Pentraxin binding to isolated rat liver nuclei

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The interaction of human C-reactive protein (CRP) and serum amyloid P-component (SAP) with isolated rat liver nuclei was studied to identify nuclear ligands for each pentraxin using the iodinated heterobifunctional thiol-cleavable cross-linking reagent sulphosuccinimidyl-2-(*p*-azidosalicylamido)-1,3'-dithiopropionate (SASD). Nuclei (100 µg of DNA) bound 21 pmol of ¹²⁵I-labelled CRP Ca²⁺-dependently at saturation with half-saturation occurring at 200 pmol of ¹²⁵I-CRP. By contrast, only 2.7 pmol of ¹²⁵I-labelled SAP was bound at saturation, with half-saturation at 50 pmol. The binding of pentraxins to nuclei is, in addition to putative chromatin binding, due to nuclear-envelope binding, where 3.2 pmol ¹²⁵I-labelled CRP binds Ca²⁺ dependently to nuclear envelopes (25 µg) at saturation, but only 0.62 pmol SAP is required to saturate. Specific photocross-linking of ¹²⁵I-2-(*p*-azidosalicylamido)-1,3'-dithiopropionate (¹²⁵I-ASD)-CRP and ¹²⁵I-ASD-SAP to nuclei revealed transfer of ¹²⁵I-photoreactive azides to nuclear-envelope proteins of 43, 46, 52 and 70 kDa. In addition, SAP binding to histones H2A, H2B, H3 and H4 was detected, whereas CRP bound only to H4. Neither pentraxin cross-linked to histone H1.

INTRODUCTION

The pentraxins are a unique family of pentameric plasma proteins comprising C-reactive protein (CRP), the classical acute-phase protein and serum amyloid P component (SAP), a normal plasma glycoprotein in man [1,2]. CRP and SAP have been highly conserved throughout vertebrate evolution and both share high sequence homology [3]. Notable are the residues 123–132 in both pentraxins, which show high sequence homology with histones H1/H5 and H4 [4].

It has been reported that SAP, and not CRP, is the protein in normal or acute-phase human serum that binds Ca²⁺-dependently to chromatin, nucleosome core particles and even DNA [5]. The binding of SAP to long chromatin is likely to be of importance, since it occurs under physiological conditions, displaces H1-type histones and solubilizes long chromatin [6]. In contrast with SAP, CRP only seems to bind, under physiological conditions, to nuclear constituents when these are immobilized on membranes or synthetic surfaces [7,8]. Immobilized ligands that CRP can bind to include histones and the U1 small nuclear ribonucleoprotein [8,9].

The potential pathophysiological relevance of these findings is extended by immunofluorescence studies showing SAP binding Ca²⁺-dependently to the nuclei of Hep-2 cells and to globular dermal deposits of nuclear material in skin biopsies from two patients with systemic lupus erythematosus [10]. CRP was shown to associate with nuclear structures within synovial cells and histiocytes in synovial biopsies from patients with rheumatoid arthritis [11].

In the present study we investigated the interaction of human SAP and CRP with rat liver nuclear proteins to identify nuclear ligands for each pentraxin using the iodinated heterobifunctional thiol-cleavable cross-linking reagent sulphosuccinimidyl-2-(*p*-azidosalicylamido)-1,3'-dithiopropionate (SASD) [12]. Rat liver nuclei were chosen as they have been well

characterized by us with respect to nuclear proteins [13]. We report that the capacity of nuclei or nuclear envelope to bind CRP specifically in the presence of Ca²⁺ is 7-fold greater than the capacity to bind SAP. Nuclear-envelope proteins to which the pentraxins cross-link are identified. SAP cross-links to more histones than CRP, and ¹²⁵I-photoreactive azide is transferred to all histones except H1.

MATERIALS AND METHODS

All reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless otherwise stated.

