Effects of Calcium, Magnesium, and Phosphorylcholine on Secondary Structures of Human C-reactive Protein and Serum Amyloid P Component Observed by Infrared Spectroscopy*

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The secondary structures of human C-reactive protein (CRP) and serum amyloid P component (SAP) in D$_2$O-based solutions in the presence or absence of calcium, magnesium, and phosphorylcholine were investigated using Fourier transform infrared spectroscopy. Quantitative analysis provided estimates of about 50% $\beta$-sheet, 12% $\alpha$-helix, 24% $\beta$-turn, and 14% unordered structure for CRP and about 54% $\beta$-sheet, 12% $\alpha$-helix, 25% $\beta$-turn, and 9% unordered structure for SAP. With both proteins significant calcium-dependent changes were observed in conformation-sensitive amide I regions assigned to each type of structure. The CRP spectrum was also affected by magnesium, but the changes differed from those induced by calcium. The SAP spectrum was not affected by magnesium. Phosphorylcholine in the presence of calcium also affected the spectrum of CRP but not the spectrum of SAP. Our present study provides the first direct comparison of the secondary structures of the pentraxins human CRP and SAP and hamster female protein (Dong, A., Caughey, B., Caughey, W. S., Bhat, K. S., and Coe, J. E. (1992) Biochemistry 32, 9364–9370). These findings suggest that the three pentraxins have similar secondary structure compositions and calcium-dependent conformational changes, but differ significantly in their responses to phosphorylcholine and magnesium. Such properties are expected to be relevant to the incompletely understood roles of these highly conserved proteins including binding to nuclear proteins, complement activation, and association with amyloids.

C-reactive protein (CRP),† serum amyloid P component (SAP), and female protein (FP) belong to a family of proteins called pentraxins (1, 2). Pentraxins, named for their cyclic configuration of five noncovalently bound identical subunits (1), are ancient proteins which have evolved at a conservative rate. Limulus CRP isolated from the hemolymph of the horseshoe crab shares about 25% amino acid sequence identity with its mammalian counterparts (3, 4), whereas 71% sequence identity is shared by mouse CRP and human CRP (5, 6). Human CRP and SAP share 50 and 72% sequence homology with hamster FP, respectively (7). The evolutionary conservation of pentraxin genes and ubiquitous presence of pentraxins in vertebrates suggest an essential function for these proteins. Although a compelling reason for the existence of these proteins has not been found, all pentraxins have Ca$^{2+}$-dependent binding activities and many are acute phase reactants (8–15). CRP and FP have a primary binding specificity for phosphorylcholine (PC) which is absent from SAP. Human CRP and SAP and hamster FP all activate complement when bound to ligands (16, 17). CRP has been demonstrated to bind substrates including chromatin (12, 14), histones (14, 15), and small nuclear ribonucleoproteins (13). These binding reactions are specific for the protein moiety and are inhibited by free PC. High affinity chromatin binding activity was also observed in SAP (18). SAP and FP have been identified as peripheral components of a variety of amyloids (19–21), and it appears that the levels of FP expression can have a profound influence on amyloid formation in vivo (22).

There have been few reports on the secondary structure of pentraxins. Knowledge on the secondary structure of pentraxins had been limited to a circular dichroism (CD) study of human CRP and SAP (23) and an infrared (IR) study of hamster FP (24) using IR. X-ray crystallographic analysis shows that the subunit of human SAP is dominated by an antiparallel $\beta$-sheet structure and contains a single short $\alpha$-helix which lies above the disulfide bridge Cys$^{96}$-Cys$^{95}$ and adjacent to the glycosylation site, Asn$^{52}$ (25). The antiparallel $\beta$-sheet structure arrangement of the SAP subunit closely resembles concanavalin A and pea lectin, despite the absence of any sequence homology between these proteins and the pentraxin (25).

In the present study, we examined the secondary structure and the Ca$^{2+}$- and PC-dependent conformational changes of human CRP and SAP in solution using IR. To avoid the aggregation found at the high concentrations ($\geq$15 mg/ml) required for obtaining a high quality infrared spectrum of protein in H$_2$O-based solution, human CRP and SAP were studied in D$_2$O-based solutions in this study. Fourier transform IR spectroscopy, aided by powerful spectral handling techniques, namely Fourier self-deconvolution (enhancement) and second-deriva-

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‡ The abbreviations used are: CRP, C-reactive protein; SAP, serum amyloid P component; FP, female protein; PC, phosphorylcholine; IR, infrared.

