Total chemical synthesis and chemotactic activity of human S100A12 (EN-RAGE)

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Abstract Human S100A12 (extracellular newly identified RAGE (receptor for advanced glycosylation end products)-binding protein), a new member of the S100 family of EF-hand calcium-binding proteins, was chemically synthesised using highly optimised 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/*tert*-butoxycarbonyl in situ neutralisation solid-phase chemistry. Circular dichroism studies indicated that $CaCl_2$ decreased the helical content by 27% whereas helicity was marginally increased by $ZnCl_2$. The propensity of S100A12 to dimerise was examined by electrospray ionisation time-of-flight mass spectrometry which clearly demonstrated the prevalence of the non-covalent homodimer (20 890 Da). Importantly, synthetic human S100A12 in the nanomolar range was chemotactic for neutrophils and macrophages in vitro.

Key words: Calcium; S100; Chemotactic; Solid-phase peptide synthesis; Mass spectrometry; Non-covalent interaction; Zinc

1. Introduction

The S100 family is comprised of closely related low molecular weight (10–14 kDa) acidic calcium-binding proteins (CaBPs), which have been associated with a range of disorders including atherosclerosis, inflammatory bowel disease, rheumatoid arthritis, delayed-type hypersensitivity, Alzheimer's disease, and cancer [1]. Myeloid S100A8 (migration inhibitory factor related protein 8 (MRP8), calgranulin A) [2], S100A9 (MRP14, calgranulin B) [2] and S100A12 (calgranulin C, p6, CAAF1, CGRP) [3,4] are found at inflammatory foci and may be released by activated neutrophils (PMN) or monocytes/macrophages. High plasma levels of A8 and A9 are indicators of inflammatory disease [5] and the proteins have anti-microbial properties in vitro [1,6,7]. Recent evidence indicates that bovine S100A12, a relatively new member of the S100 family, is a ligand for the receptor for advanced glycosylated end products (RAGE) expressed by macrophages, endothelial cells and lymphocytes [8]. It is proposed that RAGE may act as the central cell surface receptor mediating the cellular responses of S100 proteins at inflammatory sites, resulting in propagation of the host response by increased monocyte recruitment and cellular activation [8].

Human S100A12 consists of 91 amino acid residues, has two EF-hands and a predicted *C*-terminal zinc-binding site (His–X_{aa}–X_{aa}–X_{aa}–His) [9]. The EF-hand structural motif consists of a loop flanked by α-helices (helix–loop–helix), which together form a single EF-hand calcium-binding site [10]. S100A12 has 40% (65%) and 46% (70%) sequence homology (similarity) with the related myeloid-associated S100 proteins, S100A8 and S100A9 (Fig. 1), and a high degree of amino acid sequence identity (59%) with the novel murine chemotactic factor, CP10 (now known as *murine* S100A8), previously described by us [11,12]. The role of homo- and hetero-dimerisation and interactions with calcium and zinc ions in the functional properties of these S100 proteins is not well understood [13–15]. Nuclear magnetic resonance (NMR) studies with calcyclin revealed its non-covalent antiparallel homodimeric tertiary structure, and non-covalent homodimerisation was confirmed in the recently reported crystal structure of psoriasin (S100A7) [16,17].

To further investigate the role of human S100A12 in inflammation, mg quantities of the protein were first required. Development of a reliable recombinant expression system for S100A12 was difficult to establish, and we undertook the total chemical synthesis of human S100A12 using highly optimised 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/*tert*-butoxycarbonyl (Boc) in situ neutralisation chemistry to provide rapid access to this protein. Here we report the synthesis, purification and potent chemotactic activity of human S100A12. The use of electrospray ionisation time-of-flight mass spectrometry (ESI-TOF

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Abbreviations: CaBP, calcium-binding protein; CD, circular dichroism; ESI-MS, electrospray ionisation mass spectrometry; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; MRP, migration inhibitory factor related protein; NMR, nuclear magnetic resonance; RP-HPLC, reversed phase-high performance liquid chromatography; Q, quadrupole; RAGE, receptor for advanced glycosylation end products; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TOF, time-of-flight; Standard IUPAC single and triple letter codes for amino acids are used throughout

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MS) to probe non-covalent protein–protein interactions of human S100A12 is also described.