Isolation of rat liver nuclei and nuclear envelopes

Rat liver nuclei were isolated by the method of Blobel & van Potter [14] as previously described, except that washing of the nuclei with Triton X-100 was omitted, as this destroys the integrity of the nuclear membrane. Nuclei were suspended at 5 mg of DNA/ml in 0.25 M-sucrose/50 mM-Tris(pH 7.4)/5 mM-MgCl₂ and stored on ice for no longer than 1 h before use. Immediately before binding experiments nuclei were resuspended at 5 mg of DNA/ml in 10 mM-Tris (pH 7.4)/140 mM-NaCl.

Nuclear envelopes from rat liver nuclei were prepared by the method of Bornens & Courvallin [15], as previously described, and suspended at 2 mg of protein/ml [16] in 10 mM-Tris (pH 7.4)/140 mM-NaCl just before binding experiments.

Preparation and iodination of CRP and SAP

Human CRP and human SAP were prepared as previously described [17,18]. The source of CRP was acute-phase serum obtained from patients 48 h after surgery (informed consent and ethical committee approval were given), whereas the source of SAP was normal serum obtained from laboratory workers. SDS/12 % -PAGE under reducing conditions showed each protein to be a single band [19]. The protein content of CRP and

Abbreviations used: CRP, C-reactive protein; SAP, serum amyloid P-component; (S)ASD, (sulphosuccinimidyl-)-2-(*p*-azidosalicylamido)-1,3'-dithiopropionate.

SAP was, by electroimmunoassay, standardized to human CRP or SAP respectively [20].

Iodogen (Pierce Chemical Co.) was used as oxidizing agent to iodinate both CRP and SAP with Na¹²⁵I (Amersham International, Amersham, Bucks., U.K.) as previously described [17]. The specific radioactivity of CRP ranged from 2.5 to 3 μ Ci/pmol and for SAP from 4.3 to 5 μ Ci/pmol. Analysis of both proteins by SDS/PAGE, followed by autoradiography, showed no contaminating proteins.

Binding of ¹²⁵I-labelled CRP and ¹²⁵I-labelled SAP to nuclei and nuclear envelopes

For binding of ¹²⁵I-labelled CRP and ¹²⁵I-labelled SAP to nuclei or nuclear envelopes, experiments were done in triplicate and contained nuclei (100 μ g of DNA) or nuclear envelopes (25 μ g of protein) and 0–400 pmol of labelled ligand in a final volume of 200 μ l of 10 mM-Tris-(pH 7.4)/140 mM-NaCl/5 mM-MgCl₂/5% (w/v) BSA (incubation buffer) containing either 1 mM-CaCl₂ or 5 mM-EGTA. Incubations were maintained at 25 °C for 30 min, then terminated by microcentrifuging the 200 μ l of reaction mixture (10000 g; 30 s; Eppendorf model 3200 centrifuge) through a 55 μ l of silicone-oil layer (G. E. Versilube F-50, S.G. 1.05) into 0.6 M-sucrose (S.G. 1.10, 150 μ l) in a polypropylene microcentrifuge tube [21]. The Microfuge tubes were then cut below the silicone-oil layer and the bottom tip placed in tubes and counted for radioactivity in a LKB Multi Gamma Counter, model 1260 (LKB Instruments, Turku, Finland). The trapped extracellular fluid was determined by doing a 'zero-time-binding' experiment by incubating nuclei or nuclear envelopes, as described above, with radiolabelled ligand at 0 °C and centrifuging within 10 s. For each binding reaction the average of the triplicates, after subtraction of the zero-time-associated radioactivity, was used as the result. To determine the portion of total ligand that is Ca²⁺-dependently bound to nuclei and nuclear envelopes, these pelleted fractions were incubated at 25 °C for 30 min in 200 μ l of incubation buffer containing 5 mM-EGTA. The chelation reaction was terminated by spinning the mixture through an oil layer as described above. EGTA-released ligand was determined by counting for radioactivity a 150 μ l aliquot of the medium above the oil layer while the EGTA resistant ligand remaining with the pellet was determined by counting for radioactivity the bottom tip of the tube.

Iodination of SASD, derivatization of CRP and SAP with ¹²⁵I-SASD and cross-linking to nuclei and nuclear-envelope proteins

All steps before the photolytic reactions were done in a dark room under red lighting.

