The Heparin Binding Site of Protein C Inhibitor Is Protease-dependent*

Wei Li and James A. Huntington

From the Department of Haematology, University of Cambridge, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Hills Road, Cambridge, CB2 0XY, United Kingdom

Protein C inhibitor (PCI) is a member of the serpin family of protease inhibitors with many biological functions and broad inhibitory specificity. Its major targets in blood are thrombin and activated protein C (APC), and the inhibition of both enzymes can be accelerated by glycosaminoglycans, including heparin. Acceleration of thrombin and APC inhibition by PCI requires that both protease and inhibitor bind to the same heparin chain to form a bridged Michaelis complex. However, the position of the heparin binding site of APC is opposite to that of thrombin, and formation of the bridged complexes must require either radical reorientation of the proteases relative to PCI or alternate heparin binding modes for PCI. In this study, we investigate how heparin bridges thrombin and APC to PCI by determining the effect of mutations in and around the putative heparin binding site of PCI. We found that heparin binds PCI in a linear fashion along helix H to bridge thrombin, consistent with our recent crystal structure (3B9F), but that it must rotate by ~60° to engage Arg-229 to bridge APC. To gain insight into the possible modes of heparin binding to PCI, we solved a crystal structure of cleaved PCI bound to an octasaccharide heparin fragment to 1.55 Å resolution. The structure reveals a binding mode across the N terminus of helix H to engage Arg-229 and align the heparin binding site of APC. A molecular model for the heparin-bridged PCI-APC complex was built based on mutagenesis and structural data.

Protein C inhibitor (PCI) is a serpin of many potential functions in human health and disease (1, 2). It was first discovered in blood as the inhibitor of activated protein C (APC) (3) and was later found in several body fluids and tissues. In the blood plasma, PCI is the principal inhibitor of APC and thrombin bound to thrombomodulin (TM) (4) and therefore exerts a predominantly procoagulant role. On the other hand, PCI can also inhibit coagulation factors directly and with the aid of glycosaminoglycan (GAG) cofactors and thus may also play an anticoagulant role (5). However, from the association of PCI levels with certain thrombotic events (6, 7) and the finding that concentration of the PCI-APC complex is a sensitive indicator of the extent of activation of blood coagulation (8), it is clear that the principal physiological role of PCI in the circulation is inhibition of APC. Interestingly, the rate of APC inhibition by PCI is quite slow, on the order of 1000 m⁻¹ s⁻¹, and would thus be predicted to compete poorly with the other potential circulating APC inhibitors, such as α₁-antitrypsin (9). The fact that the PCI-APC complex is the marker of thrombotic events suggests that despite the low circulating PCI concentration and rate of APC inhibition, PCI is the principal physiological inhibitor of APC. This is due to the aid of GAG cofactors such as the heparan sulfates that line the vascular endothelium. Both PCI and APC bind to GAGs, and the presence of heparin in vitro accelerates the maximal rate of inhibition by over 2000-fold (when accounting for dissociation constants) (10). Thus, the mechanism of GAG activation of APC inhibition by PCI is of potential relevance for hemostasis and thrombosis.

PCI belongs to the serpin family of protease inhibitors. Members of this family share a unique mechanism where the rate of inhibition depends upon the rate of formation of the initial Michaelis complex, and final inhibition is achieved through subsequent radical conformational changes in both the serpin and the protease (for recent reviews, see Refs. 5 and 11). Several serpins are regulated by heparin and other GAGs, including antithrombin, heparin cofactor II, protease nexin 1 (PN1), plasminogen activator inhibitor 1 (PAI1), and PCI. Interestingly, all can be found in the blood and are inhibitors of thrombin (12). Sequence alignment, mutagenesis, and biochemical studies show that antithrombin, heparin cofactor II, protease nexin 1, and plasminogen activator inhibitor 1 use helix D to interact with heparin. To date, the only crystal structure of a serpin bound to heparin is for antithrombin with its specific pentasaccharide (and derivatives) (13–16). As predicted, the structures revealed a binding mode along helix D, with some contribution from the A helix, and it H is anticipated that the other heparin binding serpins will have similar modes of interaction with GAGs. However, antithrombin is unique in its ability to discriminate certain heparin sequences. All other heparin binding serpins interact nonspecifically, so there is a possibility that other modes of binding will be employed. Heparin cofactor II, for instance, is thought to utilize two separate but overlapping sites along helix D for binding to heparin and dermatan sulfate (17).