2. Materials and methods

2.1. Materials

Boc-(L)-amino acids were obtained from Novabiochem (San Diego, CA, USA) and the Peptide Institute (Osaka, Japan). Boc-amino acid-Pam-resin was purchased from Applied Biosystems (Foster City, CA, USA). Dichloromethane (DCM), diisopropylethylamine (DIEA), *N*,*N*-dimethylformamide (DMF) and trifluoroacetic acid (TFA) were obtained from Auspep (Melbourne, Australia). High performance liquid chromatography (HPLC) grade acetonitrile was purchased from BDH chemicals. HBTU was purchased from Richelieu Biotechnologies (Quebec, Canada).

2.2. Solid-phase synthesis of S100A12

Human S100A12 (TKLEEHLEGIVNIFHQYSVRKGHFDTLSKGELKQLLTKELANTIKNIKDKAVIDEIFQGLDANQDEQVDFQEFISLVAIALKAAHYHTHKE) was manually synthesised stepwise using highly optimised in situ neutralisation/HBTU activation protocols for Boc/benzyl chemistry as previously described [18,19]. The synthesis was performed on Boc-Glu(*O*-Bzl)-Pam resin using the following N^{α}- and side-chain protected amino acids: Boc-Arg(4-toluenesulphonyl), Boc-Asp(*O*-cyclohexyl)-OH, Boc-Asn(xanthyl)-OH, Boc-Glu(*O*-cyclohexyl)-OH, Boc-Gln(xanthyl)-OH, Boc-His(N_{im}-2,4-dinitrophenyl)-OH, Boc-Lys(N_{ε}-2-chlorobenzyloxycarbonyl)-OH, Boc-Ser(*O*-benzyl)-OH, Boc-Thr(*O*-benzyl)-OH, and Boc-Tyr(2-bromobenzyloxycarbonyl)-OH. Each residue was coupled for 10 min and coupling efficiencies determined by the quantitative ninhydrin assay [28]. If first coupling yields were below approximately 99.2%, re-coupling was carried out until satisfactory yields were achieved. The average yield of chain assembly was 99.6% per residue. Partial deprotection of the peptide was carried out on the resin prior to HF cleavage. Dinitrophenyl groups were removed by thiolysis (10% DIEA, 20% β-mercaptoethanol and 70% DMF). The N-terminal Boc group was removed with TFA. The resin was then washed with DMF followed by DCM and then dried under a nitrogen atmosphere. Side-chain protecting groups were removed and the peptide cleaved from the resin by treatment with anhydrous HF/*p*-cresol (9:1) for 60 min at −5 to 0°C. After evaporation of the HF, the crude peptide was precipitated, washed with diethyl ether, dissolved in 50% aqueous acetonitrile and lyophilised following aqueous dilution.

2.3. Purification of synthetic S100A12

Analytical and preparative reversed phase-HPLC (RP-HPLC) were performed on a Waters 600 HPLC system. Analytical HPLC was run on a Vydac *C*-4 column (5 μm, 4.6×150 mm, 300 Å) at a flow rate of 1 ml/min and UV absorbance monitored at 214 nm. Preparative RP-HPLC was run on a Waters *C*-4 column (5 μm, 46×150 mm, 300 Å) at a flow rate of 10 ml/min and UV absorbance monitored at 230 nm. The crude product was then dissolved in 50% buffer B (0.09% TFA in 90% acetonitrile) and eluted using a gradient system of 25–75% buffer B versus buffer A (0.1% aqueous TFA) over 50 min. Eluted fractions containing pure S100A12 were identified by analytical RP-HPLC and ESI-MS, pooled, lyophilised and stored at −78°C.