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*‡ Moore and Fasman (67) have proposed recently used the term "poly-proline II" to describe the short, flexible segments often referred to as "random coil."
tive analysis, has become a widely used method for studying the secondary structure of polypeptides and proteins (26-30). The amide I band (from 1700 to 1820 cm⁻¹), which is due almost entirely to the C=O stretching vibration of the peptide linkages that constitute the backbone structure, is known to be sensitive to small variations in molecular geometry and hydrogen bonding patterns of proteins and has been extensively used in the studies of secondary structure and conformational changes of proteins (27-31). Empirical studies have demonstrated that the infrared amide I band areas associated with a particular secondary structure (e.g., α-helix, β-sheet, turn, and unordered structures), divided by the sum of all band areas assigned to secondary structures, provide a quantitative estimation of the relative amounts of the secondary structure components which are close to those derived from x-ray diffraction data (27, 28, 32-34). The water spectral handling technique developed in our laboratory (35) enables us to obtain infrared spectra of proteins in H₂O as well as D₂O-based solutions with high quality and reproducibility. Results of our present studies indicate that human CRP and SAP are predominantly composed of antiparallel β-sheet structure, with small amounts of α-helix and unordered structures. Comparative infrared spectra analysis of human CRP, SAP, and hamster FP provide direct evidence that pentraxins have similar secondary structure folding patterns. Ca²⁺-dependent conformational changes were detected in amide I regions assigned to β-sheet, α-helix, β-turn, and unordered structure, especially the β-sheet region. The protein conformational changes induced by binding of Mg²⁺ and PC were also detected in CRP.

MATERIALS AND METHODS

Purification and Preparation of CRP and SAP—Human CRP was isolated and purified from human pleural fluid and plasmapheresis samples by affinity chromatography, gel filtration, and ion exchange chromatography as described previously (15). Human SAP was isolated and purified from human pleural fluid and plasmapheresis samples as described elsewhere (36).

The Ca²⁺-depleted CRP and SAP in D₂O (99.9%, Cambridge Isotope Laboratories, Cambridge, MA) buffer were obtained by addition of 2 ml of D₂O-based buffer with EGTA (20 mm Tris, 100 mm NaCl, 2 mm EGTA, pH 8) to 0.5 ml of CRP (2.9 mg/ml) or 0.5 ml of SAP (2.2 mg/ml) and then by concentration of the samples to about 5 mg/ml by centrifugation using a Centricon 10 microcentrator (Amicon) at 4000 g. The concentrate was diluted with D₂O-based buffer and the mixture was centrifuged at 4000 g for 24 h. To remove excess EGTA, the D₂O-based buffer without EGTA was used in the last cycle of hydrogen-deuterium exchange. The final concentrations of CRP and SAP were about 5 mg/ml. The CRP and SAP samples containing 2 mm CaCl₂, 2 mm MgCl₂, or 1 mm PC were obtained by adding concentrated stock solutions of CaCl₂, MgCl₂, or PC to the Ca²⁺-depleted protein solutions.

Infrared Measurements and Amide I Spectra Analysis—Protein solutions were prepared for infrared measurement in a CaF₂ cell (Beckman FH-01) with a 50-µm spacer. Infrared spectra were recorded at 20 °C with a Perkin-Elmer model 1800 Fourier transform infrared spectrometer equipped with a HgCdTe detector and interfaced with a Perkin-Elmer 7700 computer. For each spectrum a 1000-scan interferometer equipped with a Hg/CdTe detector and interfaced with a Perkin-Elmer 7700 data station. For comparison, the figure also includes the spectrum of hamster FP in H₂O-based solution as obtained previously (35). The similarity between the amide I and II bands of CRP and SAP suggests that the secondary structure compositions of the two proteins are very much alike. After 24 h of hydrogen-deuterium exchange, the spectra of both CRP and SAP exhibit absorbance maxima for their amide I and amide II bands at 1633 and 1455 cm⁻¹, respectively. The amide I band absorbance maximum for FP in H₂O-based solution is at nearly the same wave number as are the amide I maxima for CRP and SAP in D₂O-based solutions. Hydrogen-deuterium exchange is found to have only small effects on amide I band frequencies (27). In contrast, amide II bands, which arise mainly from an out-of-phase combination of N-H in-plane bending and C-N stretch vibrations of peptide linkages (31), are typically red-shifted by about 100 cm⁻¹ due to exchange of hydrogen by deuterium. Therefore, the 95 cm⁻¹ lower wave number of the amide II bands for CRP and SAP in D₂O-based solution compared to the amide II band of FP in H₂O-based solution is expected.