(a) **Iodination of SASD.** The method was essentially as previously described [12]. Briefly, SASD (Pierce Chemical Co.) was dissolved at 1 mg/50 μ l of dimethyl sulphoxide then diluted to 1 mg/ml with 10 mM-sodium tetraborate, pH 8.4. Iodination was carried out immediately after solubilization using Iodogen as oxidizing agent and Na¹²⁵I at 10 μ Ci/ μ g of SASD. Reactions were terminated after 30 s by removal of the reaction mixture from the reaction tube.

(b) **Derivatization of CRP and SAP with ¹²⁵I-SASD.** ¹²⁵I-SASD was coupled to CRP or SAP directly after iodination at a molar ratio of 300 mol of ¹²⁵I-SASD/1 mol of CRP (or 1 mol of SAP) for 30 min at room temperature in a final volume of 300 μ l of 100 mM-sodium tetraborate, pH 8.4, containing 140 mM-NaCl. Non-coupled ¹²⁵I-SASD and free iodine was removed from ¹²⁵I-ASD-CRP or ¹²⁵I-ASD-SAP by dialysis into 10 mM-Tris (pH 7.4)/140 mM-NaCl (5 min) using Millipore membrane filters with pore size 0.025 μ m (Millipore, Bedford, MA, U.S.A.).

(c) **Cross-linking of ¹²⁵I-ASD-CRP and ¹²⁵I-ASD-SAP to nuclei and nuclear envelopes.** ¹²⁵I-ASD-CRP (100 pmol of CRP) and ¹²⁵I-ASD-SAP (100 pmol of SAP) was incubated directly after dialysis with either nuclei (100 μ g of DNA) or nuclear envelopes (25 μ g of protein) for 30 min at 25 °C in a final volume of 200 μ l incubation buffer containing 1 mM-CaCl₂. Reactions were terminated by microcentrifuging (10000 g) nuclei for 30 s and nuclear envelopes for 60 s. The pelleted material was washed twice with incubation buffer containing 1 mM-CaCl₂, then resuspended in 200 μ l of this buffer. Samples were photolysed from above for 10 s on ice with a xenon lamp, fixed at 500 W, 9 cm from the light source. Nuclei were resuspended in 2.3 M-sucrose in 5 mM-Tris(pH 7.4)/5 mM-MgCl₂ and nuclear envelopes in 50 mM-Tris(pH 7.5)/25 mM-KCl/5 mM-MgCl₂ (TKM buffer). Nuclei and nuclear envelopes were prepared for SDS/10–15%- and SDS/10%-PAGE respectively under reducing conditions, and radiolabelled proteins were identified by autoradiography (Kodak XAR film). Remaining nuclei and nuclear-envelope samples were stored when necessary at –20 °C until required for further analysis.

Fractionation of nuclei after cross-linking of ¹²⁵I-ASD-CRP and ¹²⁵I-ASD-SAP

Nuclei which had been cross-linked with either ¹²⁵I-ASD-CRP or ¹²⁵I-ASD-SAP were fractionated according to the following protocol. Nuclei were incubated at 20 °C for 30 min in 0.25 M-sucrose in TKM buffer containing DNAase I (10 μ g/ml) and pelleted by centrifugation at 10000 g for 10 min. The DNAase I supernatant was saved and the histones extracted from the pellet with 2 M-NaCl in TKM buffer (see above). Nuclear envelopes were obtained by centrifugation of the 2 M-NaCl extract. Supernatants from the 2 M-NaCl and DNAase I extractions were dialysed and freeze-dried. Analysis of the fractions was by SDS/10%-PAGE under reducing conditions.

RESULTS

Solubility of SAP in Ca²⁺-containing media

Autoaggregation and precipitation of SAP in the Ca²⁺-containing buffers [22] used in binding experiments was prevented by the addition of BSA to a final concentration of 5% (w/v). SAP at 2.5 μ M (the maximum concentration used in binding experiments) in 1.5 mM-CaCl₂/5% (w/v) BSA could be demonstrated, by gel-exclusion chromatography (Sephacryl S200; 40 ml column), to exist as unaggregated decamers. The position at which 2.5 μ M-SAP was eluted as a single peak from the column in the absence of CaCl₂ was within 1% of that when SAP was eluted with 1.5 mM-CaCl₂/5% BSA (w/v) (results not shown).

