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Identification of potential ferric binding residues in the iron-binding protein of pathogenic *Neisseria meningitidis* through structure-based multiple sequence alignments

Beatrice Gorinsky[a,*], Robert W. Evans[b]

[a] *Department of Crystallography, Birkbeck College, Malet Street, London WC1 7HX, UK*
[b] *Division of Biochemistry and Molecular Biology, UMDS, Guy's Hospital, London SE1 9RT, UK*

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Abstract The ferric iron-binding proteins of pathogenic *Neisseria* display structural and metal-binding properties characteristic of the transferrin family. In the absence of structural data for the ferric iron-binding proteins, spacial folding templates have been derived for the meningococcal protein from complete and partial structure-based multiple sequence alignments with structurally related proteins. The templates have been used to identify a number of potential iron-binding residues. These include four residues that are identical with the iron coordinating ligands of transferrin, but only two reside within equivalent structural elements.

Key words: Ferric iron-binding protein; *Neisseria meningitidis*; Transferrin; Periplasmic binding protein; Structure prediction; Multiple sequence alignment

1. Introduction

The ferric iron-binding proteins (Fbps) and the transferrins exhibit similarities in their metal-binding and spectral properties and the nature of their coordinating ligands. The Fbps reversibly bind a single ferric ion per molecule of protein and function as periplasmic-binding proteins in the active transport of iron by pathogenic *Neisseria* [1–5]. Similarly the transferrins are a highly conserved family of vertebrate proteins that reversibly bind ferric ions, one in the N-terminal lobe and one in the C-terminal lobe of the protein [6–8]. Structural studies of the transferrins have shown that each ferric ion is octohedrally coordinated through four protein ligands, an aspartic acid residue from the c3 loop region, two tyrosine residues, from the g7 loop region and the first crossover strand, and a histidine residue from the second crossover strand, together with a synergistically bound carbonate ion, coordinated as a bidentate ligand. The carbonate ion is, in turn, coordinated by the protein through a cluster of three peptide NH groups, a threonine and an arginine residue from the f5 loop region. In the absence of structural data for the Fbps, apparent similarities in iron coordination between the two families have led to the proposition that the prokaryotic ferric iron-binding proteins are single-sited iron-binding analogues of the eukaryotic transferrins [8].

The Fbps are members of a well characterised family of periplasmic binding proteins (PBPs) that are responsible for the active transport of a wide variety of nutrients across the periplasmic space between the inner and outer cell membranes of Gram-negative bacteria [9–12]. The structures of a number of these proteins have been determined, ranging in weight from 23 to 52 kDa [13–17]. They consist of two α/β open-sheet domains formed from a single polypeptide chain and separated by a deep cleft containing the ligand-binding site. Topologically the PBPs resemble a half molecule of transferrin with two or three short peptide segments connecting domains which open and close via a hinge bending motion. As with the transferrins, ligand binding favours a 'closed' conformation, but in the absence of ligand, the 'open' and 'closed' forms of the PBPs are thought to be in equilibrium [17].

We have systematically employed complete and partial sequences of the proteins listed in Table 1 in structure-based multiple sequence alignments to derive spatial folding templates for the meningococcal Fbp (Fbp_neime). Using these templates we have identified nine 'transferrin-like' binding residues in regions adjacent to the substrate binding site of the protein, which are likely to include some or all of the ferric ion coordinating ligands of Fbp_neime. Four of these residues are identical with the iron coordinating ligands of transferrin but only two occupy equivalent positions on the protein structure.

2. Materials and methods

The nine proteins selected for these studies are listed in Table 1. Coordinates for the structures (codes 1PBP, 1SBP, 1HSL, 1LST and 1LFG, respectively) were obtained from the Brookhaven Protein Data Bank (PDB) [18]. For the purposes of this study, the individual N- and C-terminal lobes of lactoferrin (residues 1–333 and 345–691) have been treated as separate structures and assigned the codes 1LFGn and 1LFGc, respectively [19].

Structural data are not available for the two periplasmic ferric ion-binding proteins of *Neisseria meningitidis* and *Neisseria gonorrhoeae* (Fbp_neime and Fbp_neigo), or for the Fbp homologues identified in *Haemophilus influenzae* (HitA) and *Serratia marcescens* (SfuA), which are thought to perform similar iron-binding functions to the Fbps in their respective iron transport systems [20–22]. In keeping with other related homologues, the functional iron-binding residues are presumed to be conserved within the existing sequence identity [20].

The protein structures were examined using the RasMol2 molecular graphics visualisation program (copyright 1992–1994) by R. Sayle on a Silicon Graphics IRIS 4D/50GT obtained from Silicon Graphics Computer Corporation, Mountain View, California, USA. Secondary structure assignments were obtained using the Kabsch and Sander DSSP algorithm [23] as implemented in the same RasMol2 program. Functionally important residues in each structure were identified through a combination of viewing on the graphics, analysis of the polar contacts, and information in the literature.

