COMPOSITIONS AND METHODS FOR BINDING MYELIN BINDING PROTEINS

BACKGROUND

The present invention relates generally to methods for studying, detecting, and treating multiple sclerosis by using peptide analogs of proteins structurally related to human myelin basic protein.

The present invention relates generally to the fields of chemistry and medicine and more particularly to compositions of matter and methods useable for detecting and inhibiting myelin binding proteins, related human and animal subject assessment, and treatments for multiple sclerosis.

Multiple sclerosis (MS) is a chronic, inflammatory disease primarily within the white matter of the central nervous system that is manifested by relapsing neurological deficits, in particular, paralysis, sensory deficits, and visual problems. The inflammatory process is mediated by T lymphocytes, B lymphocytes, and macrophages. The demyelination of axons in MS is accompanied by a macroscopic lesions called plaques. T cell reactivity to myelin basic protein may be a critical component in the development of MS as pathogenic T cells found in lesions have restricted heterogeneity of antigen receptors (TCR).

Jansen et al., in United States patents 7,208,270 and 6,489,299, described a method for diagnosing a person having MS or being at risk of developing MS, comprising the following steps: providing a sample of a body fluid or tissue from said person, said sample containing at least one of the wild type SCF-Apoptosis-Response Gene- (wt-SARG-1-*)* protein and nucleic acids encoding wt-SARG-1, if taken from a person not having MS or a risk of acquiring MS, detecting the presence of wt-SARG-1-protein or nucleic acids encoding wt-SARG-1 in said sample and diagnosing MS or a risk of acquiring MS, if wt-SARG-1-protein or nucleic acids encoding wt-SARG-1 are not present in said sample.

Steinman et al., in United States patents 6,740,638, 6,489,299, and 6,369,033, described peptide analogues of human myelin basic protein containing residues 87-99. Residue 91 of the peptide analogues is altered from the L-lysine residue found in the native protein to any other amino acid. Steinman claimed pharmaceutical compositions of the peptide analogues are provided with claims for the peptide analogues when administered to patients with multiple sclerosis.

Gaur et al., in United States patents 6,379,670 and 6,251,396, were directed toward peptide analogs of human myelin basic protein claiming peptide analogs that were at least seven amino acids long and derived from residues 83 to 99 of human

myelin basic protein. The analogs were altered from the native sequence at least at positions 91, 95, or 97. Additional alterations were claimed at other positions and pharmaceutical compositions containing these peptide analogs were claimed to be useful for treating multiple sclerosis.

Hashim, in United States patent 4,230,696, claimed synthetic compounds of the formula. Acid addition salts thereof are disclosed wherein R.sub.1 and R.sub.5 are each independently selected from the group consisting of hydrogen, hydroxy, the residue of an amino acid and the residue of a polypeptide and R.sub.4 is selected from the group consisting of lysine and arginine residues; provided that R.sub.1 and R.sub.5 are not both hydrogen or both hydroxyl at the same time. The disclosure of intermediate compounds for preparing the compounds of the above formula and derivative compounds having the same biological activity and pharmaceutical compositions wherein the essential active ingredient is a synthetic compound of the invention were made. The compounds and compositions of the invention were claimed to be useful for the prevention, suppression, treatment, and diagnosis of multiple sclerosis.

Nye et al., in United States patent 7,041,503, claimed compositions and methods for the clinical assessment, diagnosis, and treatment of multiple sclerosis. The compositions of the invention claimed were molecules related to the 21.5 kDa fetal isoform of human myelin basic protein, and include nucleic acids and polypeptides. The inventors claimed nucleic acid molecules were useful in the efficient production of modified and unmodified 21.5 kDa myelin basic protein polypeptides, such polypeptides being useful for assaying T cells for responsiveness to myelin basic protein epitopes. The inventors claimed polypeptides of the invention were also useful as therapeutic agents that act by inducing T cell responses, including apoptosis, as a means of treating multiple sclerosis.

For convenience, the amino acid groups are referred to by abbreviations, following accepted and common practice in peptide chemistry. For example, the following abbreviations for amino acids are used, at times, throughout the following specification and claims:

SER – serine
ASN – asparagine
PHE – phenylalanine
ASP – aspartic acid
GLU – glutamic acid
TYR – tyrosine
LEU – leucine
MET – methionine

ARG – arginine
LYS – lysine
ILE – isoleusine
VAL – valine
GLY – glycine
ALA – alanine

PRIOR ART

The following publications are cited in the specification and are incorporated by reference in their entirety in all jurisdictions where this is appropriate:

PATENT DOCUMENTS

United States Patent 4,230,696 Oct. 1980 Hahim
United States Patent 6,251,396 Jun. 2001 Gaur et al.
United States Patent 6,369,033 Apr. 2002 Steinman et al.
United States Patent 6,489,299 Dec. 2002 Steinman et al.
United States Patent 6,379,670 Apr. 2002 Gaur et al.
United States Patent 6,740,638 Jun. 2004 Steinman et al.
United States Patent 7,041,503 May 2006 Nye et al.
United States Patent 7,208,270 Apr. 2007 Jansen et al.

