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Crystal Structure of the Calcium-stabilized Human Factor IX Gla Domain Bound to a Conformation-specific Anti-Factor IX Antibody*

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The binding of Factor IX to membranes during blood coagulation is mediated by the N-terminal γ-carboxyglutamic acid-rich (Gla) domain, a membrane-anchoring domain found on vitamin K-dependent blood coagulation and regulatory proteins. Conformation-specific anti-Factor IX antibodies are directed at the calcium-stabilized Gla domain and interfere with Factor IX-membrane interaction. One such antibody, 10C12, recognizes the calcium-stabilized form of the Gla domain of Factor IX. We prepared the fully carboxylated Gla domain of Factor IX by solid phase peptide synthesis and crystallized Factor IX-(1–47) in complex with Fab fragments of the 10C12 antibody. The overall structure of the Gla domain in the Factor IX-(1–47)-antibody complex at 2.2 Å is similar to the structure of the Factor IX Gla domain in the presence of calcium ions as determined by NMR spectroscopy (Freedman, S. J., Furie, B. C., Furie, B., and Baleja, J. D. (1995) Biochemistry 34, 12126–12137) and by x-ray crystallography (Shikamoto, Y., Morita, T., Fujimoto, Z., and Mizuno, H. (2003) J. Biol. Chem. 278, 24090–24094). The complex structure shows that the complementarity determining region loops of the 10C12 antibody form a hydrophobic pocket to accommodate the hydrophobic patch of the Gla domain consisting of Leu-6, Phe-9, and Val-10. Polar interactions also play an important role in the antibody-antigen recognition. Furthermore, the calcium coordination network of the Factor IX Gla domain is different than in Gla domain structures of other vitamin K-dependent proteins. We conclude that this antibody is directed at the membrane binding site in the ω loop of Factor IX and blocks Factor IX function by inhibiting its interaction with membranes.

Factor IX plays an important role in an intermediate stage of the blood coagulation cascade (1). Factor IX can be activated to Factor IXa in the presence of calcium ions either by Factor Xla or by the complex of Factor VIIa-tissue factor-bound on membrane surfaces. Factor IXa and its cofactor, Factor VIIa, assemble to form a membrane surface-bound enzyme complex that can efficiently activate Factor X to Factor Xa in the presence of calcium ions. Defective activity or deficiency of Factor IX due to mutation of the Factor IX gene is the cause of hemophilia B.

The vitamin K-dependent blood coagulation and regulatory proteins require vitamin K for the posttranslational synthesis of γ-carboxyglutamic acid, an amino acid clustered in the N-terminal Gla1 domain of these proteins. The Gla domain is a membrane binding motif that, in the presence of calcium ions, supports interaction of these proteins with phospholipid membranes that include phosphatidyserine. We have recently reported the crystal structure of prothrombin fragment 1 bound to lysophosphatidyserine (2). The serine head group binds Gla domain-bound Ca-5 and Ca-6 and Gla residues 17 and 21, fixed elements of the Gla domain-fold, predicting the structural basis for phosphatidyserine specificity among Gla domains. The x-ray crystal structure of porcine Factor IX, performed in the absence of calcium, revealed the structure of the two epidermal growth factor domains and the serine protease domain but only a small C-terminal portion of the Gla domain because of disorder in the Gla domain in the absence of metal ions (3). Efforts to crystallize intact Factor IX in the presence of calcium by our group and others have been unsuccessful. NMR studies of the Factor IX Gla domain in solution indicate a largely disordered and unstructured peptide in the absence of metal ions, but this domain folds into a stable structure in the presence of calcium ions (4–6). Based upon these studies, we have demonstrated that the phospholipid binding site is located in the ω loop, the N terminus of the Gla domain. However, the positions of calcium ions and an understanding of the coordination of calcium ions by γ-carboxyglutamic acid could not be determined except by extrapolation from the known structure of the Gla domain of prothrombin (7) and by molecular modeling and energy minimization (8).

Recently, the structure of bovine Factor IX-(1–46) bound to a snake venom protein, Factor IX-binding protein, has been crystallized in the presence of calcium and magnesium ions and in the presence of calcium alone (9). Although the emphasis of this work is on the possible role of magnesium in Gla domain binding to membrane surfaces, examination of the calcium-Factor IX-(1–46) complex revealed that the calcium coordination within the Gla domain differs from the Gla domains of other vitamin K-dependent proteins, including prothrombin (7), Factor VII (10), and Factor X (11). This may be due to perturbations of the Factor IX Gla domain structure by the bound venom FIX-binding protein, or it may represent differences in the fine structure of the γ-carboxyglutamic acid-cal-

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The atomic coordinates and structure factors (code 1NL0) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

The abbreviations used are: Gla, γ-carboxyglutamic acid; FIX, Factor IX; CDR, complementarity determining region; r.m.s.d., root mean square deviation; MES, 4-morpholineethanesulfonic acid; NMP, N-methylpyrrolidone; L, light chain; H, heavy chain; HC, heavy chain constant domain; HV, heavy chain variable domain; LC, light chain constant domain.

1 The abbreviations used are: Gla, γ-carboxyglutamic acid; FIX, Factor IX; CDR, complementarity determining region; r.m.s.d., root mean square deviation; MES, 4-morpholineethanesulfonic acid; NMP, N-methylpyrrolidone; L, light chain; H, heavy chain; HC, heavy chain constant domain; HV, heavy chain variable domain; LC, light chain constant domain.
**EXPERIMENTAL PROCEDURES**

**Materials—**10C12 Fab(α′) was a kind gift from Drs. C. J. Refino and D. Kirchofer (Genentech). Protein concentration was assayed using the Advanced Protein Assay (Cytoskeleton, Denver, CO). Mecuripapain (0.5 mg/ml) were added to 710 μl of 10C12 F(ab′)/H11032 in the presence of calcium ions (13). 10C12 was then reformatted into a F(ab′)2-like form with two Fab fragments connected by a leucine zipper. A conformation-specific antibody, 10C12, similar to those that bind prothrombin or Factor IX in the presence of calcium ions but not in the presence of magnesium ions (14–16) is a calcium-dependent and calcium-specific antibody for Factor IX. There was no binding in the presence of magnesium ions (17, 18).