PCI uses an entirely different region for binding to GAGs. Biochemical and mutagenesis studies have shown that helix D...
is not involved, but that helix H (along the back of the serpin if shown in the classic orientation) is likely to be the principal interaction site (18) (Fig. 1A). The issue is complicated by the presence of an N-terminal extension containing several basic residues, the so-called A-helix (19), which has been shown to have some influence on heparin affinity, and the presence of a large basic patch adjacent to helix H (20) (Fig. 1B). Although it appears as if the H helix is the principal GAG binding site, mutation of the basic residues on helix H only marginally affects heparin affinity or heparin acceleration of protease inhibition (18, 21, 22). In fact, it takes at least three simultaneous mutations to see an appreciable detrimental effect and removal of all four positively charged residues to knock out heparin acceleration altogether. We have recently shown that the minimum heparin length that fully occupies the heparin binding site of PCI is an octasaccharide, which fits neatly along the length of helix H (22) (Fig. 1C).

Our recent structures of native PCI (22) and the PCI-thrombin-heparin complex (23) demonstrate that heparin accelerates PCI inhibition solely by bridging proteases to PCI (i.e. not an allosteric mechanism), and biochemical studies have shown that the minimum heparin length required for effective bridging to thrombin and APC is around 14 monosaccharide units (24). The structure of the bridged PCI-thrombin complex was solved in the presence of a 14-mer heparin chain, but only a disaccharide was observed interacting with thrombin. Extending the disaccharide to a 14-mer heparin chain fully occupied both the known heparin site on thrombin and the putative binding site on helix H of PCI (Fig. 2A). These two sites were aligned perfectly so that heparin would not have to deviate from its natural linearity (25). Interestingly, unlike thrombin, which utilizes exosite II to bind heparin (26), APC uses the equivalent of exosite I (10, 27). When we overlay APC onto the PCI-thrombin structure (Fig. 2B), it is instantly clear that either the orientation of APC relative to PCI or the binding mode of heparin on PCI must change in a radical fashion. We previously proposed a co-occupation mechanism for the PCI-APC-heparin complex in which PCI and APC share a common site on heparin to relieve the electrostatic repulsion between the proteases and inhibitor (20). This mechanism requires heparin to bind across the H helix and would involve the adjacent basic site.

Why has PCI evolved a different heparin binding site when compared with other heparin binding serpins? How can heparin binding to PCI effectively bridge two proteases that have heparin binding sites opposite to each other? To address these questions, we created a panel of charge reversal mutants along and adjacent to helix H and tested their effect on the ability of heparin to accelerate thrombin and APC inhibition. We found that mutations affected bridging in a protease-dependent manner, suggesting different modes of heparin binding to PCI in the thrombin and APC complexes. Surprisingly, the mutation adjacent to helix H (R229E) resulted in more efficient bridging to thrombin but abrogated bridging to APC. To investigate the possible modes of heparin binding, we solved a crystal structure of PCI in complex with 8-mer heparin. The heparin was found bound to the N terminus of helix H, in a position that would effectively bridge APC and engage Arg-229 in the context of a longer heparin chain. We built a PCI-APC-14-mer heparin model based on the PCI-thrombin complex and our structure of PCI bound to heparin, which satisfies the mutagenesis data for PCI and APC. We propose that heparin binds to PCI on the H helix, but with a certain degree of plasticity, and can rotate on the surface of PCI with the H helix as the pivot point.

FIGURE 1. The structure and putative heparin binding site of PCI. A, a ribbon depiction of native PCI (with helix A in green, helix D in blue, helix H in cyan, β-sheet A in red, and the reactive center loop on top in yellow with P1 as a ball) is shown rotated from the classic view (with β-sheet A facing) to focus on the heparin binding site along helix H. Residues mutated in this study are shown as rods and labeled. B, the electrostatic surface (blue and red for positive and negative charge, respectively) of PCI (oriented as in A) shows that helix D is not basic and that the likely heparin binding site lies in the basic region on and adjacent to helix H. C, a close-up of the electrostatic surface (semitransparent) in the helix H region of PCI (same orientation as before), with a modeled position of a heparin octasaccharide (rods, with carbons colored yellow). The residues mutated for this study are shown as rods and labeled.
EXPERIMENTAL PROCEDURES

Materials—Size fractionated heparin was purchased from Iduron (Manchester, UK). Human thrombin and APC were purchased from Hematologic Technologies, Inc. Thrombin substrate S2238 and APC substrate S2366 were purchased from Chromogenix. All crystallization reagents were purchased from Hampton Research. Porcine pancreatic elastase was purchased from Roche Applied Science.

