Characterization of heparin binding by a peptide from amyloid P component using capillary electrophoresis, surface plasmon resonance and isothermal titration calorimetry

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Synthetic peptides based on amino-acid residues 27–38 of human serum amyloid P component represent a novel type of heparin binders as they do not contain clusters of basic amino acids or other known features associated with protein or peptide heparin binding. Here, we characterize the binding using capillary electrophoresis (CE), surface plasmon resonance (SPR), and isothermal titration calorimetry (ITC). By CE, heparin-binding activity was readily apparent for both a regular peptide and a slightly N-terminally modified form, while a sequence-scrambled peptide had no measurable binding. Dissociation constants in the 1–15 µM range were estimated, but only a minor part of the binding isothem was covered by the experiments. SPR measurements using immobilized peptides verified heparin binding, the range of the binding constants, and the reduced binding of the sequence-scrambled peptide. Structurally defined heparin oligosaccharides were used to establish that while the tetrasaccharide is too small to exhibit strong binding, little difference in binding strength is observed between hexa- and tetradeca-saccharides. These experiments also confirmed the almost complete lack of activity of the sequence-scrambled peptide. The amino-acid sequence-dependent binding and the importance of a disulfide bond in the peptide were verified by ITC, but the experimental conditions had to be modified because of peptide precipitation and ITC yielded significantly weaker binding constants than the other methods. While the precise function of the peptide in the intact protein remains unclear, the results confirm the specificity of the glycosaminoglycan interaction with regard to peptide sequence by applying two additional biophysical techniques and showing that the N-terminal part of the peptide may be modified without changing the heparin binding capabilities.

Keywords: amyloid P component; heparin binding; surface plasmon resonance; capillary electrophoresis; isothermal titration calorimetry.

Amyloid P component (AP) is a heparin-binding serum protein, which is also found outside the circulation in basement membranes, connective tissue, and in all types of amyloid deposits [1–5]. Because of its largely unknown physiological function [6], its affinity for amyloid, and possible involvement in the pathogenesis of systemic lupus erythematosus [7], the structure and activity of AP are intensely studied [8–12]. In a search for functional peptides in the AP structure with homology to known cell attachment peptides, a control peptide representing residues 27–38 of AP (AP-1) was surprisingly found to support cell attachment [13]. The peptide was subsequently found to bind heparin and other anionic carbohydrates including DNA, with apparent dissociation constants in the 0.5–10 µM range based on capillary electrophoresis (CE) and solid-phase inhibition assays [14–16]. We found that the tryptic glycopeptide corresponding to amino acids 14–38 of AP glycopeptide binds heparin [16] and that the attached carbohydrate protects against proteolytic breakdown [17]. A dissociation constant of 1.5 µM was estimated for the interaction with low molecular mass heparin [18]. The amino-acid sequence of this heparin-binding peptide does not fit any of the classical known heparin binding consensus sequences which consist of clustered basic residues [19–21] nor does it seem to conform to the turn sequences seen in the fibroblast growth factor family [22]. Thus, it may represent a new type of heparin binding sequence. The current study was undertaken to confirm and extend the results of the original study that was solely based on CE performed at pH 2.5 [14] and to get data on the interaction kinetics and thermodynamics using independent micromethods. Surface plasmon resonance measurements [23] and isothermal titration calorimetry represent alternative methods for performing binding studies involving mixtures of low molecular mass reactants and low amounts of samples [24]. These methods are therefore used in the present study together with CE to measure the strength of the AP-1
peptide interaction with heparin, to evaluate the amino-acid sequence specificity of the binding, and to assess if a minimal heparin structure necessary for binding can be defined.