We report here the crystal structure of the Factor IX Gla domain in the presence of calcium ions in complex with the 10C12 Fab fragment of a conformation-specific antibody directed against the calcium-stabilized form of the Gla domain of Factor IX. We identify the Factor IX-antibody interface that provides a structural basis to understanding the inhibition of membrane binding by this conformation-specific antibody. Furthermore, the marked similarity of the positions of bound calcium ions and the coordination network that defines the interaction of γ-carboxyglutamic acids with calcium ions in our Factor IX Gla domain structure, bound to the conformation-specific antibody at the hydrophobic patch (Leu-6, Phe-9, and Val-10) in the ω loop and the bovine Factor IX Gla domain bound to the venom Factor IX binding protein on a surface on the side of the Gla domain involving Phe-25, indicates that the differences observed between the Factor IX γ-carboxyglutamic acid–calcium coordination network and the Gla domains of other vitamin K-dependent proteins are not due to perturbation of the structure of the Gla domain by bound protein.

**Chemical Synthesis of Factor IX (1–47)—**Peptides were synthesized using Fmoc (N-(9-fluorenylmethoxycarbonyl) N-methylpyrrolidine (NMP) chemistry on an Applied Biosystems Model 430A peptide synthesizer. Amino acids were coupled as 1-hydroxybenzotriazole esters onto ρ-hydroxyethylphenoxyethylpolylysine resin preloaded with aspartic acid (Applied Biosystems, Foster City, CA). All of the residues from cycle 30 through 47 were double-coupled. Following each coupling, all of the uncoupled ɛ-N termini were acetylated. Gla residues were coupled using modified activation, coupling, and deprotection cycles. Gla (1 mmol) was dissolved in 0.4 ml of dichloromethane and 1.0 ml of N-hydroxybenzotriazole/NMP for 1 h with nitrogen agitation. Coupling onto a protected resin-bound amino acid proceeded for a total of 90 min with vigorous stirring. After removal of amino acid solution and washing with NMP, Gla residues were deprotected with 20% piperidine/NMP for 30 min. Five NMP washes were done prior to the next amino acid addition.

Cleavage of the peptide from its solid support and simultaneous side chain deprotection was performed using trifluoroacetic acid/water/thioanisole/phenol/1,2-ethanedithiol (82.5:5:5.5:2.5). The cleavage reaction was allowed to proceed for 5 h at 25 °C. The peptide was then purified on a C18 high pressure liquid chromatography column eluted with an acetonitrile/water gradient. Lyophilized Factor IX (1–47) (1.2 mg) was dissolved in 1.2 ml of sodium acetate, pH 5.5, 0.5 mM NaCl. This solution was transferred to an Eppendorf tube containing 24 μl of 20 mM CaCl₂ and then to 12 μl of 0.2 mM CaCl₂ to raise the final calcium chloride concentration to 2 mM. The reconstituted Factor IX (1–47) remained clear at room temperature indefinitely but precipitated when stored at 4 °C.

**10C12 Fab-Factor IX (1–47) Complex Formation and Crystalization—**10C12 Fab fragment (4 ml; 0.18 mg/ml) was mixed with 1 ml of Factor IX (1–47) at 0.50 mg/ml. The complex was purified on a HiLoad 16/60 Superdex 75 gel filtration column to eliminate aggregates using 0.2 mM NaCl, 2 mM CaCl₂, 0.1 mM HEPES, pH 7.5. The purified protein was concentrated in a Millipore Ultrafree concentrator with 5,000 molecular weight cutoff to a concentration of 4.0 mg/ml. Crystals of 10C12 Fab-Factor IX (1–47) complex were formed using the sitting drop vapor diffusion method at a precipitant condition of 2.3 M ammonium sulfate, 0.1 mM HEPES, pH 7.5, 5 mM calcium chloride, 2% 2-methyl-2,4-pentanediol. The crystals were rapidly soaked in mother liquor containing 25% glycerol and then flash-frozen in a liquid nitrogen stream and mounted in the goniometer for diffraction data collection.

**X-ray Data Collection and Structure Determination—**The x-ray diffraction data used to determine the crystal structure 10C12 Fab-Factor IX (1–47) complex were collected at beam lines 12x and ×25. All of the diffraction data were indexed and processed using the HKL2000 suite (19).

The structure was determined by the molecular replacement method with AMORE software (20). Homology models of 10C12 Fab heavy and light chains were built from the sequences by program FReD (21). The template PDB coordinates used for homology modeling were as follows: 2FB4L, 2IG2L, 1DCLB, 1DCLA, and 3MCG2 for the light chain, and 2IG2H, 1AQKH, 8FABD, 1DBFH, and 2FB4H for the heavy chain, respectively. This Fab model was then separated into four domains to be used as molecular replacement models: heavy chain constant domain (HC), heavy chain variable domain (HV), light chain constant domain (LC), and light chain variable domain. Models from domain HC, HV, and LC gave distinguishable AMORE molecular replacement rotational solutions with the height of first peaks approximately 16 higher than the strongest noise peak (defined as peak drop) at a resolution range of 4–Å. At the translational function step of the molecular replacement method, however, the only LC model gave discernible AMORE translation function solutions that were 1.2σ higher than the strongest noise peaks and had a correlation coefficient of 0.55 and a low R-factor of 20.9%. A resolution range of 1.53/1.34 Å for the LC model was used to search for the positions of the rest of the models, yielding translation function peak drop/correlation coefficients of 3.7σ/53.0, 4.0σ/53.9, and 7.3σ/52.9 for HC, HV, and LC chain variable domains, respectively. All of these domains assembled properly into a Fab molecule and packed well inside a crystal lattice without severe overlap with symmetry-related molecules. This Fab fragment was refined successfully to R and Rfree values of 0.305 and 0.372, respectively, by CNS (22), further confirming the correct molecular replacement solution of the Fab fragment. At this stage, the correct conformations of side chains of complementarity determining regions (CDR) were clearly visible in 3Fo – 2Fc, ω-ε-weighted electron density maps (23) and were adjusted or built-in manually using the graphic program (24). Molecular replacement using models derived from other Gla domain structures failed despite the high degree of sequence and structural homology among the known Gla domain structures. The 3Fo – 2Fc electron density maps revealed some connected density, presumably Gla domain, next to Fab CDR loops. A Gla domain model derived from the crystal structure of the Factor X Gla domain–Factor X-binding protein complex (11) was then manually positioned into this electron density. The resulting Gla domain packed well with neighboring molecules and was successfully refined with molecular dynamic simulated annealing and conjugate gradient minimization protocols together with some manual adjustment to final R and Rfree values of 0.233 and 0.270, respectively.