Helix and strand notations correspond to the equivalent secondary structure in lactoferrin [19]. Loop and turn regions are defined by the circumscribing elements of secondary structure. 'Transferrin-like'

*Corresponding author. Fax: (44) (171) 631 6803.
E-mail: b.gorinsky@iona.cryst.bbk.ac.uk

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binding residues are defined as the residues involved in the coordination of ferric ions by members of the transferrin family, namely aspartic acid, tyrosine and histidine residues.

Interatomic distances and polar interactions (hydrogen bonds, salt bridges and water bridges) between the peptide residues and bound ions or molecules were obtained using the QUANTA program provided by the Polygen Corporation, Waltham, Mass., USA on a Silicon Graphics IRIS 4D/50GT obtained from Silicon Graphics Computer Corporation, Mountain View, California, USA.

Protein sequences were obtained from the literature, the PDB and the OWL data base release 9 (A.J. Bleasby and D. Akrigg, Protein Engineering Club, Leeds University, UK) [24,25].

Structure prediction assignments were obtained using the Leeds Secondary Structure Prediction Suite, Vax version 3.0, 20 September 1988 (E. Eliopoulis, Leeds University). Data was output graphically as a consensus prediction from eight secondary structure prediction methods using a program written by Roman Laskowski [29]. Estimates of the accuracy for secondary structure predictions for the Fbp iron-binding homologues were obtained by comparing the predicted and calculated structural assignments for residues from equivalent elements of core structure in 1PBP, 1SBP, 1HSL, 1LST, 1LFGn and 1LFGc. The parameter used to measure the accuracy of the secondary structure assignments within each structural element was calculated as the total percentage of residues correctly predicted. Reliabilities were found to vary widely for the different elements, ranging from 93.5% for residues of helix 9 to 7.4% for residues of helix 7, and