Association of CRP and SAP with nuclei and isolated nuclear envelopes

The association of ¹²⁵I-labelled CRP and ¹²⁵I-labelled SAP with nuclei (100 μ g DNA) in the presence of Ca²⁺ was not saturable over the concentration range tested (Figs. 1a and 1b). Washing the nuclei with EGTA after binding of ¹²⁵I-labelled CRP and ¹²⁵I-labelled SAP in Ca²⁺ indicated that these ligands associate with nuclei through two processes: a saturable Ca²⁺-dependent process removable with EGTA, and a linear, Ca²⁺-independent, process resistant to EGTA washing (Figs. 1a and 1b). The saturable Ca²⁺-dependent association of each ligand with nuclei represents the specific binding of each ligand to nuclei. The EGTA-resistant linear-bound ligand (Figs. 1a and 1b), which was found to be quantitatively equivalent to the binding of these ligands to nuclei in the presence of EGTA and to approximate the residual binding of each ligand in the presence

Fig. 1. Binding of CRP and SAP to nuclei (100 μg of DNA) and nuclear envelope (NE) (25 μg of protein)

(a) ^{125}I -labelled CRP binding to nuclei; (b) ^{125}I -labelled SAP binding to nuclei; (c) ^{125}I -labelled CRP binding to nuclear envelope; (d) ^{125}I -labelled SAP binding to nuclear envelope. ●, Binding in 1 mM- CaCl_2 ; ○, labelled ligand released from nuclei and nuclear envelope by washing with EGTA; □, labelled ligand remaining with nuclei and nuclear envelope after washing with EGTA.

of a 100-fold excess of unlabelled ligand (results not shown) represents the non-specific binding to nuclei. The saturable Ca^{2+} -dependent binding of ^{125}I -labelled CRP to nuclei (100 μg of DNA) occurred outside of the range 0–400 pmol used in binding experiments (Fig. 1a). ^{125}I -labelled CRP binding Ca^{2+} -dependently to nuclei (100 μg DNA) at saturation was 21 pmol with half-saturation occurring at 200 pmol of ^{125}I -labelled CRP (results not shown). By contrast, the Ca^{2+} -dependent binding of ^{125}I -labelled SAP to nuclei (100 μg DNA) at saturation was 2.7 pmol, with half-saturation occurring at 50 pmol of ^{125}I -labelled SAP (Fig. 1b).

To determine whether the interaction of CRP and SAP with nuclei involves interaction with the nuclear envelope, these ligands were incubated with isolated nuclear envelopes. For this purpose 25 μg of nuclear-envelope protein was used, which is the approximate protein yield from nuclei with a DNA content of 100 μg . Ca^{2+} concentrations of ≥ 1 mM were found necessary to obtain maximum association of ^{125}I -labelled CRP or ^{125}I -labelled SAP with nuclear envelopes (results not shown). The association of these ligands in 1 mM- CaCl_2 was not saturable over the range measured (Figs. 1c and 1d), but could be shown, as was shown for binding to nuclei, on EGTA chelation to consist of a Ca^{2+} -dependent component removable with EGTA and an EGTA-resistant component (Fig. 1c and 1d). This linear Ca^{2+} -dependent association of these ligands with nuclear envelopes proved, as was shown for the Ca^{2+} -independent binding to nuclei, to be non-specific binding, whereas the saturable Ca^{2+} -dependent binding of ^{125}I -labelled CRP and ^{125}I -labelled SAP to nuclear envelopes represents specific binding. The amount of ^{125}I -labelled CRP binding Ca^{2+} -dependently to nuclear envelopes (25 μg of protein)

at saturation was 3.2 pmol with half-maximal saturation occurring at 70 pmol of ^{125}I -labelled CRP (Fig. 1c). By contrast the amount of ^{125}I -labelled SAP binding Ca^{2+} -dependently to nuclear envelopes (25 μg of protein) at saturation was 0.62 pmol, with half-saturation occurring at 90 pmol of ^{125}I -labelled SAP (Fig. 1d).