2.4. MS

Mass analysis of all synthetic peptides was firstly performed on a PE-Sciex API-III triple quadrupole (Q) mass spectrometer equipped with an ion-spray (pneumatically assisted electrospray) atmospheric pressure ionisation source (ESI-MS). Samples were injected using a syringe infusion pump at nmol concentrations at 20 μl/min, coupled directly to the ionisation source via a fused-silica capillary (75 μm i.d.×40 cm length). Sample droplets were ionised at a positive potential of 5 kV and entered the analyser through an interface plate and subsequently through an orifice (100 μm diameter) at a potential of 80 V. Full scan spectra were acquired by scanning Q1 from *m*/*z* 500–2000 Da with a scan step of 0.2 Da and a dwell time of 0.4 ms. Calculated masses were obtained from the program MacBioSpec (PE-Sciex). The data were recorded and processed using the Tune 2.5-FPU (PE-Sciex) and MacSpec 3.3 (PE-Sciex) programs on a Macintosh computer.

2.5. Endoproteinase Glu-C digest

S100A12 was digested with endoproteinase Glu-C (*Staphylococcus aureus* V8; EC 3.4.21.19) in 50 mM ammonium acetate (pH 4) with an enzyme:substrate ratio of 1:50 (w/w) at 37°C for 2 h. The crude product was analysed by positive ion HPLC/MS with a declustering potential of 50 V. Full scan mass spectra were acquired over the mass range of 400–2000 Da with a scan step of 0.1 Da and a dwell time of 0.3 ms.

2.6. ESI-TOF mass spectrometry

Mass spectra were acquired using an ESI-TOF mass spectrometer constructed at the University of Manitoba [20]. An acceleration voltage of 4 kV was used for TOF analysis. Samples were normally electrosprayed from a stainless steel needle (i.d. 120 μm) using a typical flow rate of 250 nl/min. The ESI interface conditions were mild to minimise possible disruption of non-covalent complexes, i.e. low capillary desolvation temperature and low declustering potential. S100A12 was reconstituted to 10^{-5} M and analysed in 5 mM ammonium bicarbonate (pH 7.5). The ESI-TOF spectra were acquired over the mass range of 500 to 4000 Da with an ion spray current of 0.1–0.2 μA.

2.7. Circular dichroism (CD)

Far UV CD spectra were obtained on a Jasco J-300 spectropolarimeter calibrated with (+)-10-camphorsulfonic acid at 25°C using a 0.1 cm path length. S100A12 in 10 mM phosphate buffer (pH 7.4) was analysed at a final constant concentration of 55 μM. The secondary structure was estimated by analysis of the spectrum between 190 and 300 nm. Each spectrum represents the average of 10 scans. Mean residue ellipticity $[\theta]_{MR}$ is expressed in ° cm^2)/dmol). Experimental estimates of the helical content were determined from $[\theta]_{222}$ measurements. The α-helix content was calculated using equations described by Chen [21] where % α-helix = $([\theta]_{222}/[\theta]_{max})\times 100\%$ and $[\theta]_{max} = -39\,500[1-(2.57/n)]$; n = number of residues per chain. This equation is used only as a qualitative estimate of peptide helicity.

2.8. Cell culture

Human THP-1 cell (ATCC TIB 202) monocytoid cells were routinely cultured at 37°C in 5% CO_2 in air in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 2 mM L-glutamine (Flow Laboratories, Irvine, Scotland, UK), 100 U/ml penicillin, 100 μg/ml streptomycin (Sigma, St. Louis, MO, USA) and 10% heated (56°C, 30 min) bovine calf serum (Hyclone Laboratories, Logan, UT, USA). The medium was filtered through Zetapor 0.2 μm membrane (Cuno, Meriden, CT, USA) to remove contaminating traces of endotoxin. Endotoxin levels in reagents and medium were <1 ng/ml by the chromogenic limulus amoebocyte lysate assay (Cape Cod Association Wood Hole, MA, USA).