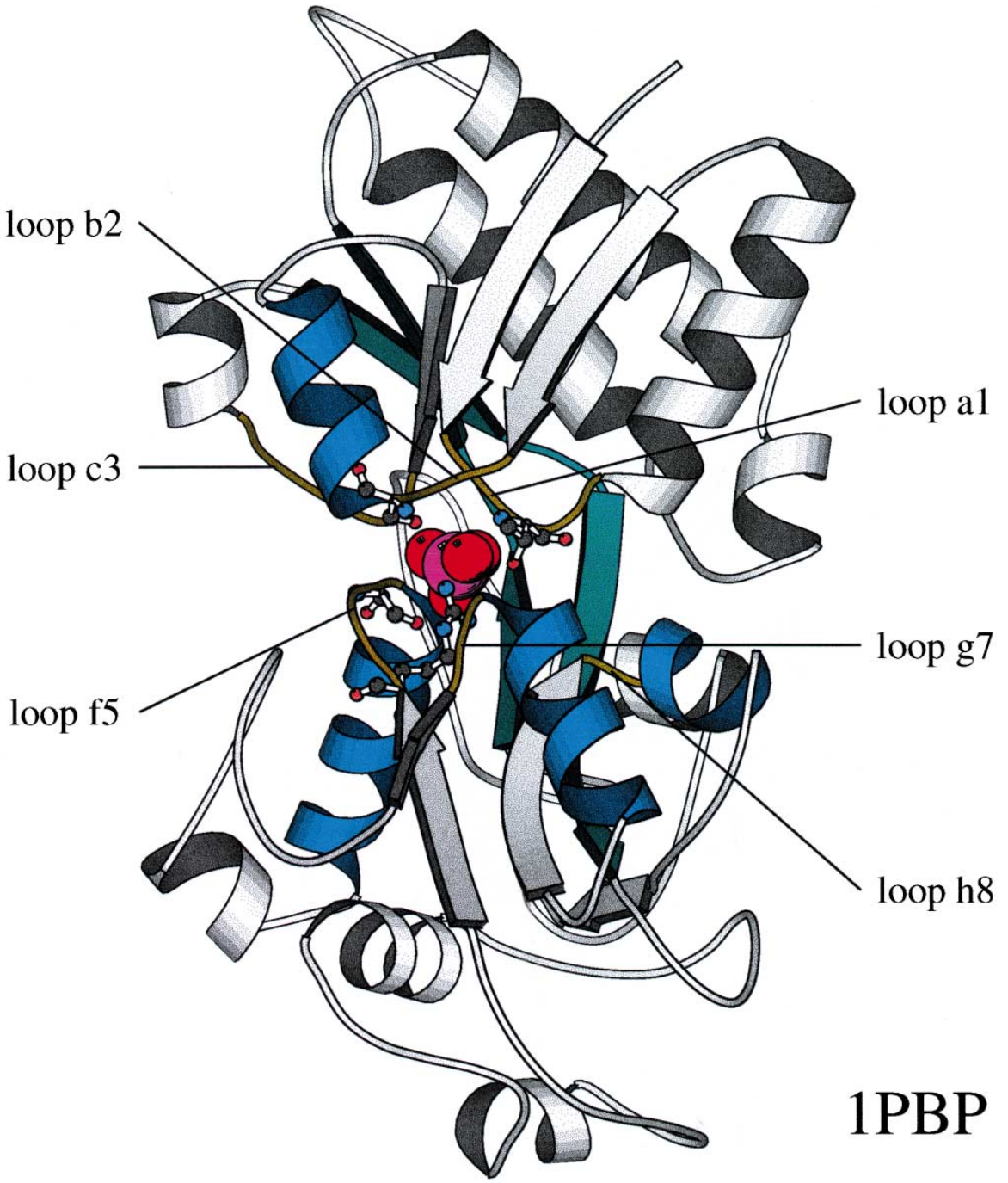


Fig. 1. A Molscript diagram of phosphate-binding protein with bound phosphate (1PBP). The phosphate anion associated with this structure is shown in CPK representation indicating where the functionally important region lies [30]. Residues interacting with the bound phosphate through side-chain groups are shown in ball-and-stick representation. Helices 2, 5, 7 and 8 are highlighted in blue [19]. Loops a1, b2, c3, f5, g7 and h8 are highlighted in yellow and the first and second inter-domain crossover strand regions are highlighted in green.

Table 1
Nine proteins employed in the structure-based multiple sequence alignments

Protein	PDB/identity code	Resolution (Å)	Source	Number of residues	Sequence identity with Fbp_neime (%)
Phosphate-binding protein	1PBP	1.90	*Escherichia coli* Thr-141/Asp mutant	321	16.1
Sulphate-binding protein	1SBP	1.7	*Salmonella typhimurium*	309	16.2
Histidine-binding protein	1HSL	1.89	*Escherichia coli*	238	23.8
Lysine-, arginine-, ornithine-binding protein	1LST	1.8	*Salmonella typhimurium*	238	18.5
Lactoferrin	1LFG	2.2	Human	691	17.5 (N-lobe) 18.2 (C-lobe)
Ferric iron-binding protein	Fbp_neime	N/A	*Neisseria meningitidis*	308	100
Ferric iron-binding protein	Fbp_neigo	N/A	*Neisseria gonorrhoeae*	308	99.1
SfuA protein	SfuA	N/A	*Serratia marcescens*	309	37.9
HitA protein	HitA	N/A	*Haemophilus influenzae*	309	69.4

from 78.9% for residues of β-strand b to 20.0% for residues of β-strand i. All structural elements with reliabilities > 87% were found to contain some correctly predicted secondary structure, whereas up to 20% of elements with reliabilities between 60% and 87% did not contain any correctly predicted secondary structure. Secondary structure in elements with reliabilities below 60% could not be predicted with any certainty.

Multiple sequence alignments were performed using the MALIGN program [26] which is based on the progressive alignment procedure of Feng and Doolittle [27,28].

3. Results and discussion

A systematic study of the PBP structures lodged in the Brookhaven database identified four members (codes 1PBP, 1SBP, 1HSL and 1LST) that exhibit a high degree of structural similarity with the individual N- and C-terminal lobes of lactoferrin (1LFGn and 1LFGc). Fig. 1 shows a MolScript diagram of one of these structures (1PBP). Similarities are

Table 2
Protein interactions with bound ligands

Protein	Substrate	a1 loop region	b2 loop region	c3 loop region		1st cross-over strand	f5 loop region			g7 loop region	h8 loop region	2nd cross-over strand
		(loop a1)	(helix 2)	(loop c3)	(helix 3)	(strand d-strand e)	(strand f)	(loop f5)	(helix 5)	(helix 7)	(strand h)	(strand i-strand j)
Phosphate-binding protein	Phosphate ion	T-10(N, OG1)[a] F-11(N)[a]	S-38(N, OG)[a]				R-135(N-H1,2)[a]	S-139 (OG)[a]	G-140(N)[a] D-141(N)[a]			
Sulphate-binding protein	Sulphate ion	D-11(N)[a]	S-45(N)[a]					S-130 (OG)[a]	G-131(N)[a] G-132(N)[a]	A-173 (N)[a]	W-192 (NE1)[a]	
Histidine-binding protein	Histidine molecule			S-70(O)[b] S-72(N)[a] S-72 (OG)[a,b]	R-77 (NH1)[a] R-77 (NH2)[c]				T-121(N)[c]		D-161 (OD1)[b]	
Lysine-binding protein	Lysine molecule	D-11 (OD2)[d]		S-70(O)[b] S-72(N)[a] S-72 (OG)[b]	R-77 (NH1,2)[a]				T-121(N)[a]		D-161(O-D1,OD2)[b]	
Lactoferrin (1LFGn)	Ferric/ carbonate ion pair				D-60 (OD1)[b]	Y-92(OH)[b]		T-117 (OG1)[a]	R-121(N-E,NH2)[a] T-122(N)[a] A-123(N)[a] G-124(N)[a]	Y-192 (OH)[b]		H-253 (NE2)[b]
Lactoferrin (1LFGc)	Ferric/ carbonate ion pair				D-395 (OD1)[b]	Y-435(OH)[b]		T-461 (OG1)[a]	R-465 (NE)[a] T-466(N)[a] A-467(N)[a] G-468(N)[a]	Y-528 (OH)[b]		H-597 (NE2)[b]

[a]Interacts with anion.
[b]Interacts with cation.
[c]Interacts with main-chain carbonyl group.
[d]Interacts with Lys(NZ) group.

