Med. **154**, 1134–1139
23 Robinson, C. V. (2002) Protein complexes take flight. Nat. Struct. Biol. **9**, 505–506
24 van Berkel, W. J., van den Heuvel, R. H., Versluis, C. and Heck, A. J. (2000) Detection of intact megaDalton protein assemblies of vanillyl-alcohol oxidase by mass spectrometry. Protein Sci. **9**, 435–439
25 Rostom, A. A., Fucini, P., Benjamin, D. R., Juenemann, R., Nierhaus, K. H., Hartl, F. U., Dobson, C. M. and Robinson, C. V. (2000) Detection and selective dissociation of intact ribosomes in a mass spectrometer. Proc. Natl. Acad. Sci. U.S.A. **97**, 5185–5190
26 Nettleton, E. J., Sunde, M., Lai, Z., Kelly, J. W., Dobson, C. M. and Robinson, C. V. (1998) Protein subunit interactions and structural integrity of amyloidogenic transthyretins: evidence from electrospray mass spectrometry. J. Mol. Biol. **281**, 553–564
27 Ashton, A. W., Boehm, M. K., Gallimore, J. R., Pepys, M. B. and Perkins, S. J. (1997) Pentameric and decameric structures in solution of serum amyloid P component by X-ray and neutron scattering and molecular modelling analyses. J. Mol. Biol. **272**, 408–422
28 Pepys, M. B., Baltz, M. L., de Beer, F. C., Dyck, R. F., Holford, S., Breathnach, S. M., Black, M. M., Tribe, C. R., Evans, D. J. and Feinstein, A. (1982) Biology of serum amyloid P component. Ann. N.Y. Acad. Sci. **389**, 286–298
29 Voet, D. and Voet, J. G. (1995) Biochemistry, Wiley, New York
30 Sobott, F., Benesch, J. L. P., Vierling, E. and Robinson, C. V. (2002) Subunit exchange of multimeric protein complexes: real-time monitoring of subunit exchange between small heat shock proteins by using electrospray-mass spectrometry. J. Biol. Chem. **277**, 38921–38929
31 Pepys, M. B., Herbert, J., Hutchinson, W. L., Tennent, G. A., Lachmann, H. J., Gallimore, J. R., Lovat, L. B., Bartfai, T., Alanine, A., Hertel, C. et al. (2002) Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. Nature (London) **417**, 254–259

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