2.9. Chemotactic activity

THP1 cells (5×10^6/ml) suspended in human Hanks' Balanced salt solution (HBSS) buffer (Sigma)/0.1% bovine serum albumin (BSA) were incubated with 5 μM calcein AM (Molecular Probes, Eugene, OR, USA) for 15 min at 37°C in 5% CO_2 in air, washed twice with HBSS and resuspended at 0.5×10^6/ml in RPMI/0.2% BSA. Assays were performed using Neuro Probe MBA-96-well Chambers with polycarbonate membrane pore size 8 μm (Neuro Probe, Bethesda, MD, USA). Various concentrations of synthetic S100A12 (10^{-8} to 10^{-13} M) diluted in RPMI/0.2% BSA (410 μl) were placed in the lower compartment and cells (300 μl) in the upper compartment. The chamber was incubated at 37°C in an atmosphere of 5% CO_2 in air for 120 min. The number of migrated cells attached to the underside of the membrane was expressed by measuring calcein fluorescence at ex = 485 nm and em = 530 nm using a CytoFluor® multi-well plate reader system (PerSeptive Biosystems, Framingham, MA, USA). Fluorescence readings were converted to cell numbers by producing a standard curve with calcein-labelled THP-1 cells. The correlation between cell number and fluorescence was high ($R = 0.97051$). C_{5a} (10^{-9} M) was used as a positive control in all experiments.

2.10. Electrophoresis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using the Bio-Rad Mini Protean II electrophoresis cell and a Tris/tricine buffer system according to standard pro-

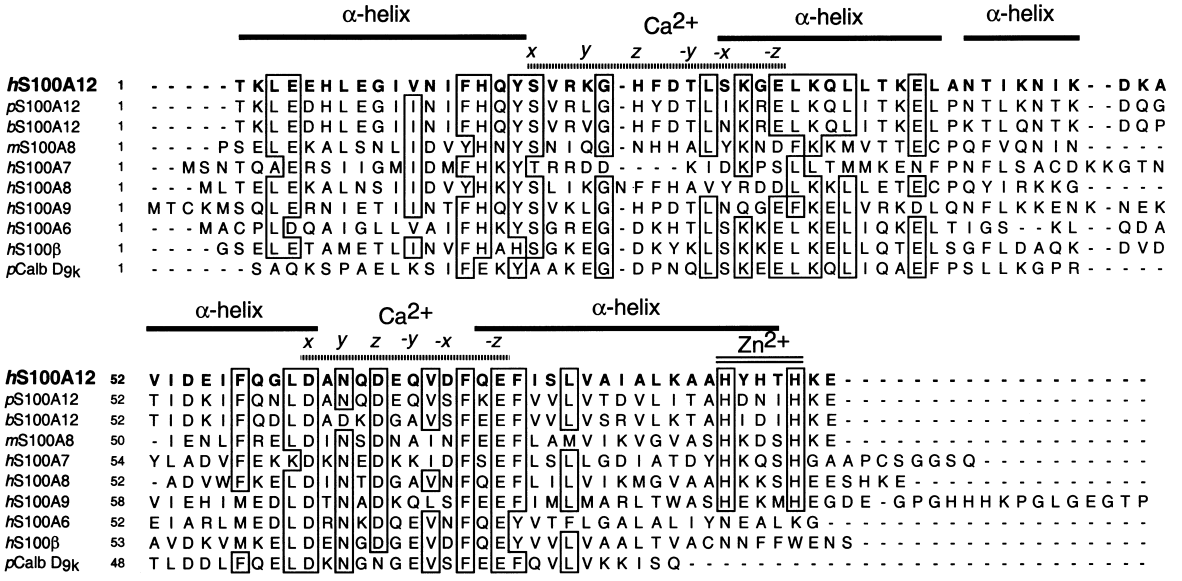

Fig. 1. Multiple sequence alignment of selected S100 proteins. S100A12 is shown bold. All sequences were obtained from the SWISS-PROT protein sequence database and aligned using CLUSTAL W analysis [39]. Numbers at the end of the sequences indicate the total number of amino acid residues, while boxed areas indicate regions of sequence homology. Predicted helical elements are indicated by a solid bar above the sequences [28]. Calcium- and zinc-binding sites are indicated by hatched and double lines, respectively. Positions x, y, z, $-y$, $-x$, and $-z$ correspond to calcium ligands. Abbreviations: Calb D_{9k}, calbindin D_{9k}; *h*, human; *m*, murine, *p*, porcine, and *b*, bovine.

cedures (Bio-Rad handbook). Proteins were separated in a 10% gel run at 100 V for 2 h and silver-stained.