For structural analysis and graphic representation, MOLSCRIPT (25), BOBSCRIPT (26), RASTER3D (27), and GRASP (28) programs were used. Hydrogen bonds and van der Waals contacts in the final model were identified using the programs HBPLUS (29) and LIGPLOT (30).
Crystal Structure of Factor IX Gla Domain

TABLE I

| Summary of X-ray diffraction data collection and model refinement for 10C12 Fab-factor IX Gla domain complex crystal |
|---------------------------------------------------------------|
| Temperature for data collection | 170 °C |
| Crystal diffraction limit | 2.10 Å |
| Total number of reflections | 241,036 |
| No. of unique reflections | 28,585 (1533) |
| Overall data redundancy | 8.4 |
| Data completeness | 81.9% (58.7)% |
| Data completeness | 81.9% (58.7)% |
| Data I (|<sup>a</sup>|<sup>b</sup>|<sup>c</sup>) | 0.050 (0.119)% |
| Data completeness | 81.9% (58.7)% |
| Model refinement | 25.7 (5.4)% |
| Protein/solvent atoms | 3640/148 |
| Calcium/sulfate ions | 6/1 |
| Resolution for refinement | 24.66–2.20 |
| R<sub>crys</sub> value | 0.233 (0.278)% |
| Estimated coordinate error | 0.270 (0.332)% for 1298 reflection (4.9%) |
| R.m.s.d. for bond lengths | 0.006 Å |
| Ramachandran plot from program PROCHECK (48) | 88.0% most favored; 11.1% allowed; 0.5% generally allowed; 0% disallowed |

<sup>a</sup> R<sub>merge</sub> = Σ(h)I( )/|Σ(h)I(h)|, where (I(h)) is the mean intensity, I(h), of reflection h.
<sup>b</sup> R<sub>crys</sub> = Σ(h)|F(h) − F(h)|/Σ(h)F(h).
<sup>c</sup> Numbers in parentheses refer to the highest resolution shell.

RESULTS

Structure of 10C12 Fab-Factor IX-(1–47) Complex—The structure of 10C12 Fab-Factor IX-(1–47) complex was determined by x-ray crystallography to 2.2 Å. The coordinates of this complex have been deposited as 1NL0 in the Protein Data bank. The current model consists of residues 1–210 of the 10C12 light chain, residues 1–126 and 135–217 of the 10C12 heavy chain, residues 1–45 of the Factor IX-(1–47), and 148 loosely attached solvent molecules (Table I). The model was refined at 2.2 Å to R<sub>crys</sub> and R<sub>free</sub> values of 0.233 and 0.270, respectively (Table I). Most of the bond angles of residues are clustered in energetically favorable (88.0%) or allowed (11.1%) regions of the Ramachandran plot. Residue Asp-152L of the 10C12 Fab fragment was clearly defined by the electron density maps but located outside of these regions. This is probably because this side chain makes hydrogen bonds to the imidazole ring of His-189L and the main chain N atom of Lys-190L, partially compensating for the high energy conformation (δΨ = 69.8°–97.0°) of its main chain. Residue 51L of 10C12 also adopts a high energy conformation (δΨ = 68.1°–45.1°), a situation commonly found in antibody structures (for example, see Ref. 31). Disorder around the heavy chain residues near residue 130 is also often observed in antibody structures. Residues 127–134 of the 10C12 heavy chain were not included in the current model because of poorly defined electron density in this region. Two C-terminal residues of the Factor IX-(1–47), residues 46 and 47, did not have well defined electron densities and were omitted from the final model.

The crystal structure of the complex of the 10C12 Fab and Factor IX-(1–47) indicates the interaction of the antibody with the N-terminal o loop of Factor IX (Fig. 1). Fab 10C12 is typical of an anti-protein antibody with six CDR loops forming a concave and undulating antigen binding surface (32). CDR loops H1, H2, H3, and L3 from this antigen binding surface form a hydrophobic pocket that binds exposed N-terminal residues of Factor IX-(1–47), resulting in an interface with a total area of 1338 Å². This interface is one of the smallest among protein-anti-protein antibody complexes (29) despite the high affinity interaction (K<sub>d</sub> 1.6 nM) between 10C12 Fab and Factor IX-(1–47) (13). Although 10C12 is a calcium-dependent conformation-specific antibody recognizing only the calcium-stabilized Factor IX-(1–47) conformer, no direct interactions between the Fab fragment of the antibody and calcium ions in the Gla domain were observed.