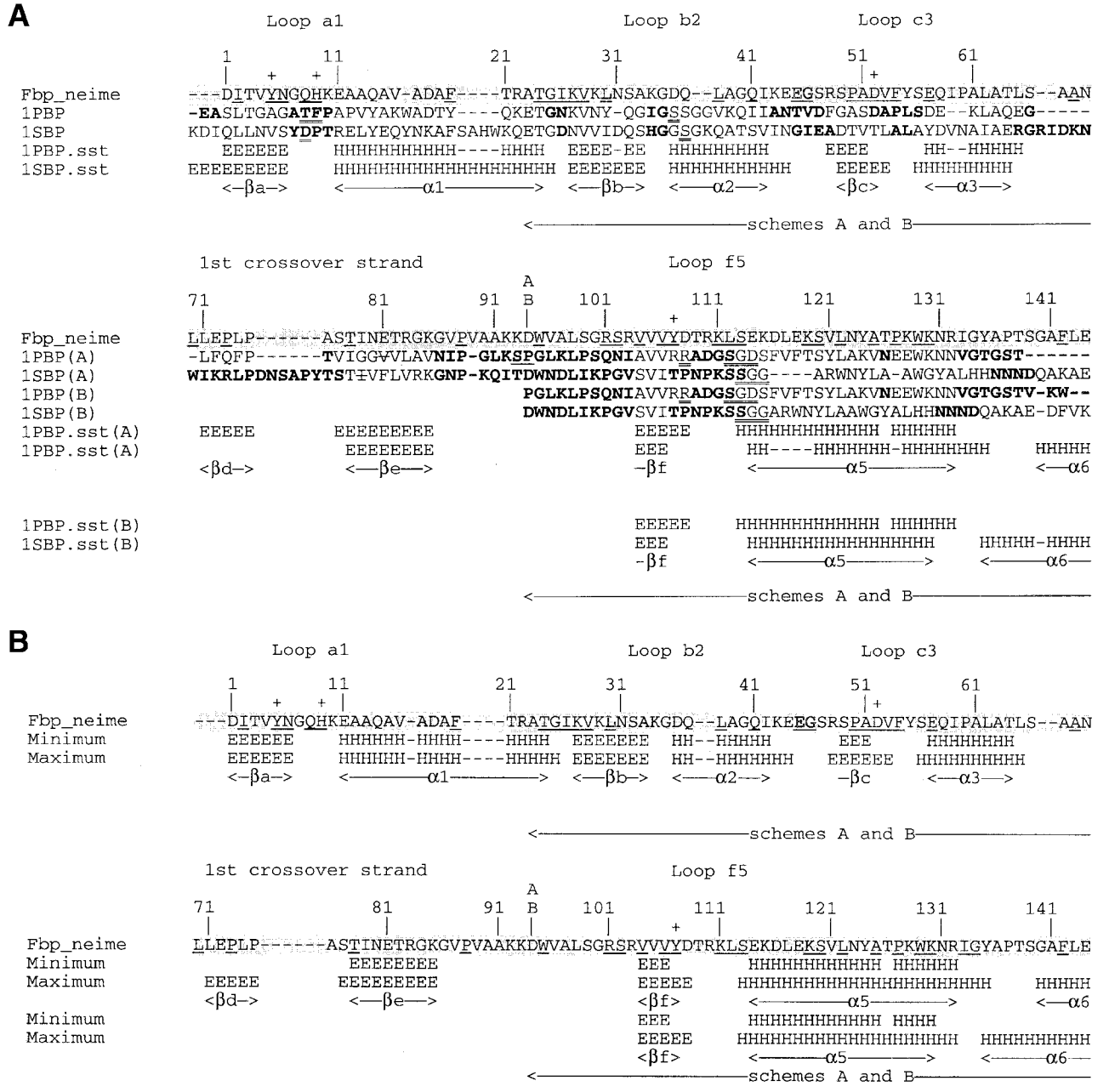
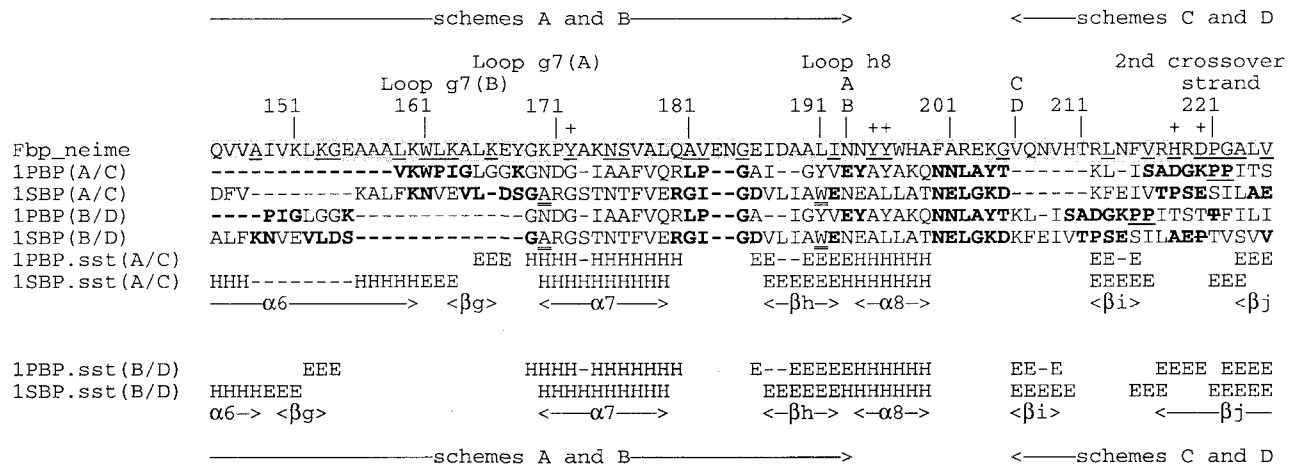