3. Results and discussion

3.1. Total chemical synthesis of S100A12

Total chemical synthesis of human S100A12 was considered the most time efficient and simple means of producing sufficient quantities of the protein for functional analysis. In general, solid-phase peptide synthesis, which was originally developed by Merrifield [22], is a facile and effective route for the rapid production of multi-mg quantities of peptides, small proteins or enzymes for immunological, biochemical or structural studies [23,24]. The technique was successfully used to synthesise many small proteins including fully active human immunodeficiency virus-1 proteinase [25,26], interleukin 8 [23,24] and phospholipase A_2 [27]. Chemical synthesis may also permit more accurate investigation of the structure–function relationships that may not be readily accomplished with recombinant proteins because of the ease of incorporation, during synthesis, of non-coded amino acids, fluorescent probes or NMR-active nuclei on specific residues.

The manual sequential chain assembly of human S100A12 (Fig. 1) was carried out by HBTU in situ neutralisation chemistry [18] using Boc/benzyl orthogonal protection. During the stepwise chain assembly, amino acid coupling efficiencies were monitored by the quantitative ninhydrin assay [28] and the average coupling yield was estimated as 99.6%. The assembly generally proceeded well except for a notable 'difficult' coupling at valine-68. This required re-coupling as the symmetric anhydride before continuation. Following sequential chain assembly, side-chain deprotection and cleavage from the resin was performed by treating with HF/*p*-cresol (9:1) for 1 h at 0°C. S100A12 was readily purified from the lyophilised crude cleaved product by C_4 RP-HPLC using a linear 1%/min gradient of 0–80% buffer B versus buffer A. Pure S100A12 eluted in 49% B as a single symmetrical peak (Fig. 2A). The manual synthesis afforded approximately 10 mg of material (>98% purity) in less than 4 weeks. The purity and integrity of synthetic S100A12 was confirmed by ESI-MS, Glu-*C* digestion and SDS–PAGE analysis. Like its porcine analogue [14], S100A12 migrated on SDS–PAGE as an 8 kDa component rather than at the expected 10.5 kDa (Fig. 2B). CaBPs often have irregular mobilities on SDS–PAGE gels [14] and a more accurate mass measurement was determined by ESI-MS. The

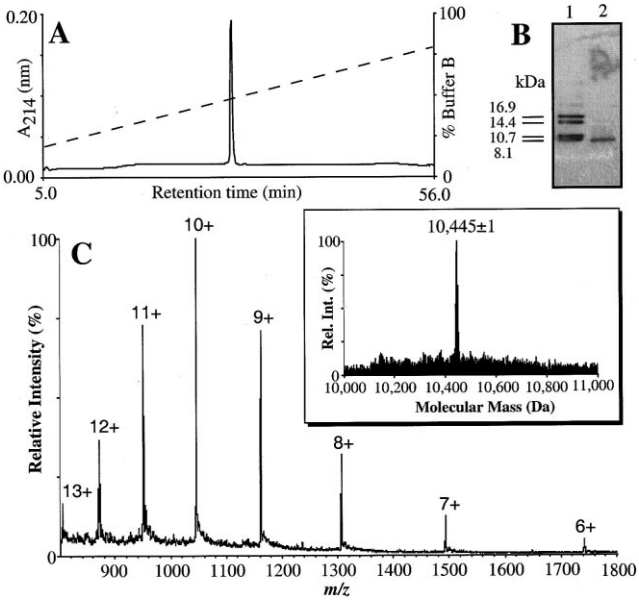

Fig. 2. Chemically synthesised human S100A12. A: C_4 RP-HPLC. B: lane 1: reference molecular mass markers of 8.1, 10.7, 14.4 and 16.9 kDa, respectively; lane 2: chemically synthesised human S100A12. C: ESI mass spectrum of S100A12 shows protonated ion species with charges 6+ to 13+ corresponding to an observed molecular mass of 10 445.4 ± 0.8 Da (calculated mass 10 444.9 Da).