These results for ^{125}I -labelled CRP and ^{125}I -labelled SAP interaction with nuclei and nuclear envelopes presented here were in good agreement for eight different experiments. The variation between experiments was less than 10%.

Derivatization of CRP and SAP with ^{125}I -SASD

Optimal derivatization of CRP and SAP with ^{125}I -SASD, iodinated at pH 9.0 with 10 $\mu\text{Ci}/\mu\text{g}$ of SASD, was achieved at a molar ratio of 300 mol of ^{125}I -SASD/1 mol of CRP (or 1 mol of SAP) (results not shown). Precipitation of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP with trichloroacetic acid in the absence of reducing agent resulted in > 99% of the radiolabel precipitating, whereas, in the presence of reducing agent, 43 and 46% of the radiolabel associated with derivatized CRP and SAP respectively precipitated. Thus 57 or 54% of the label is incorporated into the photoreactive azide of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP respectively, with the remainder of the radiolabel being associated with the protein. This radiolabelling occurs presumably by disulphide-bond exchange between SASD and the proteins. The specific radioactivity of the total ASD-protein complex was calculated to be 1×10^6 c.p.m./ μg of CRP or 1.2×10^6 c.p.m./ μg of SAP, whereas the specific radioactivity of each protein when only the label on the azide was considered was found to be 0.57×10^6 c.p.m./ μg of CRP or 0.54×10^6 c.p.m./ μg of SAP. For

Fig. 2. Autoradiograph of reducing SDS/10–15%-PAGE analysis of nuclei after incubation with ^{125}I -ASD-CRP and ^{125}I -ASD-SAP with Ca^{2+}

Lanes: 1, Coomassie Blue stain; 2, nuclei incubated with ^{125}I -ASD-SAP in the presence of a 20-fold excess of unlabelled SAP (+u.v. radiation); 3, nuclei incubated with ^{125}I -ASD-SAP (+u.v. radiation); 4, nuclei incubated with ^{125}I -ASD-CRP (+u.v. radiation); 5, nuclei incubated with ^{125}I -ASD-CRP in the presence of a 20-fold excess unlabelled CRP (+u.v. radiation); 6, nuclei incubated with ^{125}I -ASD-SAP (no u.v. radiation); 7, nuclei incubated with ^{125}I -ASD-CRP (no u.v. radiation).

Fig. 3. SDS/10%-PAGE analysis, under reducing conditions, of fractionated nuclei after cross-linking of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP with Ca^{2+}

Lanes: 1, Coomassie Blue stain of proteins released by DNAase I digestion; 2 and 3, autoradiograph of the proteins released by DNAase I from nuclei cross-linked with ^{125}I -ASD-CRP and ^{125}I -ASD-SAP respectively; 4, Coomassie Blue stain of nuclear-envelope proteins isolated from nuclei after cross-linking of either ^{125}I -ASD-CRP or ^{125}I -ASD-SAP; 5 and 6, autoradiograph of isolated nuclear envelopes from nuclei cross-linked with ^{125}I -ASD-CRP and ^{125}I -ASD-SAP respectively.

ten different coupling experiments these results did not differ by more than 7%.

Binding of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP to nuclei and isolated nuclear envelopes

The binding of 25 pmol of derivatized ligand to nuclei and nuclear envelopes was investigated, since the non-specific binding of 25 pmol of CRP or SAP to nuclei (100 μg of DNA) and isolated nuclear envelopes (25 μg of protein) in the presence of Ca^{2+} was minimal (Fig. 1). To calculate the total association of CRP or SAP with nuclei and nuclear envelopes, after binding of ^{125}I -ASD-CRP or ^{125}I -ASD-SAP, the specific radioactivity of the total ^{125}I -ASD-pentraxin complex was used. Incubation of ^{125}I -ASD-CRP (25 pmol of CRP) or ^{125}I -ASD-SAP (25 pmol of SAP) with nuclei (100 μg of DNA) resulted in a total of 2.2 pmol of CRP or 0.6 pmol of SAP becoming associated with the nuclei