It has been well documented that the absorbance maximum frequency of the infrared amide I band is determined by the predominant secondary structure in the proteins (27, 29-31, 38). For proteins in H₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29). For proteins in D₂O-based solutions, the amide I band maxima of proteins with predominant α-helix structure are usually found at 1656 ± 2 cm⁻¹ and those with predominant β-sheet structure are usually found between 1643 and 1631 cm⁻¹ (24, 29).
Effects of Ca\(^{2+}\), Mg\(^{2+}\), and PC on Pentraxins

The original, Fourier self-deconvoluted, and inverted second-derivative amide I infrared spectra of human CRP with or without Ca\(^{2+}\) and PC. CRP (---); CRP with 2 mM Ca\(^{2+}\) (-----); CRP with 2 mM Ca\(^{2+}\) plus 1 mM PC (-----). Top, the original spectra; middle, Fourier self-deconvoluted spectra; bottom, inverted second-derivative spectra. The reproducibility of all spectra was confirmed by at least two independent measurements.

Fig. 2. The original, Fourier self-deconvoluted, and inverted second-derivative amide I infrared spectra of human CRP with or without Ca\(^{2+}\) and PC. CRP with 2 mM Ca\(^{2+}\) plus 1 mM PC. The second-derivative spectra are deconvoluted, and second-derivative amide I' spectra of CRP are found between 1688, 1678, and 1655 cm\(^{-1}\), which are not amide I components, were included in the curve-fitting to avoid possible distortion. These results support the conclusion that human CRP in solution is also composed predominantly of \(\beta\)-sheet structure. Turn, \(\alpha\)-helix, and unordered structures were observed in lesser amounts (Table I). A similar, but somewhat less reproducible, secondary structure composition was also obtained using the Fourier self-deconvolution/curve-fitting method of Susi and Byler (27) (data not shown).

Fig. 3. The curve-fitting of inverted second-derivative spectra of human CRP with or without Ca\(^{2+}\) and PC. The curve-fitting procedure was carried out as described under "Materials and Methods," and the deconvoluted band frequencies are listed in Table I.

Table I: Assignments for deconvoluted infrared amide I band components of human C-reactive protein and serum amyloid P component in D\(_2\)O-based solutions

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1631 (2(^{+}), 2)</td>
<td>(\beta)-Strand</td>
</tr>
<tr>
<td>1648 Unordered</td>
<td>(\beta)-Strand</td>
</tr>
<tr>
<td>1655 (4(^{+}), 4)</td>
<td>(\alpha)-Helix</td>
</tr>
<tr>
<td>1669 (4(^{+}), 2)</td>
<td>Type III turn or (3_1) helix</td>
</tr>
<tr>
<td>1669 (7(^{+}), 71)</td>
<td>(\beta)-Turn</td>
</tr>
<tr>
<td>1678 (7(^{+}), 7)</td>
<td>(\beta)-Turn</td>
</tr>
<tr>
<td>1688 (\beta)-Turn</td>
<td>(\beta)-Turn</td>
</tr>
<tr>
<td>1695 (\beta)-Strand</td>
<td>(\beta)-Strand</td>
</tr>
</tbody>
</table>

The values in parentheses are from proteins with Ca\(^{2+}\) (marked with asterisk) and Ca\(^{2+}\) plus PC.

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\(^3\) A. Dong, P. Huang, B. Caughey, and W. S. Caughey, unpublished data.
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The values represent the percentage of the total integrated intensity of the second-derivative amide I infrared spectrum which corresponds to bands assigned to the designated secondary structure.