10C12 Fab-Factor IX Gla Interactions—Despite sequence similarities within the N terminus of the vitamin K-dependent proteins (Fig. 2), the 10C12 antibody is specific for Factor IX and does not bind to the Gla domains of other vitamin K-dependent proteins, although it does bind to Factor IX of other species. A hydrophobic pocket is formed from 10C12 Fab CDR heavy chain residues Ala-33, His-35, Ile-50, Tyr-52A, Ser-52, Lys-56, Tyr-58, Ala-98, Ala-100, Arg-100A, and CDR light chain residues Trp-91 and Phe-96 (Fig. 3) (numbered using the Kabat-Wu system (33)). Light chain residue Phe-96 and heavy chain residues Ile-50 and His-35 constitute the bottom of the pocket. The wall of the pocket is built in part by heavy chain residues Tyr-58, Lys-56, and Ser-52 and in part by light chain residue Trp-91. Heavy chain residue Ala-100 defines a protrusion in wall of the hydrophobic pocket. The presence of glycine...
instead of alanine at this residue greatly reduced the affinity between this antibody and the Factor IX Gla domain (13), illustrating the importance of the shape of this pocket. The hydrophobic pocket accommodates the antigenic determinant within Factor IX, including Lys-5, Leu-6, Phe-9, Val-10, and Gln-11. Polar interactions are limited to the electrostatic interaction between Lys-5 on Factor IX and Asp-93 and Asn-31 on the antibody light chain. Only four hydrogen bonds were found between 10C12 and Factor IX-(1–47) (Table II).

A representative electron density map of one region of the antibody-antigen interface is shown in Fig. 4. The conformations of most of the residues are well defined by the electron density map. This map illustrates a unique conformation of the 10C12 CDR3 loop of the heavy chain (residues 96H–100H), a tight turn characterized by a hydrogen bond between side chains in addition to the usual main chain hydrogen bond between i and i+3 residues.

Gln-11 of Factor IX-(1–47) interacts with all three CDR loops of the Fab heavy chain. The amide nitrogen atom of Gln-11 forms a hydrogen bond (2.49 Å) to the 10C12 heavy chain residue Thr-31. Water molecules also mediate the interaction between Factor IX and the antibody. A cavity identified in the antibody-antigen interface is occupied by a water molecule (S148).

**Crystal Structure of Factor IX Gla Domain**

Calcium ions are required for the folding of Gla domains into functional membrane binding structures. In our Factor IX-(1–47) structure, a total of six calcium ions were observed. γ-Carboxyglutamic acid plays a critical role in the coordination of calcium ions in Factor IX as it does in the other known vitamin K-dependent blood coagulation and regulatory proteins. Ca-4 is unique in that it is held in place by carboxyl groups contributed by four γ-carboxyglutamic acid residues. The calcium ion coordination for Factor IX-(1–47)-10C12 Fab is shown in Table III. Five calcium ions (Ca-1 to Ca-5) are aligned (3.7–4.0-Å spacing) at the core of the Gla domain.
Table II

| Hydrogen bonds | Factor IX-(1–47) | 10C12 | Distance Å |
|----------------|------------------|-------|------------|
| Phe-9 (O)      | Tyr-52AH (N)     | 2.98  |            |
| Gln-11 (NE2)   | Thr-31H (OG1)    | 2.49  |            |
| Lys-5 (NZ)     | Asp-93L (OD1)    | 2.81  |            |
| Lys-5 (NZ)     | Asn-31L (OD1)    | 2.74  |            |

van der Waals contacts

| Leu-6 (CB)     | Ala-100H (CB)    | 3.68  |            |
| Leu-6 (CD1)    | Trp-91L (CZ3)    | 3.76  |            |
| Leu-6 (CD1)    | Trp-91L (CE3)    | 3.47  |            |
| Leu-6 (CD2)    | Trp-91L (CH2)    | 3.50  |            |
| Leu-6 (CD2)    | Trp-91L (CZ3)    | 3.56  |            |
| Leu-6 (CD2)    | Tyr-58H (CE2)    | 3.39  |            |
| Leu-6 (CD2)    | Tyr-58H (CD2)    | 3.38  |            |
| Leu-6 (CG)     | Trp-91L (CZ3)    | 3.82  |            |
| Leu-6 (CG)     | Trp-91L (CE3)    | 3.74  |            |
| Leu-6 (CG)     | Trp-91L (CD2)    | 3.89  |            |
| Gla-8 (C)      | Lys-56H (CD)     | 3.84  |            |
| Phe-9 (CA)     | Ser-52H (CB)     | 3.73  |            |
| Phe-9 (CA)     | Ser-52H (CA)     | 3.88  |            |
| Phe-9 (CA)     | Ser-52H (CB)     | 3.89  |            |
| Phe-9 (CD1)    | Ser-52H (CA)     | 3.64  |            |
| Phe-9 (CD1)    | Ser-52H (CA)     | 3.76  |            |
| Phe-9 (CD1)    | Ile-50H (C)      | 3.74  |            |
| Phe-9 (CD2)    | Ile-50H (C)      | 3.76  |            |
| Phe-9 (CD2)    | Tyr-58H (CE2)    | 3.59  |            |
| Phe-9 (CD1)    | Tyr-58H (CE2)    | 3.20  |            |
| Phe-9 (CE1)    | Tyr-58H (CD2)    | 3.69  |            |
| Phe-9 (CE1)    | Tyr-58H (CZ)     | 3.84  |            |
| Phe-9 (CE1)    | Lys-56H (CD)     | 3.42  |            |
| Phe-9 (CE2)    | Lys-56H (CA)     | 3.41  |            |
| Phe-9 (CE2)    | Lys-56H (CB)     | 3.41  |            |
| Phe-9 (CE2)    | Lys-56H (CB)     | 3.88  |            |
| Phe-9 (CZ)     | Tyr-58H (CZ)     | 3.68  |            |
| Phe-9 (CZ)     | Tyr-58H (CE2)    | 3.40  |            |
| Phe-9 (CZ)     | Tyr-58H (CD2)    | 3.45  |            |
| Phe-9 (CZ)     | Tyr-58H (CZ)     | 3.80  |            |
| Phe-9 (CZ)     | Lys-56H (CB)     | 3.87  |            |
| Val-10 (C)     | Tyr-52AH (CD1)   | 3.70  |            |
| Val-10 (CG1)   | Ala-98H (CA)     | 3.85  |            |
| Gla-11 (CA)    | Tyr-52AH (CZ)    | 3.87  |            |
| Gla-11 (CA)    | Tyr-52AH (CE1)   | 3.60  |            |
| Gla-11 (CA)    | Tyr-52AH (CD1)   | 3.67  |            |

FIG. 4. Electron density map of the CDR3 loop of the heavy chain of 10C12 Fab and the ω loop of Factor IX (1–47). The representative electron density net is shown in cyan. The Factor IX Gla domain ω loop is shown with carbon atoms in gray. The 10C12 Fab CDR H3 loop is shown with carbon atoms in magenta. The 10C12 H3 loop forms a tight β-turn stabilized by two hydrogen bonds (red dashed line). For clarity, the electron density associated with Ca-4 and Ca-5 has been deleted.