in the presence of CaCl_2 . These results agree favourably with the respective data obtained for binding of directly iodinated CRP (25 pmol) and SAP (25 pmol) to nuclei (Figs. 1a and 1b). In the presence of a 20-fold excess of the respective unlabelled ligand, the total association of each derivatized ligand was reduced by 89 and 86% respectively. In addition, incubation of these ligands with nuclei in the presence of EGTA reduced the binding to 0.04 pmol of ^{125}I -ASD-CRP and 0.01 pmol of ^{125}I -ASD-SAP. Incubation of ^{125}I -ASD-CRP (25 pmol of CRP) and ^{125}I -ASD-SAP (25 pmol of SAP) with nuclear envelopes (25 μg of protein) resulted in quantitatively the same association of ligand as that found when directly labelled ligands are used (Fig. 1c and 1d). For 25 μg of nuclear-envelope protein, a total of 0.5 pmol of ^{125}I -ASD-CRP and 0.1 pmol of ^{125}I -ASD-SAP bound in the presence of CaCl_2 , and 0.015 pmol ^{125}I -ASD-CRP and 0.004 pmol of ^{125}I -ASD-SAP in the presence of EGTA. In addition, a 20-fold excess of the corresponding unlabelled ligand reduced the total association of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP by 90 and 89% respectively. This Ca^{2+} -dependent binding of derivatized CRP and SAP to nuclei and nuclear envelopes was reproducible, and the results of five different experiments did not vary by more than 10%.

When non-photolysed nuclei were analysed after binding of ligands in the presence of Ca^{2+} , only bound radiolabelled CRP or SAP was detected, with no cross-linking to nuclear proteins being observed (Fig. 2). A number of nuclear proteins became radiolabelled as a result of specific photocross-linking of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP. Several non-histone proteins, of molecular masses 43–70 kDa, became intensely radiolabelled, in addition to minor radiolabelling of other nuclear proteins through photocross-linking of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP (Fig. 2). In addition, photocross-linking of ^{125}I -ASD-CRP to nuclei resulted in transfer of ^{125}I -label to histone H4 and photocross-linking of ^{125}I -ASD-SAP to nuclei resulted in histones H2A, H2B, H3 and H4 becoming radiolabelled (Fig. 2). No conclusive cross-linking of either ligand to histone H1 was detected. The radiolabelling of these nuclear proteins through cross-linking of ^{125}I -ASD-CRP or ^{125}I -ASD-SAP was markedly reduced by a 20-fold excess of unlabelled ligand (Fig. 2) and was absent with EGTA in the reaction mixture (results not shown).

Fractionation of nuclei after cross-linking

Fractionation of the nuclei after photocross-linking of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP was performed to determine the localization of the radiolabelled proteins. The proteins released from these nuclei by DNAase I treatment were not found to be radiolabelled (Fig. 3). Analysis of the extracted histones proved binding of CRP to H4 and of SAP to H2A, H2B, H3 and H4, with neither ligand binding to H1 (results not shown). The nuclear-envelope fraction isolated from photolysed nuclei proved to contain the bulk of the intensely radiolabelled non-histone nuclear proteins (Fig. 3). These results were confirmed by photocross-linking ^{125}I -ASD-CRP and ^{125}I -ASD-SAP to isolated nuclear envelopes (Fig. 4). Cross-linking of ^{125}I -ASD-CRP and ^{125}I -ASD-SAP to isolated nuclear envelopes in the presence of Ca^{2+} resulted in intense radiolabelling of four envelope proteins of 43, 46, 52 and 70 kDa (Fig. 4). These correspond to the most intensely labelled non-histone proteins in envelopes isolated from nuclei after photocross-linking with ^{125}I -ASD-CRP and ^{125}I -ASD-SAP (Fig. 3). This cross-linking of CRP and SAP to isolated nuclear envelopes was photospecific, as no labelling was observed in the absence of photolysis (Fig. 4). In addition, a decrease in labelling in the presence of a 20-fold excess of unlabelled ligand (Fig. 4) and an absence of labelling in the presence of EGTA (results not shown) was observed.