<table>
<thead>
<tr>
<th>Pentraxin</th>
<th>Secondary structure</th>
<th>a-Helix</th>
<th>(\beta)-Sheet</th>
<th>Turn</th>
<th>Unordered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CRP</td>
<td></td>
<td>13.0</td>
<td>50.0</td>
<td>23.3</td>
<td>13.7</td>
</tr>
<tr>
<td>CRP/Ca(^{2+})</td>
<td></td>
<td>9.2</td>
<td>45.4</td>
<td>27.6</td>
<td>17.8</td>
</tr>
<tr>
<td>CRP/Ca(^{2+})/PC</td>
<td></td>
<td>10.3</td>
<td>45.3</td>
<td>27.3</td>
<td>17.1</td>
</tr>
<tr>
<td>Human SAP</td>
<td></td>
<td>10.1</td>
<td>55.9</td>
<td>24.9</td>
<td>9.1</td>
</tr>
<tr>
<td>SAP/Ca(^{2+})</td>
<td></td>
<td>12.8</td>
<td>52.3</td>
<td>26.0</td>
<td>8.9</td>
</tr>
<tr>
<td>SAP/Ca(^{2+})/PC</td>
<td></td>
<td>12.8</td>
<td>52.9</td>
<td>26.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Hamster FP(^{a})</td>
<td></td>
<td>9.2</td>
<td>50.8</td>
<td>28.4</td>
<td>11.6</td>
</tr>
<tr>
<td>FP/Ca(^{2+})</td>
<td></td>
<td>10.8</td>
<td>50.0</td>
<td>29.8</td>
<td>9.4</td>
</tr>
<tr>
<td>FP/Ca(^{2+})/PC</td>
<td></td>
<td>10.8</td>
<td>53.9</td>
<td>28.9</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* Data from Dong et al. (24).

SAP was similar to those estimated for human CRP and hamster FP (Table II) and is consistent with a predominantly antiparallel \(\beta\)-sheet structure in solution as well as in crystal (25). A similar secondary structure composition was also estimated using the Fourier self-deconvolution-curve-fitting method of Susi and Byler (27) (data not shown).

Calcium-dependent Amide I Spectral Changes—Calcium plays an important role in the biological function of pentraxins (8, 9, 11, 14, 15). By comparison of amide I spectra of CRP and SAP with or without Ca\(^{2+}\), significant spectral differences were consistently seen in regions assigned to \(\beta\)-sheet, \(\alpha\)-helix, \(\beta\)-turn, and unordered structures (Figs. 2 and 4). The Ca\(^{2+}\)-dependent spectral changes were seen clearly even without resolution enhancement. The most remarkable changes were detected at regions assigned to \(\beta\)-sheet and unordered structures. Upon Ca\(^{2+}\) binding, the strong bands ascribed to \(\beta\)-sheet are blue-shifted about 1 cm\(^{-1}\) accompanied by narrowing, while the bands arising from \(\alpha\)-helix are red-shifted about 1 cm\(^{-1}\) in both CRP and SAP (Table I). Furthermore, a new band at 1660 cm\(^{-1}\) appeared in the spectra of CRP, whereas a significant intensity increase at 1660 cm\(^{-1}\) band was observed in SAP. These results provided strong evidence for conformational changes due to Ca\(^{2+}\) binding in both proteins.

**Phosphorylcholine-dependent Amide I Spectral Changes**—Relatively small but fully reproducible spectral changes in the amide I region were observed in CRP following the addition of PC in the presence of Ca\(^{2+}\). The PC-dependent amide I spectral changes are concentrated between 1673 and 1640 cm\(^{-1}\), the regions assigned to turn, \(\alpha\)-helix, and unordered structures. The curve-fitting analysis revealed that the changes resulting from PC binding are mainly the frequency shift and relative intensity changes of two bands ascribed to \(\beta\)-turn structure near 1663 and 1671 cm\(^{-1}\) (Table I). Such frequency and intensity changes in \(\beta\)-turn-related bands usually suggest relative movements in main chain structures (29); in the case of CRP, the antiparallel \(\beta\)-sheet structure. A PC-dependent change is not apparent in the spectra of SAP.