MATERIALS AND METHODS

Chemicals and materials

AP-(27–38)-peptide (H-EKPLQNFTLCFR-NH₂) (AP-1), scrambled AP-(27–38)peptide (H-TRLFPKECLNQF-NH₂) (scrAP-1), and the marker peptide (Ac-PSKDH-OH) (M) were obtained from Schafer-N (Copenhagen, Denmark). Streptavidin sensor chip, CM 5 chip, NaCl/Hepes buffer, and EDC/NHS were from Biacore (Uppsala, Sweden). Low molecular weight heparin (LMW heparin, molecular mass 5000 Da), sodium salt from porcine intestinal mucosa was from Calbiochem (La Jolla, CA, USA). Gycosaminoglycan heparin and semipurified heparin (heparin pentosanoglycan) were both sodium salts of porcine intestine in origin with ≈150 U/mg, an average molecular mass ≈2 000 Da and were obtained from Celsus Laboratories (Cincinnati, OH, USA). Oligosaccharides, AUpS (1→4)-α-d-GlcPα16S(1→4)-α-d-GlcPα16S(1→4)-α-d-GlcPα16S (where n = 1, 2, 3, 4, 5), corresponding to tetrasaccharide, hexasaccharide, octasaccharide, decaasacharide, octa- and decasaccharide and tetra-decasaccharide, and AUA is 4-deoxy-α-l-threo-hexopyranosyluronic acid), were prepared from bovine lung heparin by controlled enzymatic depolymerization with heparin lyase I and purified to homogeneity as previously described [25].

Protection of peptides and characterization by MS

AP-1 peptides were S-pyridylated to protect their Cys residue; 1 mM peptide in 10 mL 0.1% (v/v) aqueous trifluoroacetic acid (Fluka, Buchs, Switzerland) was incubated with 2 mL 4,4'-dithiopyridine (Aldrich, Steinheim, Germany) by stirring at 37 °C for 1 h. The reaction product was purified by reversed phase HPLC on a preparative C18 column (22 × 250 mm, 10 μm particle size) from Vydac (Hesperia, CA, USA). A flow rate of 10 mL min⁻¹ and a 20-min gradient of 20-45% acetonitrile in 0.1% trifluoroacetic acid were used and the reaction product was identified through its distinct 280 nm absorbance that the unmodified peptides lack. An S-carboxymidomethylated AP-1 peptide was prepared in the same way by reacting 1.3 mM peptide in dilute NaCl/P seasoning with 24 mM iodoacetamide for 1 h at room temperature. The collected material was lyophilized and the peptide masses were subsequently verified by mass spectrometry on a Mariner ESI-TOF mass spectrometer equipped with a standard ion source (PerSeptive Biosystems, Framingham, MA, USA). For N-terminal sequencing, the peptides were dissolved in 30% acetonitrile, 0.2% formic acid and fragmented by collision induced dissociation (CID). The settings were: Skimmer 1 potential 50 V, quadrupole DC potential 10 V, and nozzle potential 400 V.

