Structural Characterization of the E2 Domain of APL-1, a Caenorhabditis elegans Homolog of Human Amyloid Precursor Protein, and Its Heparin Binding Site*

The amyloid β-peptide deposit found in the brain tissue of patients with Alzheimer disease is derived from a large heparin-binding protein precursor APP. The biological function of APP and its homologs is not precisely known. Here we report the x-ray structure of the E2 domain of APL-1, an APP homolog in Caenorhabditis elegans, and compare it to the human APP structure. We also describe the structure of APL-1 E2 in complex with sucrose octasulfate, a highly negatively charged disaccharide, which reveals an unexpected binding pocket between the two halves of E2. Based on the crystal structure, we are able to map, using site-directed mutagenesis, a surface groove on E2 to which heparin may bind. Our biochemical data also indicate that the affinity of E2 for heparin is influenced by pH: at pH 5, the binding appears to be much stronger than that at neutral pH. This property is likely caused by histidine residues in the vicinity of the mapped heparin binding site and could be important for the proposed adhesive function of APL-1.

Amyloid precursor protein (APP) belongs to a gene family that in mammals includes two additional members, APLP1 and APLP2 (1–3). This gene family is important for viability because mice lacking all three members die shortly after birth (4). The function of APP and APP-like proteins and why their loss causes death are, however, not clear. Rare mutations in the human APP gene are also known to cause familial Alzheimer disease (for a review, see Ref. 5). The nematode Caenorhabditis elegans has a single APP-related gene, apl-1 (6). Inactivation of apl-1 prevents proper molting, a process that allows the worm to shed old cuticles between larval stages, and causes several other morphological defects (7).

APP and APP-related proteins are type I membrane proteins. The majority of the full-length protein resides on the extracellular side of the membrane (Fig. 1A), and contains two conserved domains (E1, E2). E1 and E2 are retained in soluble forms of the protein, which are proteolytically released from the membrane by secretases. So far neither the primary sequence nor the three-dimensional structure of these two conserved domains has revealed any homology that would allow a convincing assignment of biological function to APP (8–11). There are, however, a number of experimental observations suggesting that the APP family of proteins might play a role in regulating cell adhesion. For example, most triple knockout mice developed cortical dysplasias, a condition that could result from defects in the adhesion of migrating neurons to extracellular matrix (4). Overexpression of apl-1 in the worm caused organ detachment (7), and transgenic flies expressing human APP developed a blistered-wing phenotype (12). Furthermore, in cell cultures, APP appears to concentrate to sites of adhesion (13–15).

The structure of human E2/CAPPD has been solved by x-ray crystallography and NMR (10, 11, 22). In the x-ray structure, which contains the entire E2 region, the molecule forms a head-to-tail dimer (10). This observation has led to the suggestion that E2 might mediate homophilic interactions among APP molecules. F-spondin, a secreted protein involved in neuronal development and repair, also appears to interact directly with E2 (16). Besides participating in these potentially important protein-protein interactions, E2 binds heparan sulfate proteoglycans (HSPGs), which are abundant both inside the cell and in extracellular matrix. Although E1 also binds heparin, E2 appears to have a higher binding affinity (17).

How APP interacts with HSPG, and whether this interaction is important for its proposed function in regulating cell adhesion, are currently unknown. Here we use a combination of crystallographic and biochemical techniques to characterize the heparin binding site on the E2 domain of the worm homolog APL-1.

EXPERIMENTAL PROCEDURES

Reagents—The sodium salt of sucrose octasulfate (SOS) was obtained from Toronto Research Chemicals (Canada). All

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‡ The atomic coordinates and structure factors (codes 3K66 and 3K6B) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The abbreviations used are: APP, amyloid precursor protein; PDB, Protein Data Bank; r.m.s., root mean square; PEG, polyethylene glycol; HSPG, heparan sulfate proteoglycan; SOS, sucrose octasulfate; GFP, green fluorescent protein; WT, wild type.
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other reagents were obtained from common vendors and were of the highest available grade.