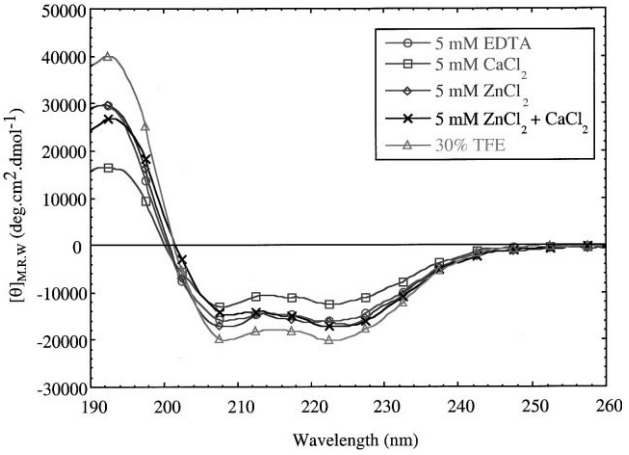

Fig. 3. Far-UV CD spectra of S100A12. Spectra acquired in the presence of 5 mM EDTA, -○-; 5 mM $CaCl_2$, -□-; 5 mM $ZnCl_2$, -◇-; 5 mM $CaCl_2/ZnCl_2$, -×-; and 30% (v/v) TFE, -△-. Protein concentration was 55 μM S100A12 in 10 mM phosphate buffer, pH 7.4, and temperature was 25°C.

observed molecular mass of 10 445.4 ± 0.8 Da, was within experimental error (0.01%) of the calculated mass: $C_{471}H_{744}N_{126}O_{142}$, 10 444.9 Da (Fig. 2C). The amino acid sequence of S100A12 was confirmed by digestion with endoproteinase Glu-*C* (*S. aureus* V8; EC 3.4.21.19) and analyses by positive ion RP-HPLC/MS at a declustering potential of 80 V. The separated peaks had *m/z* ion values which correlated well with the expected and observed molecular weights of digest fragments for the complete S100A12 amino acid sequence (Table 1).

3.2. Ca^{2+}/Zn^{2+} induced conformational changes

The structures of S100 proteins are highly conserved [10,29,30] and secondary structures are predominantly helical. Calcium and/or zinc regulate the function of several members of the S100 family [41–43]. CD was used as a qualitative gauge to investigate the potential conformational effects of these divalent cations on S100A12 secondary structure. The CD spectra of S100A12 in several environments shown in Fig. 3 exhibit minima at 208 and 222 nm, characteristic of α-helical peptides and proteins. Using the method of Chen [21] the apparent α-helical content of *apo*-S100A12 was estimated as 41%. The amount of calcium chosen for CD experiments was similar to levels in extracellular biological fluid (2–5 mM). In comparison to the *apo* form, the addition of 5 mM Ca^{2+} ions in the form of $CaCl_2$ decreased helical content by 27% indicating a conformational change upon calcium binding, and agreeing well with previous observations with other CaBPs [14]. In addition to the two calcium-binding domains, S100A12 has a predicted His–X_{aa}–His–X_{aa}–His zinc-binding motif from residues 85 to 89, which would also be expected to adopt a helical structure (Fig. 1). In this motif the His residues at positions *n* and *n*+4 are usually separated by a single helical turn [9,31]. Addition of 5 mM $ZnCl_2$ to *apo*-S100A12 only increased α-helicity by 1%. Interestingly, when zinc was added first, followed by calcium, overall helicity (42%) was substantially greater than with calcium alone, suggesting that zinc may antagonise conformational changes provoked by Ca^{2+}. Similar results were also obtained with peptides containing only the high-affinity C-terminal EF-hand domain of S100A12 and its neighbouring zinc-binding site, S100A12(54–91) and S100A12(38–91) (data not shown). When the hydrophobicity of the solution environment was increased by addition of 30% (v/v) 2,2,2-trifluoroethanol (TFE) in the absence of calcium or zinc ions, helicity increased to 49%, with no increased effect at higher concentrations. This compared well with the expected maximal S100A12 helicity of 52% predicted from sequence homology with S100A6 [17].