CE and CE-based binding assays

CE was performed on a Beckman P/ACE 2050 instrument. Electrophoresis buffer was 0.1 M sodium phosphate, pH 7.46. Detection was by UV-absorbance at 200 nm and the separation path was a 50-μm inner diameter uncoated fused silica capillary with 50 cm to the detector window and of 57 cm total length. Separations were carried out at 17 or 18 kV corresponding to a current of approximately 85 μA at a capillary temperature thermostating of 20°C. The capillary was rinsed after electrophoresis for 1 min with each of the following: 0.1 M NaOH, water, and electrophoresis buffer. Samples were pressure injected for 8 after a 2.5-min injection of water and were subjected to electrophoresis from a separate set of buffer vials than those used for prerinse. The AP-1 peptide preparation used for the CE experiments was found to contain a mixture of regular AP-1 and modified (dehydrated, see below) AP-1 (mAP-1) while scrAP-1 was homogenous. When indicated, the unmodified AP-1 peptide was purified to homogeneity by RP-HPLC using a C18 column and a 0–70% gradient of acetonitrile in 0.1% trifluoroacetic acid for 20 min at 1 mL min⁻¹. The collected material from 150 μL of 0.5 mg·mL⁻¹ peptide was dried down in a centrifugal vacuum evaporator and resolubilized in 50 μL of water. Peptide concentration was estimated by CE based on a standard curve of peak areas vs. peptide concentration using the starting AP-1 preparation with a known total peptide concentration and known distribution between mAP-1 and AP-1 (1:0.9 based on peak areas). Correlation coefficients (r²) of standard curves were >0.98. Binding assays were performed by analysis of preincubated mixtures of various concentrations of the peptides (including a marker peptide) with a constant concentration of LMW heparin. Preliminary experiments were carried out with mixtures of the scrambled and the unpurified AP-1 preparation in 10 mM sodium phosphate, pH 7.46 with or without added LMW heparin. Subsequent binding experiments were carried out by diluting a stock peptide solution at various concentrations in the 10 mM phosphate or in water and then split it into two 5-μL samples. To one sample 2 μL water was added, and to the other 2 μL LMW heparin (0.01 mg·mL⁻¹ in water) corresponding to a final heparin concentration of 0.4 μM and total peptide concentrations covering the range of 10–30 μM. Samples were incubated overnight at 4 °C and then analyzed by CE. Peptide peak areas in the incubations with heparin (representing free, unbound peptide) were converted to concentrations using the peptide standard curve and subtracted from the total concentration of peptide present in each incubation to get the amount of bound peptide. The internal marker (non-reacting peptide) was used to ensure that each set of incubations were similarly diluted and in case the marker deviated from its calculated peak area value, the total peptide concentration was adjusted accordingly.

Surface plasmon resonance (SPR) measurements

The SPR measurements were performed on a Biacore 2000 instrument operated using BIA evaluation v3.2 software. Buffers were filtered and deoxygenated and all experiments were carried out at 25 °C.

Immobilization of regular and scrambled AP peptides on carboxymethylated chips

Regular and scrambled AP-1 peptides were coupled to carboxymethylated chips (CM5 chips) as follows: the CM5 chip was activated using an injection pulse of an equimolar
mix of NHS and N-ethyl-N-(dimethylaminopropyl)carbo-
dimide (EDC) (35 μL, 5 μL/min⁻¹, final concentration
0.05 M, mixed immediately prior to injection). The surface
was then cleaned with the extraclean step available in the
instrument. The 80-μL peptide solution was then injected
manually (100 μg/mL into 3 mM citrate). Four different
pH values (4.5, 5.5, 5.5, and 6) were examined. A pH of 4.5
resulted in the immobilization of the greatest amount of
regular and scrambled AP-1 peptides. Excess unreacted sites
on the sensor surface were blocked with a 35-μL injection of
1 mM ethanolamine and the surface was again cleaned with
the extraclean program. Glycosaminoglycan heparin samples
for binding studies were prepared in 10 mM sodium
phosphate buffer, pH 7.40. Samples (15 μL) were injected
into each cell at a flow rate of 5 μL/min⁻¹ (over a
concentration range of 10–300 μM). At the end of each
cell injection, the same buffer was continuously flowed
over the sensor surface to monitor dissociation. At the end
of each run, the sensor surface was regenerated by injecting
a 10-μL pulse of 2 mM NaCl followed by reinstituting flow
with sodium phosphate buffer and the next test compound
while recording sensorgrams. Kinetic parameters were
evaluated using the BIOLINEvaluation software (ver. 3.0.2,
1999). Signals from control cells were subtracted from the
signal generated by the flow cell containing immobilized
AP-1 peptides.

Isothermal titration calorimetry (ITC)

ITC was performed on a model 4209 microtitration
calorimeter (Hart Scientific, Pleasant Grove, UT, USA).
The interaction between regular and scrambled AP-1 with
glycosaminoglycan heparin was measured. Heparin
(1.3 mM of 50 μM) was titrated with 25 10-μL injections
of peptide (1.5 mM). The samples were in 10 mM sodium
phosphate buffer at pH 6. The experiments were per-
formed at 25 °C using an external water bath. Peak
areas were analyzed using the BINDWORKS (version 1)
program from Applied Thermodynamics. The experi-
mental procedures and data fitting have been previously
described [24].