FIG. 5. Gla domain of Factor IX. The Gla domain structure shown as a stereoimage includes calcium ions 1, 2, 3, 4, 5, and 7 as black spheres), residues 5, 6, 9, and 10 in magenta, cysteines 18 and 23 in yellow, tyrosine 45 in green, Gla residue side chains and peptide backbone in Corey-Pauling-K. Hung coloring.
mains were seen in the ω loop region (31), perhaps corresponding to sequence variability in this region (Fig. 2). Some differences in the orientation of the side chains are also significant. Factor VII and Factor X Gla domains have a Gla residue, Gla-6, that bridges Ca-4 and Ca-5 with both carboxylate groups of its side chain (Fig. 8). This Gla residue, along with three other loop residues (residues 3–5), participates in a tight β-turn. In Factor IX-(1–47), the side chain of this Gla residue (Gla-7 in Factor IX numbering) rotates along its Cα-Cβ bond by −120°. Only one carboxylate group of its side chain bridges calcium ions 4 and 5. The other carboxylate group forms hydrogen bonds to the backbone nitrogen atoms at residues 3, 4, and 5. As a result, Ca-4 has one less oxygen ligand compared with the Factor VII and Factor X Gla domains. This rotation of the Gla-7 side chain stabilizes the ω loop into a unique conformation with all of the backbone nitrogen atoms in this region oriented toward the negatively charged Gla-7. This rotation of the Gla-7 side chain is not an artifact due to the antibody 10C12 binding nor is it unique to Factor IX. Bovine prothrombin fragment 1, which is similar to Factor IX in the insertion of a glycine in its N-terminal sequence (Fig. 2), also shows a Gla-7 conformation similar to that in Factor IX. Unlike Fac-

### TABLE III

Comparison of the calcium ion coordinations for Factor IX-(1–47)

|   | NMR-derived Factor IX structure (5) | Molecular Dynamics-derived Factor IX structure (8) | X-ray-derived Factor IX structure (This work) | X-ray-derived Factor IX structure (9) |
|---|------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Ca-1 | Gla-26-OE1 | Gla-26-OE1 | Gla-26-OE1 | Gla-26-OE1(eq. to OE1) |
|      | Gla-26-OE4 | Gla-26-OE4 | Gla-26-OE4 | Gla-26-OE3(eq. to OE4) |
|      | Gla-30-OE1 | Gla-30-OE1 | Gla-30-OE3 | Gla-30-OE2(eq. to OE1) |
|      | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 |
|      | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE3(eq. to OE4) | Gla-8-OE4 |
|      | Gla-30-OE2 | Gla-27-OE1 | Gla-27-OE1 | H2O 159 |
| Ca-2 | Gla-8-OE2 | Gla-8-OE2 | Gla-8-OE2 | Gla-8-OE1(eq. to OE2) |
|      | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 |
|      | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 |
|      | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE3(eq. to OE4) | Gla-8-OE4 |
|      | Gla-30-OE2 | Gla-27-OE1 | Gla-27-OE1 | H2O 159 |
| Ca-3 | Gla-7-OE3 | Gla-7-OE3 | Gla-7-OE3 | Gla-27-OE2 |
|      | Gla-6-OE1 | Gla-6-OE1 | Gla-6-OE2 | Gla-27-OE2 |
|      | Gla-27-OE2 | Gla-27-OE2 | Gla-27-OE2 | Gla-27-OE2 |
|      | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 |
|      | Gla-8-OE2 | Gla-8-OE2 | Gla-8-OE4 | Gla-8-OE2(eq. to OE4) |
|      | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 |
|      | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 | Gla-30-OE4 |
|      | Gla-17-OE1 | Gla-17-OE1 | Gla-17-OE1(eq. to OE2) | Gla-17-OE1 |
|      | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 | Gla-30-OE3 |
| Ca-4 | Gla-27-OE1 | Gla-27-OE1 | Gla-27-OE1(eq. to OE1) | Gla-27-OE2 |
|      | Gla-27-OE4 | Gla-27-OE4 | Gla-27-OE4 | Gla-27-OE4 |
|      | Gla-17-OE4 | Gla-17-OE4 | Gla-17-OE4 | Gla-17-OE4 |
|      | Gla-7-OE3 | Gla-7-OE3 | Gla-7-OE3 | Gla-7-OE3(eq. to OE1) |
|      | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4(eq. to OE2) |
|      | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3(eq. to OE3) |
|      | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2(eq. to OE3) |
|      | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4(eq. to OE4) |
|      | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 |
| Ca-5 | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4 |
|      | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2 |
|      | Tyr-1-O | Tyr-1-O | Tyr-1-O | Tyr-1-O |
|      | Ser-3-OG | Ser-3-OG | Ser-3-OG | Ser-3-OG |
|      | Gla-7-OE1 | Gla-7-OE1 | Gla-7-OE1 | Gla-7-OE3(eq. to OE1) |
|      | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4 | Gla-7-OE4(eq. to OE2) |
|      | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3(eq. to OE3) |
|      | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2(eq. to OE3) |
|      | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4(eq. to OE4) |
|      | Gla-17-OE1 | Gla-17-OE1 | Gla-17-OE1 | Gla-17-OE1(eq. to OE2) |
|      | Gla-17-OE4 | Gla-17-OE4 | Gla-17-OE4 | Gla-17-OE4(eq. to OE3) |
|      | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3 | Gla-17-OE3(eq. to OE3) |
|      | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2 | Gla-17-OE2(eq. to OE3) |
|      | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4 | Gla-8-OE4(eq. to OE4) |
|      | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 | Asn-2-OD1 |
| Ca-6 | Gla-21-OE1 | Gla-21-OE1 | Gla-21-OE1 | Gla-21-OE2(eq. to OE1) |
|      | Gla-21-OE4 | Gla-21-OE4 | Gla-21-OE4 | Gla-21-OE2(eq. to OE4) |
| Ca-7 | Gla-15-OE2 | Gla-15-OE2 | Gla-15-OE2 | Gla-15-OE1(eq. to OE2) |
|      | Gla-20-OE1 | Gla-20-OE1 | Gla-20-OE1 | Gla-20-OE1(eq. to OE3) |
|      | Gla-20-OE3 | Gla-20-OE3 | Gla-20-OE3 | Gla-20-OE3(eq. to OE4) |
|      | Gla-15-OE4 | Gla-15-OE4 | Gla-15-OE4 | Gla-15-OE4 |
|      | Gla-30-OE1 | Gla-30-OE1 | Gla-30-OE1 | Gla-30-OE2 |
|      | Gla-30-OE2 | Gla-30-OE2 | Gla-30-OE2 | Gla-30-OE4 |
|      | Gla-33-OE3 | Gla-33-OE3 | Gla-33-OE3 | Gla-36-OE2 |
|      | Gla-33-OE4 | Gla-33-OE4 | Gla-33-OE4 | Gla-36-OE3 |
| Ca-9 | Gla-36-OE4 | Gla-36-OE4 | Gla-36-OE4 | Gla-36-OE1 |
|      | Gla-40-OE3 | Gla-40-OE3 | Gla-40-OE3 | Gla-40-OE1 |
The crystal structure of Factor IX Gla domain was determined to understand the calcium-ligand network and its interaction with Factor VIIIa. The antibody 10C12 was identified from a human scFv library and showed specificity for Factor IX but not Factor VIIIa. The antibody was reconfigured into a F(ab')2 format and was shown to be functionally active, prolonging the partial thromboplastin time and inhibiting platelet-mediated blood coagulation.