Fig. 2. (A) Sequence alignment of Fbp_neime, 1PBP and 1SBP used for deriving the Fbp_neime template. 1PBP and 1SBP residues from regions classified as 'strand' or 'helix' structure in the Molscript outputs are depicted in normal lettering while regions classified as 'coil' or 'turn', and the first and last residues of consecutive structural elements not separated by 'coil' or 'turn' residues, are depicted in bold lettering. Residues interacting with the bound ligand through main-chain or side-chain groups are doubly underlined. Non-interacting residues from the crossover strands of 1PBP and 1SBP, that most closely approach the bound ligand, are denoted by barred lettering. Fbp_neime residues conserved between Fbp_neime, Fbp_neigo, SfuA and HitA are underlined. The nine putative Fbp_neime iron-binding residues are marked by a +. The secondary structure of 1PBP and 1SBP, as defined in the program DSSP [23], is shown by the lines 1PBP.sst and 1SBP.sst where H denotes helix and E is beta structure. The underlined residues SP and PP in the 1PBP sequence denote residues Ser-90 and Pro-121 and Pro-216 and Pro-251 respectively and identify points from which structurally variable regions have been deleted in the alignment. (B) Consensus secondary structure assignments for the Fbp_neime residues as defined from the alignment of 1PBP and 1SBP sequences. The minimum consensus shows the positions where H and E are common across both known 3D structures for each position in the Fbp_neime alignment and the maximum consensus shows the positions where H and E are present in one or both of the known 3D structures.

particularly evident in the relative orientations of helices 2, 5, 7 and 8 which extend out from the substrate-binding cleft [19]. The four PBPs selected bind either small anions or zwitterions (1PBP and 1SBP bind oxyanions, 1LST and 1HSL bind amino acids), while 1LFGn and 1LFGc bind ferric/carbonate ion pairs.

The six structures share a common core structure which has been individually modified and embellished. The relationship between the structurally conserved core regions and the structurally variable regions of the proteins was determined through a systematic study of their structural differences and similarities. Details of residue-substrate interactions in the six structures are set out in Table 2 and reveal the structural equivalence of many of the coordinating residues. Sub-



Scheme 1.

strate-binding residues are present on regions adjacent to the binding cleft, from among the a1, b2, c3, f5, g7, h8 loop regions and the two interdomain crossover strands, all of which must be regarded, in the first instance, as potential sites for iron-binding residues of Fbp_neime [19].

We identified potential substrate-binding regions in the Fbp_neime sequence using structure-based multiple sequence alignments to derive spatial folding templates for the meningococcal protein, compatible with the available structural data. These templates were obtained from a series of complete and partial multiple sequence alignments performed with different combinations of sequences using the MALIGN package [26]. Both multiple sequence data and information from structural and prediction data were incorporated in the alignment procedure. Where appropriate, ranges of sub-optimal alignments were also explored. Using the templates potential substrate-binding regions of Fbp_neime were identified and screened for 'transferrin-like' binding residues conserved between the four iron-binding homologues Fbp_neime, Fbp_neigo, HitA and SfuA. The f5 loop region was additionally screened for conserved residue clusters capable of binding a synergistic anion, since there appears to be a general propensity for this region to bind negative groups (Table 2). Sequence alignments for Fbp_neime, 1PBP and 1SBP are set out in Fig. 2 and the alignment conditions are listed in Table 3.

3.1. Threading on the a1, b2 and c3 loop regions in the first domain

This segment of polypeptide chain contains three potential substrate-binding loop regions, a1, b2 and c3 following β-strands a, b and c [19]. Threading the aligned Fbp_neime sequence onto the 1PBP backbone chain superimposes two conserved 'transferrin-like' binding residues, His-9 and Asp-52, on residues Phe-11 from the a1 loop and Asp-56 from the c3 loop of 1PBP. Phe-11 and Asp-56 are located adjacent to the phosphate-binding site of 1PBP and both side chains extend in the general direction of the binding cleft. The side chains of His-9 and Asp-52 may be similarly orientated towards the binding cleft in Fbp_neime, despite the fact that homologous structure-based alignments with proteins of low sequence homology, cannot be relied upon to align all structurally equivalent residues with absolute precision. A third conserved 'transferrin-like' binding residue, Tyr-5, appears to be a less satisfactory candidate iron ligand, as it is located at the edge of loop a1, and aligns with Ala-7 of 1PBP and Val-8 of 1SBP, which are well removed from their respective binding sites.