RESULTS

Heparin binding of AP-1 peptides characterized by CE

By using CE, it was possible to separate the sequence-
scrambled version of AP-1 from the regular version at a pH
of 7.46 (Fig. 1). In addition, with this particular preparation
of regular AP-1, we observed a modified form of AP-1
(mAP-1), represented by the first peak in the electrophore-
Gram. Mass spectrometry showed that the preparation
contained approximately equal amounts of two com-
ponents with monoisotopic masses of 1603.13 and 1585.34.
The mass of 1603.13 is in agreement with the theoretical
monoisotopic mass of 1602.78 for AP-1. By amino-acid
sequencing using MS, we found that the mass deficit of
approximately 18 in the modified peptide corresponds to the
first two residues (results not shown). This is compatible
with N-terminal Glu and Lys side chains cyclizing and
losing water in the process. The MS-observed fragmen-
tation pattern of the two N-terminal amino-acid residues of
mAP-1 supported this notion (data not shown).

Heparin reactivity was subsequently assessed by adding
heparin (200 μM) to the electrophoresis buffer (Fig. 1B).
Both the regular AP-1 peptide and the N-terminally
modified form (both at ≈75 μM in the sample) disappeared
from the peak profile, i.e. they both bind heparin while the
scrambled AP-1 peptide (156 μM in the sample) was not
affected. The binding peptides complex with the highly
negatively charged heparin and therefore migrate much
slower than nonbound peptides. A defined complex peak
was not seen. This indicates that the peptide-heparin
complexes dissociate during the time of the experiment.
However, the interaction is not so weak that a dynamic
equilibrium exists during the analysis allowing all molecules
to spend the same fraction of time in complexes (which
would give regular shifts in peak positions; see reviews on
the theory of affinity electrophoresis [26]). An additional
reason for not obtaining one defined complex peak may be
that the population of binding sites on heparin is hetero-
geneous.

A direct binding assay was then carried out using
preincubated samples and CE to simultaneously separate
the three peptides and quantify the amount of free peptide
at different concentrations. This is a different set-up than the
one shown in Fig. 1 because samples are pre-equilibrated.
The analyses showed peaks representing the fraction of
Characterization of a novel heparin-binding peptide (Eur. J. Biochem. 269) 2863

Heparin-binding AP-1 peptides that are not bound depending on the total peptide concentration in the equilibrium mixture. The data in Fig. 2 are the peptide peak areas in the sample mixture at different peptide concentrations incubated with a constant amount of LMW heparin or water (controls). Thus, the differences in the positions of the fitted straight lines are indicative of the amount of bound peptide. It is clear that binding of heparin by the scrambled peptide is very low while there is a robust binding by the two other peptides as also suggested by the preliminary assay (Fig. 1). However, the variability of the measurements leading to relative standard deviations in excess of 25% in repeated experiments makes reliable binding constant estimates difficult (Fig. 3). By performing the experiments with purified AP-1 and by incubating the samples in the absence of salt (water instead of phosphate buffer), we obtained less scattered data, as illustrated by the data points (asterisks) in Fig. 3 that were obtained using 0.4 μM heparin. When fitting single-site binding hyperbolas, Kₐ values around 1 μM were obtained for the less precise data obtained with the peptide mixture in dilute phosphate. With the 0.4 μM heparin data obtained in water and using purified AP-1, a better curve fit (r² > 0.98) was obtained that gave a Kₐ value of 16 μM, which is in good agreement with data previously obtained using pH 2.5 citrate as the electrophoresis buffer [14]. While we can conclude that the mAP-1 does not appear to bind heparin differently from AP-1 and that no binding can be detected with the scrambled AP-1 peptide (despite the fact that this peptide has the same amino-acid composition as AP-1), the data cover only a limited part of the binding curve because of peptide solubility problems and because of the limits of detection offered by UV-based detection in CE. The data therefore required support from independent micromethods for binding assays such as SPR and ITC.