Fig. 4. Autoradiograph of SDS/10%-PAGE analysis, under reducing conditions, of nuclear envelopes after incubation with ^{125}I -ASD-CRP and ^{125}I -ASD-SAP with Ca^{2+}

Lanes: 1, Coomassie Blue stain; 2, nuclear envelopes incubated with ^{125}I -ASD-CRP (+ u.v. radiation); 3, nuclear envelopes incubated with ^{125}I -ASD-CRP in the presence of a 20-fold excess of unlabelled CRP (+ u.v. radiation); 4, nuclear envelopes incubated with ^{125}I -ASD-SAP (+ u.v. radiation); 5, nuclear envelopes incubated with ^{125}I -ASD-SAP in the presence of a 20-fold excess of unlabelled SAP; 6, nuclear envelopes incubated with ^{125}I -ASD-CRP (no u.v. radiation); 7, nuclear envelopes incubated with ^{125}I -ASD-SAP (no u.v. radiation).

The nuclear-envelope protein of 46 kDa that becomes intensely radiolabelled through cross-linking of pentraxins has the same molecular mass as that of the major nucleoside-triphosphatase of the rat liver nuclear envelope. This enzyme has been identified by photoaffinity labelling as a 46 kDa protein and has been implicated in the nuclear transport of RNA [24]. It may thus well be a component of the nuclear-pore complex. A glycoprotein of 46 kDa has also been identified as a component of the nuclear-pore complex [25], although its identity with the envelope ATPase has not been established. Binding of the pore complex by CRP would conceivably facilitate entry into the nucleus, which must occur in order for labelling of the histones to occur.

Our data show the complexity of pentraxin interactions with nuclear constituents. Each pentraxin shows a predilection for certain ligands, depending on experimental conditions. Owing to the variety of ligands for pentraxins, specific affinities cannot be calculated from these data. However, given our data and the findings of Butler *et al.* [6], SAP emerges as the major chromatin-binding protein, whether in solution or intact in nuclei. More disparate ligands have been identified for CRP, mostly by displaying chromatin on synthetic surfaces [7–9]. The precise physiological relevance of these pentraxin–ligand interactions still needs to be defined, but it is likely that, at sites of tissue necrosis, nuclear ligands can be liberated, in which case these pentraxin interactions could be of importance.

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DISCUSSION

We show that, when ^{125}I -ASD-SAP is cross-linked to nuclei, Ca^{2+} -dependent specific radiolabelling of all four core histones occurs. This could either be due to direct SAP binding to histones or to DNA binding adjacent to the core histones, with secondary transfer of the ^{125}I -photoreactive azide. Our failure to show cross-linking to H1 is in agreement with the finding of Butler *et al.* [6] that SAP binding to native long chromatin displaces H1.

CRP binding to immobilized chromatin and histones with considerable binding to H1, H2A and H2B, without appreciable binding to H3 and H4, has previously been observed [8]. In addition, co-precipitation of CRP with isolated histones in physiological Ca^{2+} concentrations has been demonstrated [23]. In contrast, our data indicate specific cross-linking only to H4 when ^{125}I -ASD-CRP is bound to nuclei in the presence of Ca^{2+} . This shows how dependent CRP binding is on the conformational state and molecular context of histones.

We identify a new class of nuclear ligands for pentraxins, namely nuclear-envelope proteins. The quantitative binding of CRP to isolated nuclear envelopes far exceeded that of SAP. Photoaffinity labelling has shown both pentraxins to bind Ca^{2+} -dependently and photospecifically to proteins of 43, 46, 52 and 70 kDa of isolated nuclear envelope and the envelope of intact nuclei. No cross-linking of the pentraxins to these nuclear proteins was observed when the binding took place in the presence of a Ca^{2+} chelator. The precise identity of the pentraxin-binding nuclear-envelope proteins has not been established. However, one of the four intensely labelled proteins has a molecular mass identical with that of a CRP-binding nuclear protein of 70 kDa identified as a component of the UI small nuclear ribonucleoprotein particle [9]. Alternatively, radiolabelling of the 70 kDa nuclear-envelope protein could indicate pentraxin binding to lamin A, a 70 kDa component of nuclear lamina associated with the inner surface of the nuclear membrane.

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