**Magnesium-induced Amide I Spectral Changes**—Fig. 6 presents an overlay of the second-derivative amide I spectra of CRP and SAP with or without Mg\(^{2+}\). Significant spectral changes involving regions assigned to the \(\beta\)-sheet and \(\alpha\)-helix.
Effects of $Ca^{2+}$, $Mg^{2+}$, and $PC$ on Pentraxins

structures were observed in CRP, whereas a complete superimposition between the spectra obtained with or without $Mg^{2+}$ was observed in SAP. The spectral changes induced by $Mg^{2+}$ in CRP differ markedly from that induced by $Ca^{2+}$, especially at the $\beta$-structure region. While binding of $Ca^{2+}$ shifted the strong $\beta$-structure-related band at 1631 cm$^{-1}$ toward the blue, binding of $Mg^{2+}$ shifted the band slightly to the red. This finding suggests that significant conformational changes can be induced by binding either $Ca^{2+}$ or $Mg^{2+}$, the role played by $Ca^{2+}$ may not be duplicated by $Mg^{2+}$.

**DISCUSSION**

Comparison of Secondary Structures of Human CRP, Human SAP, and Hamster FP—It has been considered likely that the pentraxins CRP, SAP, and FP have similar protein structures on the basis of their high sequence homology (5-7) and similar pentameric structures (1, 2). A detailed comparative study of the secondary structures of pentraxins has not been conducted. However, using far-UV CD spectroscopy, Young and Williams (23) estimated similar $\alpha$-sheet and $\alpha$-helix structures for human CRP and SAP. However, using second-derivative Fourier transform-IR spectroscopy Dong et al. (24) estimated that hamster FP contains much lower amounts of $\alpha$-helix than suggested by Young and Williams (23) for CRP and SAP (11% for FP compared with 34% for CRP and SAP). The present study shows that human CRP and SAP and hamster FP have similar amide I spectral patterns (Fig. 7). The spectrum of each protein exhibits a very strong low wave number $\beta$-structure component near 1634 cm$^{-1}$ and a weak high wave number $\beta$-structure component near 1695 cm$^{-1}$ as well as a weak band arising from $\alpha$-helix and a weak band arising from unordered structure. Quantitative analysis (Table II) revealed that human CRP and SAP and hamster FP are all predominantly composed of antiparallel $\beta$-sheet structure (50-55%) with small amounts of $\alpha$-helix (9-13%) and unordered (9-18%) structures. These results support the idea that pentraxins have similar secondary structure folding patterns. Nevertheless, there are notable differences among the three pentraxins. Human SAP and hamster FP both exhibit a $\beta$-structure-related band near 1625 cm$^{-1}$, which has been attributed to the "exposed" or strongly hydro-

gen-bonded $\beta$-sheet structure (46, 47). No similar band near 1625 cm$^{-1}$ was found in the spectrum of human CRP. Under identical conditions, the major $\beta$-structure component of SAP exhibits a slightly higher frequency ($\sim$1 cm$^{-1}$) than that of CRP (Table I). Furthermore, human CRP contains a considerably higher percentage of unordered structure than either human SAP or hamster FP (Table II).

We should mention here that the 34% $\alpha$-helix structure estimated for human CRP and SAP in solution by Young and Williams (23) using far-UV CD spectroscopy is apparently an overestimation on the basis of the recent x-ray crystallographic analysis of human SAP (25). This overestimation of $\alpha$-helical content by far-UV CD may have resulted from the aromatic side chain clusters formed in these proteins (24, 48, 49). The amino acid composition of human CRP derived from the complementary DNA sequence shows that it contains 8 Tyr, 13 Phe, and 6 Trp (5, 50). The location of many of these residues sequentially close to each other suggests they may form aromatic side chain clusters.

Several methods have been developed in the past few years on the basis of empirical studies to estimate the relative contributions of different types of secondary structures in proteins from their infrared amide I spectra (32, 33, 51-53). These methods can be classified into two categories. First are the deconvolutional methods including Fourier self-deconvolution/curve-fitting (27, 32) and second-derivative analysis (33). Second are the semi-deconvolutional methods including the partial least-squares analysis (51), factor analysis (52), and data base analysis (53). The advantages and disadvantages of these methods have been discussed in recent review articles (54, 55). However, due to the empirical nature and uncertainty in data processing and interpretation associated with each of these methods, the evaluation should ultimately be based on how successful these methods are in estimating the secondary structure of a protein prior to any high resolution information becoming available. The excellent agreement between the secondary structure of pentraxin hamster FP estimated on the basis of IR-second-derivative analysis (24) and recent x-ray crystallographic analysis of human SAP (25) provides further support for the use of the refined IR-second-derivative analysis (24, 35) as a reliable technique for estimating protein secondary structure despite its inherent shortcomings, namely the potential interference of weak positive side lobes associated with the second derivative. IR-second-derivative analysis is particularly useful for the detection of subtle changes in structure such as the effects of $Ca^{2+}$, $Mg^{2+}$, and $PC$ observed in this study. Similar secondary structure compositions for CRP and SAP were also obtained using the Fourier self-deconvolution/curve-fitting method of Susi and Byler (27) (data not shown).