3.3. S100A12 homodimerisation by ESI-TOF MS

Insights into macromolecular interactions of S100 proteins are traditionally obtained using X-ray crystallography [32] or NMR [16,17]. Although these techniques provide considerable structural information, they are time consuming and require multi-mg quantities of pure proteins. Recent developments in ESI-MS provide significant advances to rapidly investigate non-covalent interactions using nmol quantities of proteins [33–35]. Information on the intrinsic propensity of S100 proteins to dimerise provides valuable insight into the molecular mechanisms by which specific S100 proteins may interact with, and alter the function of selective target proteins. A pertinent example is the observation of S100A8 homodimers by mild ESI-MS analysis [36].

ESI-TOF MS analysis is well suited for oligomerisation studies as it requires low quantities of proteins and can analyse large complexes with relatively high *m/z* values, >3000 [34] in a much shorter time frame than NMR or X-ray crystallography studies which require relatively high protein solubility (mM) or good diffracting crystals. Macromolecular interactions may arise from at least four types of relatively weak forces: ionic interactions, hydrogen bonds, the hydrophobic effect or van der Waals forces. Their weak nature makes most gas-phase complexes relatively fragile and gentle ionisation conditions are required to minimise complex dissociation [35]. Although ESI-MS satisfies this criterion and can now give insight into non-covalent interactions, caution must

Table 1
Endoproteinase Glu-*C* human S100A12 digest fragments observed by LC/MS analysis

Position	Experimental mass	Calculated mass	S100A12 fragment sequence
1–4	489.3	489.28	TKLE(E)
5–8	526.2	526.24	(E)EHLE(G)
32–34	387.5	387.25	(E)LKQ(L)
68–84	1834.5	1834.02	(Q)VDFQEFISLVAIALKAA(H)
32–39	971.6	971.60	(E)LKQLLTKE(L)
40–55	1784.0	1784.00	(E)LANTIKNIKDKAVIDE(I)
56–65	1119.5	1119.52	(E)IFQGLDANQD(E)
67–72	764.3	764.33	(E)QVDFQE(F)
9–31	2631.4	2631.36	(E)GIVNIFHQYSVRKGHFDTLSKGE(L)
73–91	2148.2	2148.18	(E)FISLVAIALKAAHYHTHKE

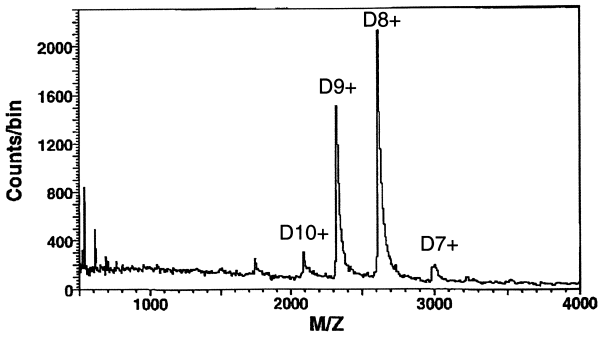

Fig. 4. ESI-TOF MS spectrum of S100A12. The major signals observed in the mass spectrum were due to the protonated ion species with charges 7+ to 10+ of the S100A12 dimer. S100A12 was dissolved in 5 mM ammonium bicarbonate (pH 7.5) and analysed at a concentration of 10^{-5} M. ESI-TOF MS spectra were acquired over the mass range of 500–4000 Da with an ion spray current of 0.1–0.2 μA.

be taken to select experimental conditions which yield *m/z* values representing non-covalent complexes in solution. Failure to observe a complex could be due to many factors, including inappropriate ionisation conditions, i.e. high declustering potential, pH, or solvent environment, making it difficult to use the technique as definitive evidence against oligomeric or non-covalent interactions. However, if suitable mass spectrometric conditions are achieved, the chemical and solvent parameters which potentially influence non-covalent interactions can be studied with confidence [34,37].