**DISCUSSION**

Conformation-specific antibodies directed at calcium-stabilized structures of the vitamin K-dependent proteins have been useful probes of protein structure. They have identified regions that undergo ligand-induced conformational transitions and have served as solid phase immunoaffinity reagents that offer facile release of the antibody-protein complex by the addition of EDTA. These antibodies have provided novel inhibitors of protein-membrane interaction.

**Calcium Ion Coordination**—We have identified six bound calcium ions in the Factor IX Gla domain of the human Factor IX-(1–47)-10C12 antibody complex. We used high concentrations of ammonium sulfate during crystallization of this complex and thus observed occupancy in only the higher affinity binding sites. In contrast, Shikamoto et al. (9) observed eight calcium ions in their crystal structure of bovine Factor IX-(1–46) in the Factor IX-(1–46)-FIX-binding protein complex. These crystals were prepared in Tris-HCl, pH 8.0, 14% polyethylene glycol, and 5 mM CaCl₂, a system in which the free calcium ion concentration would be much higher than in ammonium sulfate. However, our recent efforts to crystallize the human Factor IX-(1–47)-10C12 antibody complex using polyethylene glycol and 5 mM CaCl₂, a system in which the free calcium ion concentration would be much lower than in ammonium sulfate. However, our recent efforts to crystallize the human Factor IX-(1–47)-10C12 antibody complex using polyethylene glycol were unsuccessful. We believe that the lower affinity sites, including Ca-6 and Ca-8, were not occupied by calcium ions under our crystallization conditions because of the reduced free calcium concentration. The suggestion that Ca-6 is a low affinity site is significant because we have shown that the serine head group of lysophosphatidylserine displaces water from the coordination sphere of Ca-6 (2). The ternary complex of Ca-6, phosphatidylserine, and Factor IX may be character-
ized by tighter binding of calcium than this complex in the absence of phosphatidylserine. Ca-8, coordinated by Gla-36 and Gla-40, has no apparent functional role because elimination of these γ-carboxyglutamic acid residues does not alter the biological assays tested (34).

The positions of the six common calcium ions bound to the Factor IX Gla domain in both our human Factor-(1–47)-10C12 complex and in the bovine Factor-(1–46)-FIX-binding protein complex are nearly identical (9). Comparison of the positions of these calciums, including Ca-1 to Ca-5 and Ca-7, revealed a r.m.s.d. of 0.203 Å compared with the overall r.m.s.d. of 0.467 Å for 44 Gla domain Ca atoms. Furthermore, the coordination of these calcium ions in these two independent structures is nearly identical (Table III). Of 31 oxygen ligands to 6 calcium ions, all are identical in both structures with the exception of one. These results emphasize that the specific γ-carboxyglutamic acid-calcium network in Factor IX differs from other Gla domains.

Nine calcium ions were modeled into our NMR-derived Factor IX-(1–47)-calcium complex structure (5) as refined by molecular dynamics simulation with initial calcium positions determined by a genetic algorithm (8). Comparison of the calcium coordination of Factor IX-(1–47)-calcium from x-ray crystallography with models of Factor IX-calcium in solution derived by extension of the prothrombin structure or molecular dynamics approaches revealed some excellent predictions and several examples where modeling proved less successful. For example, the coordination of Ca-4 by carboxyl groups from Gla-27, Gla-7, and Gla-17 was predicted in the molecular dynamic analysis (Table III). The role of the carboxyl groups of Gla-17 and the hydroxyl O of Tyr-1 in coordinating Ca-5 was correctly predicted, but a role for the Gla-21 carboxyl was missed. Furthermore, a Gla-7 carboxyl group was proposed as a ligand but this was not observed in the crystal structure. These observations emphasize that the prothrombin calcium coordination, albeit similar, is not an adequate model for the Factor IX-calcium complex. In addition, molecular dynamic approaches can generate low energy models but the fine structure predicted is only a first approximation of the calcium coordination network present in Factor IX determined experimentally.