**APL-1 E2 Constructs and Protein Purification**—Based on the human E2 structure and sequence alignment (10), we initially designed six constructs that differed only by several residues at both N and C termini. After subcloning into pET28a (Novagen), the proteins were overexpressed in BL21(DE3) cells (Stratagene). Unfortunately, crystals derived from these constructs were either twinned in morphology, or of low resolution (8 Å). Re-analysis of APL-1 sequence suggested that its most C-terminal helix (αF) might be longer and that sequence downstream of αF might form additional secondary structures. Thus a longer construct (APL-1<sub>134–472</sub>) was made, ligated into pET28a in-frame with an N-terminal His tag, and overexpressed in BL21(DE3). The His-tagged recombinant protein was purified using a Ni-nitrilotriacetic acid column (Invitrogen). After cleaving off the His tag by thrombin, the protein was concentrated and passed through a Superdex G-200 size exclusion column (Amersham Biosciences) to remove aggregates. The longer construct eventually produced suitable crystals for diffraction analysis. All mutants of E2 used in this study were based on the APL-1<sub>134–472</sub> construct and generated by site-directed mutagenesis using the QuikChange kit (Stratagene).

**Crystallization, X-ray Diffraction Data Collection, and Structural Determination**—All initial crystallization screens were performed in sitting drops using 96-well Hampton crystallization trays. The best crystals were obtained from construct APL-1<sub>134–472</sub> at room temperature: 1 μl of protein solution (20 mg/ml E2 or Se-Met-substituted E2, 20 mM Tris, pH 7.0, 0.5 mM NaCl) was mixed with 1 μl of freshly prepared well solution (20% PEG 3350, 15% isopropyl alcohol, 100 mM HEPES pH 7.5), and equilibrated with the same well solution in the dark; rod-like and needle-like crystals typically formed within 3 days. They were cryo-protected in 25% ethylene glycol before freezing. The final data set used for phasing were merged from 10 mm HEPES, 0.5 mM NaCl, 1 mM EDTA, pH 7.0 at a flow rate of 0.5 ml/min. Weight-average molecular weight was determined using ASTRA software as described (46) that used a Rayleigh-Debye-Gans light scattering modeling (see Equation 1), which related the amount of scattered light to the concentration and weight average molecular weight of solute and second virial coefficient,

\[
K^*c/R(\theta) = 1/M_w + 2A_2c
\]  
(Eq 1)

where \(R(\theta)\) is the intensity of excess scattered light at an angle \(\theta\), \(c\) is the concentration of the solute, \(M_w\) is the weight average molecular weight of the solute, \(A_2\) is the second virial coefficient, \(K^*\) is an optical parameter equal to \(4\pi n^2(n/dn/dc)^2/\lambda_0N_A\), \(n\) is the refractive index, \(dn/dc\) is the refractive index increment for the solute, \(N_A\) is Avogadro’s number, and \(\lambda_0\) is the wavelength of the scattered light.

The hydrodynamic radius \(R_h\) was measured on a DynaPro dynamic light scattering detector in batch mode at 1 mg/ml (Wyatt Corp.). The intensity correlation function was analyzed by the method of cumulants (38) and regularization (39) to extract \(R_h\).

**Protein Stability**—The thermal stability test was performed on an AVIV Model 215 CD spectrometer. The protein sample was kept in phosphate-buffered saline buffer at a concentration of 0.3 mg/ml. Protein unfolding was monitored by a loss of rotation of polarized light at 222 nm upon heating (4 to 95 °C with one-degree increment and 6-min equilibration time). To ensure formation of an intramolecular disulfide bond in E371K/D342C/S362C, the purified mutant protein was diluted to 10 μg/ml with ice-cold phosphate buffer containing 500 mM NaCl and 2 mM mercaptoethanol. 1.4 mM reduced and 0.7 mM oxidized glutathione, and then 5% PEG 600, were added. Air was bubbled through a sparger into the protein solution for 2 days at 4 °C. The oxidized protein was repurified by a Ni<sup>2+</sup> column.
Construction and Microinjection of Plasmids to Generate Transgenic Worms—To generate a plasmid containing the \( \text{apl-1(yn32)} \) E371K mutation, DNA from \( \text{yn32} \) animals were amplified with primers APP22 (5'-H11032-AAGCAGTGCAAGACCAAG-AAG-3'/H11032); the product was digested with SgrA1 and SphI (New England Biolabs) and subcloned into APL-1HSGFP (7). To generate a plasmid containing the \( \text{yn32} \) E71K/D342C/S362C triple mutations, we digested the pET28a construct containing these mutations with BamHI and SphI and subcloned the fragment into APL-1HS to generate APL-1(E71K/D342C/S362C) (7); the APL-1(E71K/D342C/S362C) construct was digested with BglII and SacII, and subcloned into the APL-1HSGFP construct to generate APL-1(E71K/D342C/S362C)::GFP (7). Constructs were microinjected at 50 ng/\( \mu \)l as described (40). Extrachromosomal arrays were integrated into the genome by UV irradiation (Stratalinker; 300 J). Confocal images were taken on a Zeiss LSM 710.