Effects of Hydrogen-Deuterium Exchange on Amide I Band Frequencies—Unlike many other proteins studied in $D_2O$-based solutions (27, 34, 56), the frequencies for the majority of deconvoluted components of CRP and SAP appear not to be significantly affected by hydrogen-deuterium exchange except for the bands ascribed to the $\alpha$-helix and unordered structures (Fig. 5) when compared with the spectrum of hamster FP in $H_2O$-based solution (24). The band frequencies assigned to different types of secondary structures in human CRP and SAP deviate somewhat from values reported for deuterated proteins (27, 32). Especially noteworthy is our assignment of the high wave number $\beta$-structure component to the band near 1695 cm$^{-1}$ instead of the band at 1675 ± 4 cm$^{-1}$ (27, 32). This assignment is supported by the infrared study of concanavalin A (46), a protein with similar secondary structure to SAP (25). Arron and co-workers (46) have shown that the deconvoluted amide I spectrum of concanavalin A exhibits a band ascribed to the high wave number $\beta$-structure component at 1694 cm$^{-1}$ in

![Diagram](image_url)
both H$_2$O and D$_2$O solutions. They attributed the lack of isotopic effects on frequencies of $\beta$-structure-related bands to low hydrogen-deuterium exchange rate of $\beta$-sheet structure. Assuming that the frequencies of the deconvoluted band component for the $\alpha$-helix structure of human CRP and SAP in H$_2$O-based solutions are similar to the corresponding band frequency in hamster FP, which is most likely true judged on the basis of the large percentage of amino acid sequence homology (7) and the similarity in secondary structure compositions (Table II), the $\alpha$-helix structure of SAP seems to be more affected by hydrogen-deuterium exchange than that of CRP (Fig. 7). In comparison with hamster FP, a red shift of 0.02 cm$^{-1}$ (from 1657 to 1652 cm$^{-1}$) in band frequency assigned to $\alpha$-helix was observed in human SAP, whereas only about 2 cm$^{-1}$ red shift (from 1657 to 1655 cm$^{-1}$) was observed in human CRP after 24-h hydrogen-deuterium exchange. This difference may be because the $\alpha$-helical structure of SAP is more exposed to solvent than the $\alpha$-helical structure of CRP.

**Table III**

<table>
<thead>
<tr>
<th>Pentraxin</th>
<th>Calcium</th>
<th>Magnesium</th>
<th>Phosphorylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CRP</td>
<td>+++</td>
<td>++</td>
<td>$\pm$</td>
</tr>
<tr>
<td>Human SAP</td>
<td>+++</td>
<td>$-$</td>
<td>+++</td>
</tr>
<tr>
<td>Hamster FP</td>
<td>+++</td>
<td>$-$</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Data from Dong et al. (24).

Both CRP and SAP bind Mg$^{2+}$, especially in the presence of Ca$^{2+}$, prevents their study due to the apparent structural and functional importance of Ca$^{2+}$ binding in pentraxins, many attempts have been made to localize the possible binding site(s) by searching for the amino acid sequence resemblance between pentraxins and the recognized consensus pattern in EF-handed (related to the E and F helices of parvalbumin) Ca$^{2+}$-binding domains of parvalbumin and calmodulin (3, 4, 24, 60, 61). The recent x-ray crystallographic analysis (25) revealed that the Ca$^{2+}$-binding site of human SAP consists of two loops from distant peptide segments and coordinates two calcium ions in a manner similar to the Ca$^{2+}$-binding sites of concanavalin A (62, 63) and pea lectin (64). Thus the Ca$^{2+}$-binding site of human SAP (and possibly pentraxins in general) differs markedly from the recognized consensus pattern of amino acid residues in the EF-handed Ca$^{2+}$-binding proteins (65, 66).

REFERENCES

Effects of Ca$^{2+}$, Mg$^{2+}$, and PC on Pentraxins