Recombinant PCI—Recombinant PCI, lacking the N-terminal 17 residues (as described previously (22)), was used throughout as control and as the template for all mutations. The PCI mutants were generated by site-directed mutagenesis as described previously (23). Reactive center loop-cleaved PCI was generated from recombinant material by incubation with porcine pancreatic elastase (1:50 w/w ratio) at room temperature for 48 h. The cleavage was checked on SDS-PAGE, and cleaved PCI was purified by heparin-Sepharose chromatography.

Protease Inhibition Assays—Both thrombin and APC inhibition reactions were carried out in 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, containing 0.1% polyethylene glycol 8000 and 0.2% bovine serum albumin. 5 mM CaCl₂ was included in the APC assays. PCI and its variants were used at 50–100 nM in the thrombin assay and 100–200 nM in the APC assay. When heparin was used, it was preincubated with PCI at 800 nM for the 16-mer, 400 nM for the 24-mer, and 16.7 μg/ml for unfractionated heparin. Final concentrations of thrombin and APC were 5 and 10 nM, respectively. At time intervals between 15 s and 10 min, reactions were stopped by adding a large volume and excess of substrate (containing 1 mg/ml Polybrene in the reactions with heparin), and residual protease activity was measured by reading change in absorbance at 405 nm. Data were analyzed by plotting the natural log of the fractional residual enzyme activity versus time and fitting the data to a linear regression using the software Prism. Second-order rate constants of inhibition were calculated by dividing the slope by the inhibitor concentration.

Crystallization, Data Collection, and Refinement—Cleaved PCI at 3.4 mg/ml in 50% glycerol was preincubated with 8-mer or 10-mer heparin (1:2.5 molar ratio), and crystal trials were established. Initial screens generated many hits for the heparin complexes, and the best condition for the cleaved PCI-8-mer heparin complex was determined to be 0.12 M Li₂NO₃ and 12% polyethylene glycol 3350. Data were collected at Daresbury Synchrotron Radiation Source (Warrington, Cheshire, UK) station 14.1 from a single flash-cooled crystal and processed using Mosflm, Scala, and Truncate (28). The structure was solved by molecular replacement using the program Phaser (29) with 1QLP as the search model. Refinement was conducted using the programs CNS (30) (version 1.2), and XtalView (31) was used for model building. Molecular images were created using PyMOL (32).

RESULTS

Heparin Accelerated Thrombin Inhibition by PCI Reverse Charge Mutants—We generated reverse charge Glu mutants for residues along helix H (Lys-266, Arg-269, Lys-270, Lys-273) and in the adjacent basic patch (Arg-229) and tested the effect of these mutations on the ability of heparin to accelerate thrombin inhibition. The basal rates (in the absence of heparin) were unaffected by the mutations (Table 1), and the presence of full-length heparin accelerated the inhibition of thrombin to the same degree for the variants as for the control (data not shown). This is in agreement with a previous report that showed that single mutations along helix H to Ala have no effect and that double mutations R269A/K270A only reduced the heparin effect by ~2-fold (21). To observe an effect of the mutations, we repeated the experiment with shorter heparin chains and found that for both 16-mer and 24-mer heparins, the mutations along helix H only reduced the heparin-accelerated rate of thrombin inhibition by a maximum of 2-fold. However, based on the reduction in the accelerated rate of thrombin inhibition, the...
four H helix mutants can be ranked in order of importance 273 > 269 > 266 > 270. This is in agreement with the structure of the PCI-thrombin-heparin complex that showed the alignment of the heparin binding site of thrombin with the H-helix of PCI. The fact that no single mutation had a significant effect on heparin bridging indicates that there is some degree of plasticity in the position of heparin along helix H. Unexpectedly, the R229E mutation resulted in a 2–3-fold increase in the acceleration of thrombin inhibition using the 16-mer and 24-mer heparins. This suggests that thrombin is bridged more effectively when heparin binding is limited to helix H, consistent with multiple modes of heparin binding to PCI.