**SPR measurements of peptide–heparin interaction**

A strong nonspecific binding between streptavidin surface (control cell) and AP-peptides precluded useful SPR results from experiments where AP-1 peptides were flowed over a biotinylated heparin surface. The Kₐmax, observed in the

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**Fig. 2.** CE-based quantitative binding assays of preincubated heparin-peptide samples demonstrate that AP-1 and mAP-1 peptides bind heparin while the scrambled AP-1 does not. Shown are peak area values for each of the free (unbound) peptides in the mixture after incubation with water (circles) or with LMW heparin (filled circles) as a function of the total peptide concentration. A stock solution consisting of 125 μM scrambled peptide and 62 μM of each of the AP-1 and the mAP-1 peptides and 41 μM of the marker peptide in 10 mM phosphate, pH 7.46 was further diluted in 2 x 20-μL aliquots and to one aliquot was added 4 μL LMW heparin (final concentration: 3.2 μg) while the other received the same volume of water. After incubating overnight at 4 °C the samples were analyzed by CE at 17 kV.

**Fig. 3.** CE-derived binding curves and binding constant estimates. Shown are bound peptide as a function of free peptide for sets of experiments performed at different constant LMW heparin concentrations (circles, 1.6 μM heparin; diamonds, 0.8 μM heparin; and asterisks, 0.4 μM heparin). Also, the experiment at 0.4 μM heparin was performed with purified AP-1 alone and took place in the absence of phosphate buffer while the 1.6 and 0.8 μM data are from experiments performed with a mixture of the three peptides in the presence of 10 mM phosphate, pH 7.46.
sensogram of the control cell without heparin was higher than in the rest of the flow cells in these experiments, indicating a peptide-streptavidin interaction (data not shown). Instead of immobilizing the heparin ligand, we therefore immobilized the peptides through amine coupling. The peptide preparations used for these experiments were homogeneous as verified by mass spectrometry (data not shown). The successful immobilization of AP peptides was confirmed by the observation of a 2500 resonance unit (RU) increase in the chip for regular peptide and 3200 RU for the scrambled.

Sensograms for the binding of heparin to immobilized regular and scrambled AP-1 peptides are shown in Fig. 4. The initial part of these curves represents a buffer flowing past the sensor surface. The second and rising part of the curves correspond to the response of the sensor surface observed as a sample injection volume flows past the immobilized peptides. The final portion of the curves correspond to the dissociation of bound heparin after the sample volume has passed the sensor chip and empty buffer flows past the sensor surface again. The observed rate constant, $k_{obs}$, was evaluated from each trace and used to calculate the association rate constant ($k_a$). A plot of $k_a$ as a function of ligand concentration yields a slope of the association rate constant, $k_a$. The ratio $k_{off}/k_{on}$ generates the overall dissociation constant ($K_d$).

Kinetic analysis of the interaction between regular AP-1 peptide and heparin, afforded a $k_{off}/k_{on} = 1.4 \times 10^{-3}$ s$^{-1}$ for $K_d$ of 1.6 $\mu$m. Identical calculations for scrambled AP-1 peptide and heparin yielded a $k_{off}/k_{on} = 1.9 \times 10^{-2}$ s$^{-1}$ for $K_d$ of 38 $\mu$m. The binding of regular peptide is over 20-fold stronger than scrambled peptide. Thus, the data agree with the CE analyses with respect to the difference in binding between the regular and scrambled AP-1 and shows that even in the event of blocking of amino groups on the peptide (through the immobilization) the binding is strong.

**SPR measurements of peptide interaction with sized oligosaccharides**

The approach used for measuring the binding of heparin was also used in an attempt to define a minimal binding structure of heparin using sized oligosaccharides. Homogeneous, structurally defined heparin oligosaccharides of the general formula: \( \Delta UAp2S \) (1-4)\( \alpha \)-GlcNp6S6S(1-4)\( \alpha \)-IdoAp2S(1-4L)\( \alpha \)-GlcNp6S6S, where $n = 1$, tetrasaccharide (X2); $n = 2$, hexasaccharide (X3); $n = 3$, octasaccharide (X4); $n = 4$, decasaccharide (X5); $n = 5$, dodecasaccharide (X6); $n = 6$, tetradecasaccharide (X7) were used.