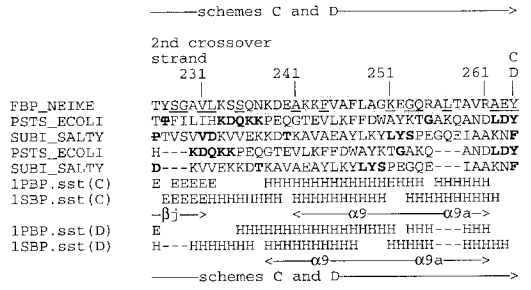
3.2. Threading in the first crossover strand

The first inter-domain crossover strand consists of a stretch of extended structure corresponding to β-strands d and e in 1LFGn and 1LFGc [19]. Threading the Fbp_neime residues

Table 3
Multiple alignment data

Fbp_neime residues		% Sequence identity		Sequences in alignments	Sequence weighting
		with 1PBP	with 1SBP		
1–30		26.7	16.7	Fbp_neime 1PBP 1SBP	1:2:1
21–66		22.2	15.2	Fbp_neime 1PBP 1SBP	1:2:1
42–75		25.0	14.7	Fbp_neime 1PBP 1SBP	1:1:1
71–100		20.7	36.7	Fbp_neime 1PBP[a] 1SBP	2:1:1
94–134	scheme A	22.0	25.0	Fbp_neime 1PBP[a] 1SBP 1LST 1HST	2:1:1:1:1
94–134	scheme B	22.0	17.1	Fbp_neime 1PBP[a] 1SBP 1LST 1HST	2:1:1:1:1
120–193	scheme A	26.5	22.6	Fbp_neime 1PBP 1SBP 1LST 1HST	2:1:2:1:1
129–193	scheme B	22.5	18.4	Fbp_neime 1PBP 1SBP 1LST 1HST	2:2:2:1:1
185–264	scheme C	14.1	14.9	Fbp_neime 1PBP[b] 1SBP 1LST 1HST	1:2:2:1:1
185–264	scheme D	12.7	17.6	Fbp_neime 1PBP[b] 1SBP	1:1:2

[a]Residues 98–120 deleted from alignments.
[b]Residues 217–250 deleted from alignments.



Scheme 2.

on the 1SBP backbone reveals a lack of any 'transferrin-like' binding residues in this section of the Fbp_neime sequence.

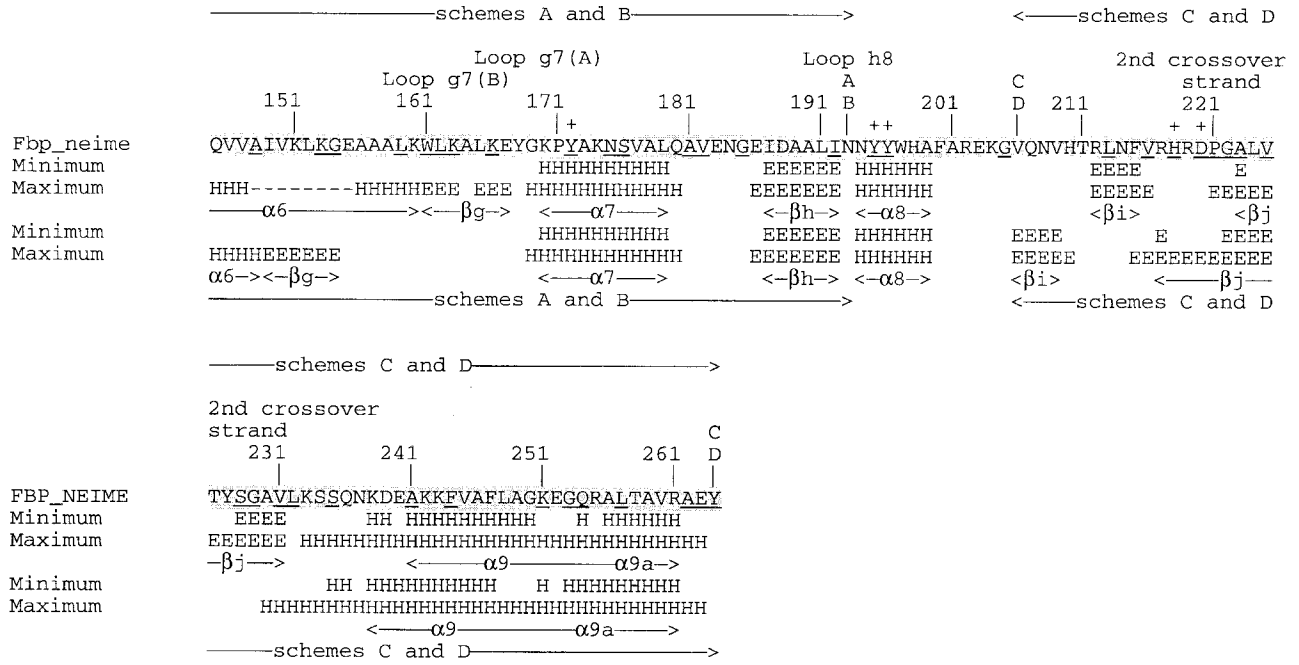
3.3. Threading on the f5, g7 and h8 loop regions in the second domain