**Heparin Accelerated APC Inhibition by PCI Reverse Charge Mutants**—As for thrombin, none of the mutations affected the rate of APC inhibition in the absence of heparin. However, in the presence of either 24-mer or full-length heparin, all reverse charge mutants decreased the rate of APC inhibition when compared with control PCI (Table 2). The charge reversal mutations on helix H marginally reduced the heparin affinity per se because the opposite effect is seen for the bridging of thrombin. Rather, the R229E mutation appears to influence the mode of heparin binding. The order of importance of the residues on helix H is also strikingly different, 270 > 266 > 273 > 269, and correlates with the distance from the most important residue, Arg-229, when plotted onto the structure of PCI (Fig. 1, A and C). Lys-270 is on the side of helix H facing Arg-229; Lys-273 and Lys-266 are oriented toward to top surface of helix H, whereas Arg-269 is on the opposite side of helix H and is pointing away from Arg-229.

**Crystal Structure of PCI Bound to Heparin**—We set out to investigate possible heparin binding modes of heparin on PCI by crystallizing native PCI in complex with short chain heparins (8-mer and 10-mer). After many screens, we managed to obtain a PCI-heparin co-crystal in a low ionic strength condition but determined by SDS-PAGE that the PCI had been cleaved in the reactive center loop. Because native and cleaved PCI have similar affinities for 8-mer heparin (22, 24), we decided to generate cleaved PCI for crystallization trials using porcine pancreatic elastase. With cleaved PCI, we readily obtained high quality isomorphous crystals in complex with 8-mer and 10-mer heparins. Several data sets were collected of the 8-mer and 10-mer complexes, and the best yielded a high quality structure to 1.55 Å resolution. Statistics for data processing and refinement are given in Table 3.

We found one complex of cleaved PCI and heparin in the asymmetric unit. The conformation of recombinant cleaved PCI solved here (Fig. 3A) is essentially equivalent to the original structure of plasma-derived cleaved PCI (20), with a root mean

### Table 1

**Modes of Heparin Binding to PCI**

| Mutant   | No heparin | +16mer | +24mer | +Full length heparin |
|----------|------------|--------|--------|----------------------|
|          | Rate constant (×10^3 M^-1 s^-1) | -Fold increase | Rate constant (×10^3 M^-1 s^-1) | -Fold increase | Rate constant (×10^3 M^-1 s^-1) | -Fold increase |
| Wild type | 3.09 ± 0.1 | 1.35 ± 0.1 | 4.4 | 4.54 ± 0.25 | 14.7 | 6.38 ± 0.51 | 634.6 |
| R229E    | 3.00 ± 0.17 | 3.62 ± 0.36 | 12.1 | 7.80 ± 0.74 | 26.0 | 3.09 ± 0.14 | 252.0 |
| K266E    | 4.09 ± 0.22 | 1.35 ± 0.19 | 3.3 | 3.42 ± 0.37 | 8.4 | 4.18 ± 0.08 | 447.7 |
| R269E    | 3.54 ± 0.04 | 1.14 ± 0.00 | 3.2 | 2.86 ± 0.16 | 8.1 | 0.87 ± 0.07 | 104.3 |
| K270E    | 2.65 ± 0.19 | 1.07 ± 0.01 | 4.0 | 2.95 ± 0.26 | 11.1 | 2.08 ± 0.05 | 255.2 |
| K273E    | 3.20 ± 0.08 | 0.70 ± 0.10 | 2.2 | 2.24 ± 0.05 | 7.0 | 0.43 ± 0.10 | 52.8 |