Western Blots—Approximately 500 \( \mu \)l of packed worms were lysed for each strain, and extracts were electrophoresed and Western blotted as described (7): blots were incubated with a 1:5000 dilution of anti-APL-1EXT antisera and immunodetected; to ensure equal loading, total protein amount for the extracts was determined by a Bradford assay. The protein amounts were also confirmed by gel staining with Coomassie Blue; in addition, blots were subsequently stripped and reprobed with 1:500 anti-actin antisera (Sigma) (data not shown).

Heparin Binding—Various E2 mutants were loaded onto a 1-ml heparin column (Amersham Biosciences) mounted on the AKTA FPLC system. The binding buffer was either 20 mM sodium acetate (pH 5.0) or 20 mM Tris-HCl (pH 7.0), and the elution buffers contained additional 2 M or 1 M NaCl, respectively. The amounts of salt required to elute the protein were recorded in S/m \( ^{-1} \) by the internal conductivity probe, and later converted to molar concentrations for ease of comparison.

PDB Accession Numbers—The coordinates and structure factors have been deposited in the PDB with the following accession numbers: 3K66 and 3K6B.
RESULTS

Structure of the APL-1 E2 Domain—Because our initial attempt to solve the structure by molecular replacement using the human E2 coordinates failed, we resorted to Se-Met-substituted protein to solve the phase problem (Table 1). The 3.3 Å resolution experimental map derived from SAD phases was clear enough for us to trace the majority of the polypeptide backbone. Missing regions, primarily loops between helices, and most of the side chains were added later to the model when the calculated phases were improved and used to generate maps.

The *C. elegans* E2 is 34% identical in sequence to the human E2 (Fig. 1B). Therefore, as expected, the two structures appear similar (Fig. 2). The most prominent feature of E2 is a long α-helix (αC) that spans the entire length of the molecule, connecting its two halves (supplemental Fig. S1): the N-terminal portion of αC forms an anti-parallel coiled-coil with αB that constitutes one structural subdomain, and the C-terminal half of αC forms a four-helix bundle with αD, αE, and αF that constitutes the other subdomain. Near the center of the molecule, there is a short cleft formed between αB and αD, where a number of conserved and basic residues are clustered.

When the worm and human E2 structures were superimposed, it became apparent that the angle between the two subdomains had changed. To facilitate the comparison between the two structures, we divided E2 in the middle of αC at residue 327 (441 for APP751). Previous proteolytic analysis of human APP indicated that this region of αC could partially unfold and become cleavable by trypsin (11). The N-terminal subdomains were superimposable with an r.m.s. deviation of 0.89 Å for 62 Ca atoms from helices αB (APL-1 residues 249–278) and αC (residues 295–326) (Fig. 2A). The C-terminal subdomains were superimposable with an r.m.s. deviation of 1.10 Å (1.71 Å when compared with the NMR structure; Ref. 11) for 75 Ca atoms from helices αC (APL-1 residues 327–347), αD (residues 358–384), and αE (residues 393–419) (Fig. 2B). For APL-1, the electron density features were clearer in the turn between αD and αE, and at the C terminus, where two helical turns and a stretch of extended structure were added to the short αF observed in human APP (green in supplemental Fig. S1). This addition shows that the C terminus of E2 folds back to project the juxtamembrane region toward the midportion of E2. Although in both APL-1 and APP the central helix αC is continuous, when superimposed on the C-terminal subdomain, the N-terminal half of APL-1 appears to have bent outwards by about 26 degrees (Fig. 2B). It is not yet clear whether this bending is due to sequence difference or results from crystal packing, or simply reflects intrinsic conformational flexibilities of the molecule. We have previously noticed that proteins with similarly arranged coiled-coil domains, such as spectrin and α-catenin, can also bend near the middle of a long connecting helix (18, 19).

APL-1 E2 Is Monomeric in Solution—E2 has a calculated molecular mass of 28.9 kDa. It eluted from the size-exclusion column with an apparent molecular mass around 50 kDa. This result may indicate that E2 could form dimers in solution (10). However, the elongated shape of the molecule renders this interpretation unreliable: for example, based on the crystal structure, the radius of gyration for a monomeric E2 was calculated to be around 26 Å (20), a value that corresponded roughly to that of a spherically shaped 50-kDa protein.