Fine Structure of the ω Loop—Although the polypeptide backbone of the ω loop in the Gla domain of the vitamin K-dependent proteins demonstrates marked structural similarity, the amino acid sequences in these regions exhibit both similarity and some critical differences. It would appear that the fine structure of the ω loop is defined by interaction of these critical side chains. We have demonstrated the special role of Gla-7 in Factor IX in orienting the polypeptide backbone and stabilizing the positions of the amide nitrogens by hydrogen bonding. This function is quite different from the homologous γ-carboxyglutamic acid residue in Factor X. We suspect that these structural differences play critical functional roles in the assembly of these proteins on membrane surfaces and during protein complex formation during blood coagulation. These structural differences appear significant and are not induced by the bound antibody because the bovine Factor-(1–46)-FIX-binding protein complex shares a similar structure.

Factor IX Specificity of 10C12 Antibody—Despite the sequence similarity, 10C12 antibody recognizes only Factor IX-(1–47) but not the Gla domains of other coagulation proteins. The Factor IX-(1–47)-10C12 structure provides a structural basis for this specificity. Table II shows all of the hydrogen bonds and van der Waals interactions between the Factor IX-(1–47) and 10C12 Fab. Of four hydrogen bonds, the Lys-5 side chain of the Gla domain forms two hydrogen bonds to the light chain of 10C12 Fab. One of the hydrogen bonds is between oppositely charged Lys-5 of the Gla domain and Asp-93L of the 10C12 Fab. This interaction is stronger than a normal hydrogen bond (4.5 versus 2–3 kcal/mol). Lys-5 is unique to Factor IX. All of the other Gla domain sequences have a hydrophobic residue at the corresponding position (Fig. 2). Thus, this lysine residue constitutes a major structural determinant targeted by 10C12 Fab. Hydrophobic interactions play an important role in Factor IX Gla domain-10C12 antibody binding and contribute to antibody specificity. CDR loops of 10C12 antibody form a hydrophobic pocket to accommodate the hydrophobic residues Leu-6, Phe-9, and Val-10. Although Leu-6 is conserved among Gla domains, a bulky hydrophobic residue at position 9 is unique to Factor IX.

Structural Basis of Hemophilia B Mutations in the Factor IX Gla Domain—The crystal structure of the Factor IX-(1–47) provides a framework to analyze the structural and functional consequences of naturally occurring genetic alterations in patients with hemophilia B, particularly those involving the ω loop. Naturally occurring point mutations of the Factor IX Gla domain in which the normal amino acid is substituted by another amino acid are known to occur at residues 2–9, 12, 17, 18, 20, 21, 23, 25–27, 29, 32, 33, 38, 41, 43, 45, and 46 (Ref. 41 and www.kcl.ac.uk/ip/petergreen/fix2.html), and the structure of the Gla domain of Factor IX will facilitate the understanding of structure-function relationships.

A hemophilia mutation at residue 2 (Asn → Ile) reduces circulating Factor IX antigen level to ~60% but lowers the functional activity of the mutant Factor IX to 1% of that in wild type Factor IX (42). Our Factor IX Gla structure indicates that Asn-2 is a structurally important residue. Its side chain forms hydrogen bonds to Gla-8 and Gla-27 and is a ligand for Ca-4. As the N terminus anchors one end of ω loop, the mutation of this residue will lead to destabilization of the ω loop.

Plasma from a naturally occurring hemophilia B mutant, lysine 5 → glutamate, has normal antigen levels but low Factor IX activity (43). Our Factor IX-(1–47) structure shows that Lys-5 is a surface-exposed residue and its side chain does not have a structural role in stabilizing the Gla domain. We speculate that this side chain participates in binding the phosphate group of phosphatidylserine in a manner analogous to the role of Lys-3 in prothrombin (2). Based upon the in vivo endothelial binding characteristics of recombinant forms of Factor IX mutated at residue 5, Gui et al. (44) have suggested that this residue is important for binding to collagen IV.

Gla-7 coordinates both the backbone of the ω loop and calcium 4 and 5. Naturally occurring mutations at this position greatly reduce activity, presumably by disrupting the integrity of this region.

Implication for the Mechanisms of Gla Domain Anchoring on Cell Surfaces—The Gla domains of vitamin K-dependent proteins mediate the binding of blood coagulation proteins to membrane surfaces. The Gla domains in these coagulation proteins share a high degree of sequence similarity, but their binding affinity for membrane surfaces vary over three orders of magnitude with dissociation constants ranging from nanomolar to micromolar (45). The presence of phosphatidylserine in the membrane is necessary for maximal Gla domain binding to phospholipid vesicles. Gla domains in the absence of calcium ions are largely disordered (3, 6, 39, 46). Calcium ions induce conformational changes in the Gla domain and are necessary for the Gla domain to fold properly. A common structural feature of functional Gla domains is the clustering of N-terminal hydrophobic residues into a hydrophobic patch and the exposure of this hydrophobic patch to solvent. Although this hydrophobic patch mediates Gla domain interaction with the
cell surface membrane and represents a major component of the phospholipid binding site, the specificity for phosphatidylserine is defined by electrostatic interactions between the phosphoserine head group and arginine and lysine residues in the Gla domain of prothrombin. We suspect that Lys-5 in Factor IX plays a role analogous to Lys-3 in prothrombin where, along with Arg-10, the positively charged amino acids bind to the glycerol phosphate backbone and the carboxyl group of the serine interacts with Ca-5 and Ca-6 (2).