This segment of polypeptide chain contains three potential substrate-binding loop regions, f5, g7 and h8 following β-strands f, g and h [19]. Alignment of the Fbp_neime sequence with residues of 1PBP and 1SBP between loop f5 and g7 can be rationalised in terms of two alternative schemes (see Schemes 1–3). The first (scheme A) is based on the premise of structural equivalence for residues Trp-129 in Fbp_neime, Trp-156 in 1PBP and Trp-141 in 1SBP, while the second (scheme B) is based on the premise of structural equivalence for residues Asn-131 in Fbp_neime, Asn-158 in 1PBP and Asn-148 in 1SBP. Scheme A translates into an extended helix 6 region, while scheme B translates into an extended g7 loop region, similar to that present in 1LFGn and 1LFGc [19].

Both schemes identify the same set of conserved residues in the Fbp_neime sequence. Tyr-107, a 'transferrin-like' binding residue, aligns adjacent to Arg-135, a substrate-binding ligand in 1PBP, located at the far edge of the f5 loop region. Three consecutive conserved residues on the same loop region, Lys-111, Leu-112 and Ser-113, overlap with similar clusters of substrate-binding residues in 1PBP and 1SBP. Their conservation may arise from structural constraints imposed by the first turn of helix 5 or may be associated with synergistic anion binding. Tyr-107 is not a particularly convincing candidate iron ligand. It is more bulky and less flexible than the closely aligned Arg-135 in 1SBP, which has to adopt a fully extended conformation in order to interact with the substrate. Added to this, if Tyr-107 proved to be a coordinating ligand, it is unlikely that residues from the f5 loop region would also bind a synergistic anion. In contrast, threading Fbp_neime residues onto loop g7 of 1SBP identifies a single conserved 'transferrin-like' binding residue (Tyr-172), close to the substrate coordinating residue, Ala-173, which is close to the binding cleft. Two conserved 'transferrin-like' binding residues, Tyr-195 and Tyr-196 are similarly located on loop h8, the site of the substrate coordinating residue, Trp-192 in 1SBP. However, limits on the relative orientations of neighbouring bulky side chains will prevent both residues coordinating the ferric ion simultaneously.

3.4. Threading in the second crossover strand

The second inter-domain crossover strand exhibits a high degree of structural variability, and sequence alignments for this region are highly sensitive to the input data, which made it necessary to extend this alignment through to helix 9. Helix 9 was identified from secondary structure predictions on the four iron-binding homologues, Fbp_neime, Fbp_neigo, HitA and SfuA. Alignment of the Fbp_neime sequence with residues of 1PBP and 1SBP in this region can be rationalised in terms of two alternative schemes, dependant on whether Thr-226 (scheme C) or Pro-221 (scheme D) are located centrally on the second crossover strand. Both schemes align two conserved 'transferrin-like' binding residues, His-218 and Asp-220, with crossover strand residues. In scheme C His-218 and Asp-220 align with residues at the edge of the strand, but in



Scheme 3.

scheme D they align with residues central to the strands of 1PBP and 1SBP. Accordingly either Asp-220 or His-218 may be potential iron-binding residues, providing that the central segment of the second crossover strand passes within binding distance of the Fbp_neime ferric ion. The probability of any 'transferrin-like' binding residue on the second crossover strand of Fbp_neime is, however, markedly reduced by the lack of such a residue on the first crossover strand and by the absence of any substrate-binding residues on the crossover strands of the four PBPs.

The lack of conserved 'transferrin-like' binding residues on the first inter-domain crossover strand of Fbp_neime effectively eliminates the possibility of identical metal ion coordination in the Fbps and the transferrins. However, the similarities in metal binding and spectroscopic characteristics shared by members of both families suggest that the residues in their immediate iron coordination spheres are similar [8]. A total of nine conserved 'transferrin-like' binding residues have been identified in the Fbp_neime sequence, in regions bordering the substrate-binding site, which are likely to include some or all of the ferric ion coordinating ligands of Fbp_neime. Modelling studies based on the alignment data suggest that some of these residues are more promising than others.