To accurately determine the oligomeric state of E2 in solution, we performed analytical ultracentrifugation and laser light scattering experiments (Fig. 3). A comparison between the sedimentation coefficient distributions at three E2 concentrations

![FIGURE 2. Structural comparison between APL-1 and APP.](image-url)
is shown in Fig. 3A. The van Holde-Weischet integral distribution plot showed identical sedimentation coefficient distributions for all three concentrations, suggesting that little or no oligomerization was occurring. To confirm the composition and identify the molecular weights, a global genetic algorithm/Monte Carlo analysis was performed (Fig. 3B), which showed a major species with a molecular mass of 31.9 (25.4, 33.9) kDa and a frictional ratio of 1.47 (1.39, 1.67) (in parenthesis: 95% confidence intervals). As expected for particles smaller than 1:20 of the incident laser wavelength (633 nm), the static scattering signal was independent of molecular shape and yielded an average molecular mass of 29.3 kDa for E2 (Fig. 3C). Dynamic light scattering measurement determined a hydrodynamic radius of 3.0 ± 0.3 nm, which for a molar mass of 29.3 kDa yielded frictional ratio of 1.46. Taken together, these biophysical measurements strongly suggested that E2 was monomeric in the solution. Further evidence about the oligomeric state of E2 was provided in the supplemental data and Fig. S2.

**The E371K Mutation Destabilizes E2 Structure**—We previously found a single mutation (E371K) within the E2 region of APL-1 that caused a lethal phenotype (incorrectly labeled previously as E372K (7)). Although the mutation could have affected ligand binding and the release of soluble forms of APL-1 from the membrane by secretases (11, 16), a shortened construct of APL-1 bearing the E371K mutation that corresponded to secretase-cleaved protein (thus constitutively secreted) failed to rescue the lethal phenotype (data not shown).

It was initially difficult to rationalize the effect of E371K mutation on protein structure because the corresponding residue in APP (Gln-483) was exposed on the surface (10, 11). After solving the structure of APL-1, we found that Glu-371 was buried by the two turns of α-helix added to short αF mentioned above (green in supplemental Fig. S1). The carboxylate group of Glu-371 formed a salt bridge with Arg-439, and was hydrogen-bonded to Trp-435, both from the extended αF (Fig. 4A). Replacing Glu-371 with a positively charged lysine was expected to disrupt these interactions. To test whether E371K could have destabilized the protein structure, we compared thermal unfolding curves of the wild-type and mutant E2 proteins. As shown in Fig. 4B, the wild-type protein showed two transitions, which were likely caused by non-cooperative unfolding of the two subdomains (between which there were little interactions). The N-terminal subdomain was probably responsible for the first and lower temperature transition because it was composed mainly of only two α-helices. The E371K mutation, which occurred in the C-terminal subdomain (supplemental Fig. S1), affected the higher temperature transition, lowering it by about ten degrees.

In the transgenic animal, the mutant APL-1 protein accumulated to a very low level in intact worms (Fig. 4, C and D). This did not appear to be solely caused by the reduction of protein stability. At the temperature the worm was grown (20 °C), both wild-type and E371K E2s should have acquired a folded conformation (the mutant E2 unfolded around 42 °C). Based on the observation that insect APP homologs all appeared to have an intramolecular disulfide bond bridging αC and αD,4 we introduced a disulfide bond to the same location in an attempt to restore E2 stability (supplemental Fig. S1). The triple mutant (D342C/S362C/E371K), after oxidization, seemed to be as stable as the wild-type protein (Fig. 4B). Nonetheless, the full-length APL-1 bearing the triple mutation still failed to rescue the phenotype in the worm (0/3 rescued lines), suggesting that

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4 R. Lauffer, personal communication.
The altered conformation in E371K must have triggered another mechanism for its clearance in vivo.

The Structure of the E2-SOS Complex—How heparin interacts with E2 is not known. An earlier study used synthetic peptides to map the heparin binding site (17). The result could now be projected onto the known crystal structures, and suggested that helices αB and αD, located separately in the two subdomains, were probably both involved (Fig. 5A). Later mutagenesis experiment confirmed that residues on αD affected heparin binding (10).