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REFERENCES
1. Furie, B., and Furie, B. C. (1988) Cell 53, 505–518
2. Huang, M., Rigby, A. C., Morelli, X., Grant, M. A., Huang, G., Furie, B., Seaton, B., and Furie, B. C. (2003) Nat. Struct. Biol. 10, 751–756
3. Brandstetter, H., Bauer, M., Huber, R., Lollar, P., and Bode, W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9796–9800
4. Freedman, S. J., Blostein, M. D., Baleja, J. D., Jacobs, M., Furie, B. C., and Baleja, J. D. (1995) J. Biol. Chem. 270, 7980–7987
5. Soriano-Garcia, M., Padmanabhan, K., de Vos, A. M., and Tulinsky, A. (1992) Biochemistry 31, 2554–2566
6. Li, L., Darden, T. A., Freedman, S. J., Furie, B. C., Furie, B., Baleja, J. D., Smith, H., Hickey, R. G., and Pedersen, L. G. (1997) Biochemistry 36, 2132–2138
7. Shikamoto, Y., Morita, T., Fujimoto, Z., and Mizuno, H. (2003) J. Biol. Chem. 278, 24990–24994
8. Banner, D. W., D’Arcy, A., Chene, C., Winkler, F. K., Guha, A., Konigsberg, W. H., Nemerson, Y., and Kirchhofer, D. (1996) Nature 380, 41–46
9. Mizuno, H., Fujimoto, Z., Atoda, H., and Morita, T. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7230–7234
10. Zhou, Y., Morais-Cabral, J. H., Kaufman, A., and MacKinnon, R. (2001) Nature 414, 43–48
11. Suggett, S., Kirchhofer, D., Hass, P., Lipari, T., Moran, P., Nagel, M., Judice, K., Schroeder, K., Tom, J., Lowman, H., Adams, C., Eaton, D., and Devaux, B. (2000) Blood Coagul. Fibrinolysis 11, 27–42
12. Lewis, R. M., Furie, B. C., and Furie, B. (1983) Biochemistry 22, 948–954
13. Borowski, M., Furie, B. C., Bauminger, S., and Furie, B. (1986) J. Biol. Chem. 261, 14899–14975
14. Liebman, H. A., Furie, B. C., and Furie, B. (1987) J. Biol. Chem. 262, 7605–7612
15. Refino, C. J., Himber, J., Burcklen, L., Moran, P., Peek, M., Suggett, S., Devaux, B., and Kirchhofer, D. (1999) Thromb. Haemostasis 82, 1188–1195
16. Refino, C. J., Jeet, S., DeGuzman, L., Bunting, S., and Kirchhofer, D. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 517–522
17. Orwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
18. Navaza, J. (1994) Acta Crystallogr. Sect. A 50, 157–163
19. Guex, N., and Peitsch, M. C. (1997) Electrophoresis 18, 2714–2723
20. Brungger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, S. S., Kuwaski, 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
21. Read, R. J. (1996) Acta Crystallogr. Sect. A 42, 140–149
22. Jones, T. A., Zou, J.-Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
23. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
24. Eannouf, R. M. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 938–940
25. Merritt, E. A., and Bacon, D. J. (1997) Methods Enzymol. 277, 502–524
26. Nicholls, A., Sharp, K. A., and Honig, B. (1991) Proteins 11, 281–296
27. McDonald, I. K., and Thornton, J. M. (1994) J. Mol. Biol. 238, 777–783
28. Wallace, A. C., Laskowski, R. A., and Thornton, J. M. (1995) Protein Eng. 8, 127–134
29. Huang, M., Syed, R., Stura, E. A., Stone, M. J., Stefanko, R. S., Ruf, W., Edgington, T. S., and Wilson, I. A. (1998) J. Mol. Biol. 283, 873–894
30. Wilson, I. A., and Stanfield, R. L. (1994) Curr. Opin. Struct. Biol. 4, 857–867
31. Wu, T. T., and Kabat, E. A. (1970) J. Exp. Med. 132, 211–250
32. Gillis, S., Furie, B. C., Furie, B., Patell, H., Huberty, M. C., Switzer, M., Foster, W. B., Scoble, H. A., and Bond, M. D. (1997) Protein Sci. 6, 185–196
33. Furie, B., and Furie, B. C. (1979) J. Biol. Chem. 254, 9766–9771
34. Tai, M. M., Furie, B. C., and Furie, B. (1980) J. Biol. Chem. 255, 2790–2795
35. Wojcik, E. G., Cheung, W. F., van der Berg, M., van der Linden, J. K., Stafford, D. W., and Bertina, R. M. (1996) Biochim. Biophys. Acta 1382, 91–101
36. Liebman, H. A., Limentani, S. A., Furie, B. C., and Furie, B. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 5879–5883
37. Sunnerhagen, M., Forsen, S., Hoffren, A. M., Drakenberg, T., Teleman, O., and Stenflo, J. (1995) Nat. Struct. Biol. 2, 504–509
38. Kelbert, L. R., Chan, J. C., Christiansen, W. T., and Castellino, F. J. (1996) Biochemistry 35, 7093–7099
39. Giannelli, F., Green, P. M., Sommer, S. P., Poon, M. C., Ludwigs, M., Dhillon, K. S., Gouseks, M., Yoshikawa, A., Figueiredo, M. S., and Brownlee, G. G. (1998) Nucleic Acids Res. 26, 265–268
40. Giannelli, F., Green, P. M., High, K. A., Sommer, S., Poon, M. C., Ludwigs, M., Dhillon, K. S., Reitsma, P. H., Goossens, M., Yoshikawa, A., and Brownlee, G. G. (1993) Nucleic Acids Res. 21, 3075–3087
41. Rowley, G., Saad, S., Giannelli, F., and Green, P. M. (1995) Genomics 30, 574–582
42. Gui, T., Lin, H. F., Jin, D. Y., Hoffman, M., Straight, D. L., Roberts, H. R., and Stafford, D. W. (2002) Blood 100, 153–158
43. McDonald, J. F., Shah, A. M., Schwalbe, R. A., Kisel, W., Dahlback, B., and Nelsestuen, G. L. (1997) Biochemistry 36, 5120–5127
44. Park, C. H., and Tulinsky, A. (1986) Biochemistry 25, 3977–3982
45. Luzzatti, V. (1967) Acta Crystallogr. 5, 802–810
46. Laskowski, R. A., MacArthur, M. W., Mass, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
Crystal Structure of the Calcium-stabilized Human Factor IX Gla Domain Bound to a Conformation-specific Anti-factor IX Antibody
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