### Table 2

**Heparin accelerated APC inhibition by PCI and its variants**

| Mutant   | No heparin | +24mer | +Full length heparin |
|----------|------------|--------|----------------------|
|          | Rate constant (×10^3 M^-1 s^-1) | -Fold increase | Rate constant (×10^3 M^-1 s^-1) | -Fold increase |
| Wild type | 1.01 ± 0.19 | 7.40 ± 0.34 | 73.6 | 6.38 ± 0.51 | 634.6 |
| R229E    | 0.96 ± 0.07 | 0.27 ± 0.01 | 2.8 | 0.36 ± 0.04 | 37.6 |
| K266E    | 1.22 ± 0.05 | 6.23 ± 0.06 | 50.8 | 3.09 ± 0.14 | 252.0 |
| R269E    | 0.93 ± 0.08 | 7.67 ± 0.43 | 82.2 | 4.18 ± 0.08 | 447.7 |
| K270E    | 0.83 ± 0.04 | 2.19 ± 0.03 | 26.3 | 0.87 ± 0.07 | 104.3 |
| K273E    | 0.82 ± 0.06 | 4.31 ± 0.10 | 52.8 | 2.08 ± 0.05 | 255.2 |

### Table 3

**Data processing, refinement and model (3DY0)**

| Crystal                  | Space group | P2₁, |
|--------------------------|-------------|------|
| Cell dimensions ( Å )     | a = 61.05, b = 49.45, c = 64.97 |
| Reflections in working/free set | 167116 | 3471.1-1.55 |
| Total reflections         | 12301 |
| Unique reflections        | 49709 |
| Solvent content (%)       | 5422 |
| Multiplicity              | 3.4 |
| Completeness (%)          | 2.3 |
| R-factor (Å²)             | 16.7 |
| Revisions statistics      | 2.7 |
| R-factor/R_merge (%)      | 95.8 |
| Root mean square deviation of bond(Å)/angles (˚) from ideality | 72.7 |
| Ramachandran plot; residues in | 19.7/21.7 |
| Most favored region (%)   | 18.9 |
| Additionally allowed region (%) | 362.3/357 |
| Generously allowed region (%) | 6078/313 |
| Disallowed region (%)     | 26.93-1.55Å |
| Reflection statistics     | 1.65-1.55Å |
| R-factor/R_merge (%)      | 0.005/1.3 |
| Root mean square deviation of bond(Å)/angles (˚) from ideality | 0.005/1.3 |
| Ramachandran plot; residues in | 0.005/1.3 |
| Most favored region (%)   | 90.7 |
| Additionally allowed region (%) | 8.4 |
| Generously allowed region (%) | 0.9 |
| Disallowed region (%)     | 0.0 |

---

**The Journal of Biological Chemistry**

**Volume 283 • Number 51 • December 19, 2008**

---

**JOURNAL OF BIOLOGICAL CHEMISTRY**

---

---
square deviation of ~1 Å for the 355 equivalent Cα atoms. The density for heparin was of high quality for the middle three of the five modeled monosaccharide units (Fig. 3B). PCI interacted with heparin in three crystallographically related positions, only one of which had contacts with residues on helix H (Fig. 3, A and B). The pentasaccharide binding to this site buries a total of 353 Å² and makes hydrogen bond and salt-bridge contacts with residues on the N terminus of helix H and on the loop containing Arg-229. Lys-270 is only making a water-mediated hydrogen bond with heparin, due to the internal orientation of the side chain. However, it is in close proximity to heparin, and there is evidence in the electron density for an alternate conformation of Lys-270 oriented toward heparin. Likewise, Lys-266 is in proximity to make multiple contacts with heparin but cannot be fully modeled into electron density. The orientation and position of the heparin fragment in this structure are surprising, but when the fragment is elongated to a 14-mer, it overlaps with Arg-229 and interacts nicely with the known heparin binding site of APC (Fig. 4A).

**DISCUSSION**

According to our mutagenesis data, thrombin and APC are bridged to PCI using two alternate heparin binding modes. Mutation of Arg-229 to Glu effectively reduces the available binding modes and forces heparin to bind in a linear fashion along helix H. This is the preferred mode for bridging thrombin, as seen in the recent crystal structure (3B9F), and therefore mutations of residues in the 37-loop are intermediate in their effects, showing a modest increase in basal and decrease in heparin-accelerated rates (27). These data suggest a classic bridging interaction principally involving the 70–80-loop of APC, with the 37-loop also contributing. Binding of PCI to heparin may also reduce the electrostatic repulsion with the 60- and 37-loops, further contributing to the acceleration through a co-occupation mechanism as proposed previously (20). Our model is consistent with these findings and puts them into structural context.