In ESI-TOF MS experiments, human S100A12 (10^{-5} M) was reconstituted and analysed in 5 mM ammonium bicarbonate (pH 6.5). The ESI-TOF mass spectrum showed protonated ion species with charges of 7+ to 10+ corresponding to the non-covalent S100A12 homodimer (20 890 Da); (Fig. 4) with no other multimeric forms evident. The S100A12 monomer contains no cysteine residues, so the formation of dimeric structures must clearly result from non-covalent interactions. The clear domination of dimers in the spectrum may indeed indicate specific interactions (Fig. 4).

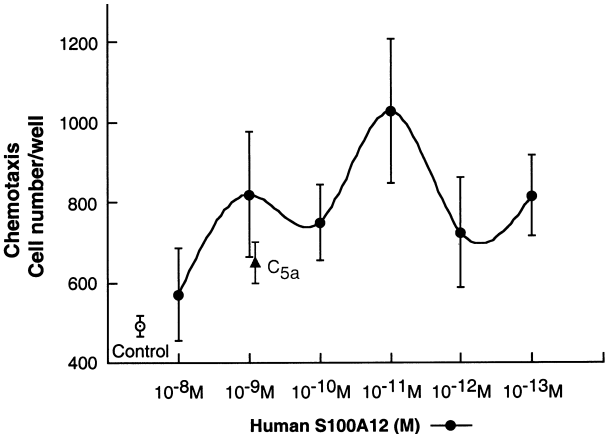

Fig. 5. Chemotactic response of THP1 cells to chemically synthesised S100A12. Mean migration of triplicate assays from two experiments ± S.D., of THP1 monocytic cells to varying molar concentrations of S100A12 (●). Control represents random migration of cells toward RPMI/0.2% BSA (-○-). Positive migration of cells to the classical chemoattractant C5a (10^{-9}M, -▲-) is given for comparison.

3.4. Bioactivity

Bovine S100A12 is chemotactic for monocytes but because S100 sequence homologues do not necessarily represent functional homologues [38], human S100A12 was tested for chemotactic activity. Chemically synthesised human S100A12 (10^{-10} and 10^{-12} M) was indeed chemotactic for human monocytoid THP1 cells (Fig. 5). Importantly, the activity was as potent as the classical chemoattractant, C5a, but occurred at markedly lower molar concentrations. S100A12 over the assayed concentration range produced a bell-shaped activity curve that is typical of all chemoattractants [38,44]. At high levels and full receptor occupancy, the cells fail to move because of the requirement for a concentration gradient and low receptor occupancy [44]. Human neutrophils and HL60 cells differentiated to neutrophils also responded and intraperitoneal injection of human S100A12 into mice elicited an inflammatory infiltrate (manuscript in preparation). The activity of the synthetic human protein was approximately 2–3 orders of magnitude more potent than reported for the native bovine protein tested using human monocytes [8]. Differences in potency may be due to sequence differences within the two proteins, particularly within the hinge region located between the EF-hands which is the chemotactic domain of murine S100A8 [40].

4. Conclusion

Chemical synthesis was a rapid and effective method of producing multi-mg quantities of functionally active human S100A12, a 10 kDa CaBP. Preliminary structural studies using CD analysis indicated a conformational change in the presence of calcium, which was antagonised by zinc ions. ESI-TOF MS analysis was used to study oligomerisation, and *m/z* values corresponding to non-covalent S100A12 dimers predominated. If the native state of S100A12 is dimeric, it may adopt a tertiary structure analogous to calcyclin and psoriasin which form a non-covalent anti-parallel homodimer [17], and this may provide insights into its functional form. Results presented here demonstrate the utility of ESI-MS and ESI-TOF MS for identifying non-covalent interactions. We confirmed that human S100A12, like its bovine analogue [8], was chemotactic for human monocytoid cells and significantly more potent.

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