Comparison of the local secondary structure in the binding regions of 1PBP, 1SBP, 1LFGn and 1LFGc demonstrates a similarity in the positions of Asp-52 from loop region c3 and Tyr-172 from loop region g7 of Fbp_neime, modelled on the 1PBP and 1SBP alignments, and two of the iron coordinating residues of lactoferrin, Asp-60 (395) from loop region c3 and Tyr-192 (528) from loop region g7. In the absence of appropriate binding ligands on the crossover strands, corresponding to His-253 (597) and Tyr-92 (435) of lactoferrin, we would speculate that His-9 from loop region a1 and Tyr-195 or Tyr-196 from loop region h8 should be suitably placed in the Fbp_neime sequence to substitute in this role. The combination of His-9, Asp-52, Tyr-172 and either Tyr-195 or Tyr-196 as ferric iron-binding ligands should not preclude synergistic anion binding on the neighbouring f5 loop region of the protein.

In the absence of structural data, threading based on homologous structure-based alignments is an effective method for constructing spacial templates from related proteins with low sequence homology. The method provides a logical means of identifying limited numbers of putative iron-binding residues, which can subsequently be investigated experimentally using site-directed mutagenesis. However, it lacks the accuracy that is needed for a detailed study of substrate interaction.

References

[1] Berish, S.A., Mietzner, T.A., Mayer, L.W., Genco, C.W., Holloway, B.P. and Morse, S.A. (1990) J. Exp. Med. 171, 1535–1546.
[2] Mietzner, T.A., Luginbuhl, G.H., Sandstrom, E.C. and Morse, S.A. (1984) Infect. Immun. 45, 410–416.
[3] Mietzner, T.A., Barnes, R.C., Jeanlouis, Y.A., Shafer, W.M. and Morse, S.A. (1986) Infect. Immun. 51, 60–68.
[4] Mietzner, T.A., Bolan, G., Schoolnik, G.K. and Morse, S.A. (1987) J. Exp. Med. 165, 1041–1057.
[5] Berish, S.A., Chen, C.-Y., Mietzner, T.A. and Morse, S.A. (1992) Mol. Microbiol. 6, 2607–2615.
[6] Baker, E.N., Rumball, S.V. and Anderson, B.F. (1987) Trends Biochem. Sci. 12, 350–353.
[7] Gerstein, M., Anderson, B.F., Norris, G.E., Baker, E.N., Lesk, A.M. and Chothia, C. (1993) J. Mol. Biol. 234, 357–372.
[8] Nowalk, A.J., Tencza, S.B. and Mietzner, T.A. (1994) Biochemistry 33, 12769–12775.
[9] Ames, G.F.L. (1986) Annu. Rev. Biochem. 55, 397–425.
[10] Adams, M.D. and Oxender, D.L. (1989) J. Biol. Chem. 264, 15739–15742.
[11] Quiocho, F.A. (1990) Philos. Trans. R. Soc. London B 326, 341–351.
[12] Chen, C.-Y., Berish, S.A., Morse, S.A. and Mietzner, T.A. (1993) Mol. Microbiol. 10, 311–318.
[13] Spurlino, J.C., Lu, G.-Y. and Quiocho, F.A. (1991) J. Biol. Chem. 266, 5202–5219.
[14] Luecke, H. and Quiocho, F.A. (1990) Nature 347, 402–406.
[15] Pflugrath, J.W. and Quiocho, F.A. (1985) Nature 314, 257–260.
[16] Yao, N., Trakhanov, S. and Quiocho, F.A. (1994) Biochemistry 33, 4769–4779.
[17] Oh, B.-H., Pandit, J., Kang, C.-H., Nikaido, K., Gokcen, S., Ames, G.F.-L. and Kim, S.-H. (1993) J. Biol. Chem. 268, 11348–11355.
[18] Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rogers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[19] Anderson, B.F., Baker, H.M., Dodson, E.J., Norris, G.E., Rumball, S.V., Waters, J.M. and Baker, E.N. (1987) Proc. Natl. Acad. Sci. USA 84, 1769–1773.
[20] Sanders, J.D., Cope, L.D. and Hanson, E.J. (1994) Infect. Immun. 62, 4515–4525.
[21] Angerer, A., Gaisser, S. and Braun, V. (1990) J. Bacteriol. 172, 572–578.
[22] Angerer, A., Klupp, B. and Braun, V. (1992) J. Bacteriol. 174, 1378–1387.
[23] Kabsch, W. and Sander, C. (1983) Biopolymers 22, 2577–2637.
[24] Bleasby, A.J. and Wootton, J.C. (1990) Protein Eng. 3, 153–159.
[25] Bleasby, A.J., Akrigg, Y. and Attwood, T.K. (1994) Nucleic Acids Res. 22, 3574–3577.
[26] Johnson, M.S., Overington, J.P. and Blundell, T.L. (1993) J. Mol. Biol. 231, 735–752.
[27] Johnson, M.S. and Dolittle, R.F. (1986) J. Mol. Evol. 23, 267–278.
[28] Dolittle, R.F. and Feng, D.-F. (1990) Methods Enzymol. 183, 659–669.
[29] Laskowski, R.A. (1992) Ph.D. Thesis, University of London.
[30] Kraulis, P.K. (1991) J. Appl. Cryst. 24, 946–950.