**Model of the Heparin-bridged PCI-APC Complex**—We were able to build a model of the PCI-APC-heparin complex by simply overlaying APC onto the thrombin component of the PCI-thrombin-heparin structure (Fig. 2B). We observed a contiguous positively charged surface stretching from the N terminus of helix H to the known heparin binding site of APC, with Arg-229 occupying a strategic position in the middle of the basic patch. The position of heparin observed in our crystal structure provides a good candidate for the mode of binding, which bridges the PCI-APC complex because its linear elongation from a pentasaccharide to a tetradecasaccharide is sufficient to link the heparin binding sites of the two proteins (Fig. 4A). This is consistent with the report that 14-mer heparin is the minimum length capable of accelerating the inhibition of APC by PCI (24).

The model is also in agreement with the mutagenesis data on APC. APC has three basic regions that affect the interaction with PCI: the 37-loop, including lysines 37, 38, and 39; the 60-loop, including lysines 62 and 63; and the 70–80-loop, including arginines 74 and 75 and lysine 78 (Fig. 4B). In our model, only the 60-loop is in close proximity to the basic surface of PCI. This interface would predictably be repulsive, and accordingly, charge neutralization or reversal mutations improve the basal rate of APC inhibition by PCI but have minimal effect on the heparin-accelerated rate (27). In contrast, mutations of residues in 70–80-loop have no effect on the basal rate but totally abolish the heparin enhancement (10). Mutations of residues in the 37-loop are intermediate in their effects, showing a modest increase in basal and decrease in heparin-accelerated rates (27). In contrast, mutations of residues in 70–80-loop have no effect on the basal rate but totally abolish the heparin enhancement (10). Mutations of residues in 70–80-loop have no effect on the basal rate but totally abolish the heparin enhancement (10). In contrast, mutations of residues in 70–80-loop have no effect on the basal rate but totally abolish the heparin enhancement (10).
Heparin thus appears to be able to bridge the PCI-thrombin complex by binding along helix H and the PCI-APC complex by binding across the H-helix, which would indicate that heparin binding on PCI is nonspecific and exhibits an unexpected degree of plasticity. This could be explained by a mechanism where heparin binds principally to helix H but can satisfy the electrostatic interactions in several different orientations with the N terminus of helix H as the pivot point.

Because heparin binds weakly to PCI in potentially several orientations, it is quite challenging to obtain a single crystal structure that reveals the principal heparin binding mode of PCI. Crystal contacts typically contribute 3–6 kcal/mol of free energy (33) and can thus easily displace weak binding heparin from a preferred binding site. This is what we suspect has happened in our structure of cleaved PCI co-crystallized with an 8-mer heparin fragment. This nudging of heparin may have occurred in our structure of cleaved PCI co-crystallized with an tetradecasaccharide allowing the bridging of PCI (bottom) to APC (top). The position of heparin nicely complements the contiguous basic groove extending from the N terminus of helix H to the known heparin binding site on APC. A, a close-up of the PCI:APC interface reveals the role of heparin in relieving electrostatic repulsion with the 60- and 37-loops and the body of PCI. The region primarily responsible for bridging is the 70–80-loop (indicated).

REFERENCES

1. Geiger, M., Zechmeister-Machhart, M., Uhrin, P., Hufnagl, P., Ecke, S., Priglinger, U., Xu, J., Zheng, X., and Binder, B. R. (1996) Immunopharmacology 32, 53–56

2. Geiger, M. (2007) Thromb. Haemostasis 97, 343–347

3. Marlar, R., and Griffin, J. H. (1980) J. Clin. Investig. 66, 1186–1189

4. Rezaie, A. R., Cooper, S. T., Church, F. C., and Esmon, C. T. (1995) J. Biol. Chem. 270, 25336–25339

5. Rau, J. C., Beaulieu, L. M., Huntington, J. A., and Church, F. C. (2002) J. Thromb. Haemost. 5, Suppl. 1, 102–115

6. Meijers, J. C., Marquart, J. A., Bertina, R. M., Bouma, B. N., and Rosendaal, F. R. (2002) Br. J. Haematol. 118, 604–609