The crystallization condition of human APP E2 (−2 M NaCl) had prevented binding of heparin in the preformed crystals, and hindered structural characterization of the E2-heparin complex (10). After obtaining APL-1 E2 crystals using PEG as the precipitant, we tried to soak SOS and various heparin polysaccharides into them. Difference Fourier analysis based on data obtained from an SOS-soaked crystal had revealed new electron density near the suspected heparin binding site, which might correspond to the bound ligand (supplemental Fig. S3). Limited by data quality (Table 1), the density lacked sufficient details to allow an unequivocal fit of the ligand. Nevertheless, the size and planar shape of the density suggested that it corresponded to one of the two sugar rings in SOS. We tentatively modeled the glucose moiety of SOS into the density because protrusions from the central planar region of the density seemed to originate from a six-membered ring structure (glucose) instead of five (fructose). According to this model, the axial (1→2) glycosidic bond would raise the disordered fructose moiety away from the protein surface, and the sulfate substituent on the 6-OH group of the glucose could reach the density observed near the N terminus of helix αB.

At least two features of the complex are noteworthy. First, the sulfated sugar was found near the midportion of the molecule, between the two structural subdomains of the density seemed to originate from a six-membered ring structure (glucose) instead of five (fructose). According to this model, the axial (1→2) glycosidic bond would raise the disordered fructose moiety away from the protein surface, and the sulfate substituent on the 6-OH group of the glucose could reach the density observed near the N terminus of helix αB.

The sulfated sugar was found near the midportion of the molecule, between the two structural subdomains (Fig. 5A). There was no density near the two conserved basic residues (Arg-368, Lys-372) previously found to affect heparin binding to human APP (10). These observations suggested that the intersubdomain region could constitute a major site for binding negatively charged ligands. Secondly, the new binding site did not resemble any known heparin binding sites in that it lacked lysine and arginine side chains (Fig. 5B) (for a review of protein structures that interact with heparin, see Ref. 21): instead, the negatively charged sulfate groups of SOS appeared to interact mainly with the side chains of Asn-246 and His-376; the sulfate group on C6...
also appeared to form hydrogen bonds with backbone amide groups near the N terminus of αB and was probably stabilized by the dipole moment of this helix.

**Mutagenesis Mapping of the Heparin Binding Site**—The relevance of the newly identified intersubdomain site in heparin binding was tested by mutagenesis (Table 2). At neutral pH, the wild-type E2 was eluted from a heparin column by 0.46 mM NaCl. When the two conserved basic residues on αD (Arg-368, Lys-372) were substituted by alanines, the protein could be eluted from the same column by 0.39 mM salt, reinforcing the earlier hypothesis that these two residues were involved in heparin binding (10). Nevertheless, when Asn-246 was singly replaced by alanine, the protein affinity for heparin was reduced further. This was unexpected, because it was generally believed that charge-charge interactions played a dominant role in protein-heparin interactions (21); the N246A mutation did not modify the charge of the protein, whereas the R368A/K372A double mutation removed two positive charges. The reduced binding of the N246A mutant could result either directly from the loss of a critically important side chain as suggested by Fig. 5B, or indirectly from a conformational change in the loop that harbored the N246A mutation. In either case, the mutagenesis data confirmed the relevance of the observed SOS site in heparin binding, and showed that, in addition to αD in the C-terminal subdomain, residues near the N terminus of αB in the N-terminal subdomain were also involved (Fig. 5B). We have examined the effect of the mutations at a lower pH (see below), and a similar trend was observed (Table 2).

Besides Asn-246, we have also mutated His-248 and His-376 to alanines. In our model, the side chain of His-248 was hydrogen-bonded to the ring oxygen (O5) of the sugar (Fig. 5B). Like N246A, the H248A mutation also reduced heparin binding quite significantly, whereas H376A only had a moderate effect. The bigger effect of the H248A mutation further supported the importance of the protein structure in the N-terminal subdomain of E2 that constituted the “right-hand” side of the SOS-binding pocket as shown in Fig. 5.

We made another mutation, H248P, to assess the contribution of the backbone amide group to heparin binding. The binding affinity of H248P was similar to that of H248A, suggesting that, after deleting the histidine side chain, the additional removal of a backbone amide group did not appear to further reduce binding.