Sensograms for the binding of immobilized regular AP-1 peptide to heparin oligosaccharides are shown in Fig. 5. The ratio $k_{off}/k_{on}$ was again used to calculate the dissociation constant ($K_d$) from each trace (Table 1). With the scrambled peptide on the surface the oligosaccharides gave a much weaker signal and no $K_d$ could be calculated from the weak binding that was observed (Fig. 5B).

With the exception of the tetrasaccharide, which showed relatively weak binding, little molecular mass dependence was observed for the interactions with the regular AP-1 peptide. However, all of the heparin oligosaccharides bound slightly weaker to the regular AP-1 peptide than in the SPR experiments with full size heparin (Fig. 4). The main difference in the binding of AP-1 to oligosaccharides vs. intact heparin appears to be a 5–10 times slower off-rate for intact heparin. This may be the result of multivalent interactions between a single heparin chain and multiple AP-1 peptides on the surface of the biosensor chip that are not possible in the case of the smaller heparin oligosaccharides. This multipoint attachment will slow the off rate as observed. The peptide sequence specificity of the binding is clearly illustrated by the absence of a measurable interaction with the scrambled peptide. For the regular AP-1 peptide, the data agree with the results of the CE measurements by yielding 2–10 $\mu$m $K_d$ values.

**ITC measurements**

ITC directly measures the heat released (or absorbed) from ligand binding to a macromolecule. The titration curves obtained showed considerable noise, possibly due to precipitation of the peptide-carbohydrate complex at the high concentrations required for these measurements (data not shown). Despite these problems, $K_d$ values of $2 \times 10^{-3}$ and $1 \times 10^{-2}$ M were calculated for regular AP-1 and scrambled AP-1, respectively. These values again demonstrate the sequence specificity of the interaction even
DISCUSSION

The current study confirms and extends our initial observation using affinity CE [14] by applying two additional biophysical techniques to confirm the specificity of this interaction with regards to peptide sequence. Furthermore, our study uses structurally defined heparin oligosaccharides to establish that while the tetrascarbohydrate is too small to exhibit strong binding, little difference in binding strength is observed between hexa- and tetradeeca-saccharides. Also, the CE experiments demonstrate the novel finding that the N-terminal portion of the peptide may be partly modified without apparent effect on heparin-binding capabilities. The $K_d$ values for AP-1 peptide binding to heparin measured by the ITC method differ significantly from the SPR and CE measurements. These differences might result from the differences in experimental conditions associated with each measurement [25]. Measurement of $K_d$ values are very method dependent [27], SPR values involve the immobilization of one of the binding partners, i.e. it is a two-phase measurement. While affinity CE and ITC are both solution phase binding assays; a reduced pH value was required in ITC to maintain peptide solubility at the high concentrations required in this method to observe measurable heats of binding. The difference in pH is expected to markedly affect the $K_d$ value measured. A large difference between ITC and SPR binding constant measurements has also been reported on heparin’s interaction with heparin-binding growth-associated molecule (HB-GAM) [28]. In the present study, it is important to point out that in each type of measurement, the natural peptide is being compared to the scrambled sequence peptide under identical conditions. In all cases, the natural peptide binds over 10-fold tighter than the scrambled sequence peptide. SPR demonstrated that an oligosaccharide length of greater than a tetrascarbohydrate size gave optimal binding. It is interesting to note that practically no interaction of sequence scrambled AP-1 peptide to heparin oligosaccharides could be measured using SPR, suggesting that this is indeed a sequence-specific interaction, in agreement with the CE measurements of the affinity. The current experiments also support the CE-based estimates of binding constants [14], the importance of the C-terminus [16], and the dependency of binding on the presence of an S-S bridge [18]. Because separation and binding assessment take place in one operation in CE, a mixture of three peptides could be evaluated directly for binding while the purified components had to be used in the SPR and ITC approaches.