7. Carroll, V. A., Griffiths, M. R., Geiger, M., Morro, C., Furlan, M., Lammle, B., and Binder, B. R. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 114–118

8. Kolbel, T., Strandberg, K., Mattiasson, I., Stenflo, J., and Lindblad, B. (2006) J. Vasc. Surg 43, 935–939

9. van der Meer, F. J., van Tilburg, N. H., van Wijngaarden, A., van, d. L. I., van der Meer, K., van Tilburg, N. H., and van Wijngaarden, A. (1997) Proc. Natl. Acad. Sci. U. S. A 94, 14683–14688

10. Johnson, D. J., and Huntington, J. A. (2003) Biochemistry 42, 8712–8719

11. Johnson, D. J., Li, W., Adams, T. E., and Huntington, J. A. (2006) EMBO J. 25, 6149–6157

12. Garg, H. G., Linhardt, R. J., and Hales, C. A. (2005) in Chemistry and Biology of Heparin and Heparan Sulfate (Garg, H. G., Linhardt, R. J., and Hales, C. A., eds) pp. 367–398, Elsevier, Oxford

13. Jin, L., Abrahams, J. P., Skinner, R., Petitet, M., Pike, R. N., and Carrell, R. W. (1997) Proc. Natl. Acad. Sci. U. S. A 94, 14683–14688

14. Johnon, D. J., and Huntington, J. A. (2003) Biochemistry 42, 8712–8719

15. Johnon, D. J., Li, W., Adams, T. E., and Huntington, J. A. (2006) EMBO J.
16. Li, W., Johnson, D. J., Esmon, C. T., and Huntington, J. A. (2004) Nat. Struct. Mol. Biol. 11, 857–862
17. Tollefsen, D. M. (1994) Ann. N. Y. Acad. Sci. 714, 21–31
18. Neese, L. L., Wolfe, C. A., and Church, F. C. (1998) Arch. Biochem. Biophys. 355, 101–108
19. Kuhn, L. A., Griffin, J. H., Fisher, C. L., Greengard, J. S., Bouma, B. N., Espana, F., and Tainer, J. A. (1990) Proc. Natl. Acad. Sci. U. S. A 87, 8506–8510
20. Huntington, J. A., Kjellberg, M., and Stenflo, J. (2003) Structure (Camb.) 11, 205–215
21. Shirk, R. A., Elisen, M. G., Meijers, J. C., and Church, F. C. (1994) J. Biol. Chem. 269, 28690–28695
22. Li, W., Adams, T. E., Kjellberg, M., Stenflo, J., and Huntington, J. A. (2007) J. Biol. Chem. 282, 13759–13768
23. Li, W., Adams, T. E., Nangalia, J., Esmon, C. T., and Huntington, J. A. (2008) Proc. Natl. Acad. Sci. U. S. A 105, 4661–4666
24. Pratt, C. W., and Church, F. C. (1992) J. Biol. Chem. 267, 8789–8794
25. Mulloy, B., Forster, M. J., Jones, C., and Davies, D. B. (1993) Biochem. J. 293, 849 – 858
26. Carter, W., Cama, E., and Huntington, J. A. (2004) J. Biol. Chem. 280, 2745–2749
27. Friedrich, U., Blom, A. M., Dahlback, B., and Villoutreix, B. O. (2001) J. Biol. Chem. 276, 24122–24128
28. Leslie, A. W. G. (1992) Joint CCP4 and ESF-EACMB Newsletter on Protein Crystallography, Number 26, Daresbury Laboratory, Warrington, UK
29. McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C., and Read, R. J. (2005) Acta Crystallogr. Sect. D Biol. Crystallogr. 61, 458–464
30. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
31. McRee, D. E. (1992) J. Mol. Graph. 10, 44–46
32. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific LLC, San Carlos, CA
33. Drenth, J., and Haas, C. (1992) J. Cryst. Growth 122, 107–109
34. Zechmeister-Machhart, M., Hufnagl, P., Uhrin, P., Xu, J., Geiger, M., and Binder, B. R. (1996) Immunopharmacology 32, 96–98
35. Wakita, T., Hayashi, T., Yuasa, H., Nishioka, J., Kawamura, J., and Suzuki, K. (1998) FEBS Lett. 429, 263–268