**Binding between E2 and Heparin Is pH-dependent**—We measured the binding of APL-1 E2 to heparin under two pH conditions (Fig. 6). At neutral pH, the protein could be eluted from the heparin column by about a half-molar salt, a result similar to those obtained previously using human APP E2 under slightly higher pH conditions (pH 7.4–7.8) (10, 17). Nevertheless, when the pH was lowered to 5, a salt concentration at least 3× higher was required to elute the protein, suggesting that the binding between E2 and heparin had become stronger. The difference was significant, and surprising, because most proteins utilized lysines and arginines to interact with the negatively charged sugar, and protonation states of these two residues did not change when pH was lowered from 7 to 5 (titration of sulfate and carboxylate groups on the heparin also did not occur here, and their protonation was expected to weaken the binding). The only chemical group that might change its charged state in this pH range, and potentially strengthen the binding, was the side chain of histidines. Therefore, our binding experiment raised the possibility that E2 heparin binding site contained histidine residues, and that protonated histidines might play an important role in stabilizing the heparin complex (another possibility was that pH triggered a conformational change in the protein). Of the 34 conserved residues in the E2 region of APP and APP-like proteins, four were histidines (His-248, His-324, His-376, and His-383; see Ref. 10). They were all located adjacent to the SOS binding site (Fig. 7), and therefore could potentially contribute to the pH dependence observed here. The conservation of these four histidines seemed to suggest that heparin binding, and its sensitivity to pH, could be important for a common function for the APP family of proteins.

**DISCUSSION**

Based on the biophysical measurements and biochemical evidence provided in this report, it seems clear that the E2 domain of worm APL-1 has to be monomeric in solution. Although the *in vivo* oligomeric state of APL-1 is not known, there is some evidence suggesting that human APP can form dimers in cells (23, 24). The E2 domain of human APP crystallized as dimers (10). The monomeric APL-1 E2 structure described here could be due to differences between human and worm proteins. Another possibility, as we have hypothesized...
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FIGURE 7. Heparin interacted with a surface groove near four conserved histidines. Residues found by mutagenesis to affect heparin binding are highlighted. The four conserved histidine residues are colored in blue. SOS is shown in yellow. Two short heparin oligosaccharides are modeled into the binding site by superimposing one of the glucosamine residues (residue 4) onto SOS: the structure of the tetrasaccharide (green) is based on the complex with FGF (44), whereas that of the pentasaccharide (red) is based on the complex with annexin (45). The pentasaccharide contacted both Arg-368 and Lys-372.

(10), is that monomeric and dimeric species of human E2 may co-exist in equilibrium in solution, and crystallization has only captured the dimeric form of the protein.

Mutagenesis data support the idea that the observed SOS site may represent a major binding site for heparin. Surprisingly, the site itself lacks permanently charged lysine and arginine residues. There are a few histidine residues inside, or near, the binding site. The observed dependence of binding affinity on pH suggests that protonation of the histidines plays an important role in the interactions with the negatively charged ligand. This feature is uncommon, but not unprecedented. Another example is HPRG, a plasma glycoprotein that uses tandem repeats of a short sequence rich in histidines to interact with heparin (25). The difference here is that the cluster of conserved histidines is brought together only in a folded protein, and is thus not obvious in the primary sequence. Besides the identified binding pocket, there may be additional contact points between heparin and the protein along helices $\alpha B$ and $\alpha D$, which harbor several lysine and arginine residues (e.g. Arg-368, Lys-372 on $\alpha D$) facing the same side of the molecule (17). In Fig. 7, we have superimposed glucosamine moieties of two known heparin structures onto the modeled glucose ring of SOS (44, 45), which illustrates the range of flexibilities that the oligosaccharide may have when one of its glucosamine residues is fixed inside the identified binding pocket. It must be emphasized, however, that the details of heparin binding are still unknown, and there is yet no data showing that the glucosamine moiety of heparin indeed binds to the described SOS site.

The binding of E2 to HSPG is expected to influence the interaction between the cell and extracellular matrix, and thus may have a direct role in regulating adhesion. Although it now seems possible that this interaction could be affected by pH changes brought about by pathological conditions such as hypoxia, ischemia, or inflammation (26–28), one may also wonder how acidic pH in various intracellular compartments, like Golgi and endosome (29), might affect the binding of APP to heparin-like molecules, and the trafficking of APP through these compartments under normal conditions (30): for example, upon endocytosis, while many receptor-ligand interactions become weakened in the acidic endosomes (31), APP will acquire a tighter binding to HSPGs internalized from the cell surface (32). How this influences its proteolytic degradation to amyloidial and non-amyloidial fragments will remain an interesting question for future studies.

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