Model simulations of the AP-1 peptide structure show that a random coil or $\beta$ sheet form are more favored. A search for this sequence by using BLAST (Basic Local Alignment Search Tool) at NCBI (National Center for Biotechnology Information) shows that it is only found within serum amyloid P protein (SAP) [11]. This sequence exists in a random L-shaped conformation in which all the basic residues (i.e. Lys28 and Arg38) and hydrogen-bonding residues (i.e. Gin31 and Asn32) lie on the same face (Fig. 6). The only known heparin-binding motif [29,30] that this sequence conforms weakly to is the one proposed by Margalit et al. [21], where a critical distance of $\sim 20 \AA$ between basic amino acids is proposed to be important for interactions with heparin, irrespective of whether the site folds into an $\alpha$ helix or a $\beta$ sheet.

![Fig. 5. SPR sensorgram of regular (A) and scrambled (B) AP-1 peptide-heparin oligosaccharides interaction. The oligosaccharides shown are: (X2) tetrascarbohydrate (X3) hexascarohdrate (X4) octascarbohydrate (X5) decaascarbohydrate (X6) dodecaascarohdrate, and (X7) tetradecaascarohdrate. Kinetic values calculated from the experiments with the regular peptide are listed in Table 1.](image)

![Table 1. Kinetic measurement of the binding of heparin oligosaccharides to the AP-1 peptide immobilized on the surface of an SPR chip (see Fig. 5).](table)

<table>
<thead>
<tr>
<th>Heparin oligosaccharide</th>
<th>$k_{on}$ (m$^{-1}$s$^{-1}$)</th>
<th>$k_{off}$ (s$^{-1}$)</th>
<th>$K_d$ (μM)</th>
<th>$R_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrasaccharide</td>
<td>$0.5 \times 10^3$</td>
<td>$17 \times 10^{-3}$</td>
<td>34</td>
<td>75</td>
</tr>
<tr>
<td>Hexasaccharide</td>
<td>$1.6 \times 10^3$</td>
<td>$7 \times 10^{-3}$</td>
<td>4.4</td>
<td>200</td>
</tr>
<tr>
<td>Octasaccharide</td>
<td>$1.6 \times 10^3$</td>
<td>$18 \times 10^{-3}$</td>
<td>11</td>
<td>250</td>
</tr>
<tr>
<td>Decasaccharide</td>
<td>$1.7 \times 10^3$</td>
<td>$7 \times 10^{-3}$</td>
<td>4.1</td>
<td>240</td>
</tr>
<tr>
<td>Dodecasaccharide</td>
<td>$1.4 \times 10^3$</td>
<td>$6.8 \times 10^{-3}$</td>
<td>4.8</td>
<td>250</td>
</tr>
<tr>
<td>Tetradecasaccharide</td>
<td>$2.9 \times 10^3$</td>
<td>$6.0 \times 10^{-3}$</td>
<td>2.1</td>
<td>250</td>
</tr>
</tbody>
</table>
A measurement of the distance between the Lys and Arg in AP-1 shows that they are ≈27 Å apart while the same residues are separated by 11 Å in the random conformation of the scrambled peptide. Modeling studies on SAP (Fig. 6) suggest that the N-terminal portion of the AP-1 peptide [EKPLQN (27–32)] is surface exposed and is the most likely portion of the peptide sequence to be involved in heparin binding. In contrast, peptide studies clearly demonstrate that the C-terminus [FTLCFRR (33–38)] is the most important portion of AP-1 for heparin binding [16,17]. Additional studies will be required to understand whether a conformational change, resulting from Ca²⁺ binding to SAP, may explain the differences between experimental results on the AP-1 peptide and modeling studies on SAP.

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REFERENCES