OTHER PUBLICATIONS

Protein Data Bank ID: 1CBS.
Kleywegt, G.J.; Bergfors, T.; Senn, H.; Le Motte, P; Gsell, B; Shudo, K.; Jones, T.A.; "Crystal structures of cellular retinoic acid binding proteins I and II in complex with all-trans-retinoic acid and a synthetic retinoid." *Structure* (1994) 2:1241

Protein Data Bank ID: 1LPJ
Folli, C.; Calderone, V.; Ramazzina, I.; Zanotti, G.; Berni, R.; "Ligand binding and structural analysis of a human putative cellular retinol-binding protein." *J. Biol. Chem.* (2002) 277: 41970

Protein Data Bank ID: 2WUT
Majava, V.; Polverini, E.; Mazzini, A.; Nanekar, R.; Knoll, W.; Peters, J.; Natali, F.; Baumgartel, P.; Kursula, I.; Kursula, P.; "Structural and functional characterization of human peripheral nervous system myelin protein P2." *PLOS One (2010) 5: E300*

Protein Data Bank ID: 3NR3

Ugochukwu, E.; Pilka, E.; Phillips, C.; Yue, W.W.; Krojer, T.; Von Delft, F.; Bountra, C.; Arrowsmith, C.H.; Weigelt, J.; Edwards, A.; Kavanagh, K.L.; Crystal structure of human peripheral myelin protein 2., Structural Genomics Consortium (SGC)

BRIEF SUMMARY OF THE INVENTION

A first aspect of the invention is directed to a method for binding myelin binding proteins in any of: a human subject; an animal subject; a human derived substance and an animal-derived substance, said method comprising the step of: administering to the subject or applying to the animal-derived substance an effective amount of a MS-BLOCK Peptide or derivative thereof or combination thereof.

In some embodiments, the MS-BLOCK Peptide or derivative thereof or combination thereof is used to detect multiple sclerosis.

In some embodiments the MS-BLOCK Peptide or derivative thereof or combination thereof is labeled with a detectable compound.

In some embodiments, the method is carried out to bring about at least one therapeutic effect or diagnostic effect selected from the group consisting of: detecting myelin binding proteins; inhibiting multiple sclerosis; treating or preventing multiple sclerosis related disease.

In some embodiments. the method is for treating multiple sclerosis.

In some embodiments, the MS-BLOCK Peptide or derivative thereof has the following general formula: X-SBNFBBOUK-Z, where:

"S" is either the amino acid SER or ASN;

"B" is either the amino acid ASP or GLU;

"N" is the amino acid ASN;

"F" is the amino acid PHE;

"O" is either the amino acid TYR or the amino acid LEU;

"U" is either the amino acid LEU or amino acid MET;

"K" is the amino acid LYS;

"X" are N-terminal amino acids preceding the amino acids SBNFBBOUK-Z, and

"Z" are any C-terminal amino acids following X-SBNFBBOUK.

In some embodiments, the MS-BLOCK Peptide or derivative thereof has the following general formula: X-RKLGJK-Z, where:

"R" is the amino acid ARG;

"X" is the amino acid LYS;

"L" is an amino acid selected from the group of LEU, ILE and VAL;

"G" is the amino acid GLY or ALA

"J" is three, four or five amino acids,

"X" are any N-terminal amino acids preceding the amino acids RKLGJK-Z, and

"Z" are any C-terminal amino acids following X-RKLGJK.

In some embodiments, the J is selected from the group of peptides consisting of: ASN-LEU-ALA, ASN-LEU-LEU. VAL-ARG-LEU, LYS-LEU-LEU, GLY-MET-ALA, and

VAL-ALA-ALA-ALA-SER.

In some embodiments, the MS-BLOCK Peptide or derivative thereof or combination thereof comprises a multimeric MS-BLOCK Peptide.

In some embodiments, the multimeric MS-BLOCK Peptide is bound to an oligomerizing substance and the method is carried out for treating a multiple sclerosis.

In some embodiments, the oligomerizing substance is selected from the group consisting of: peptides, small molecules, and cross-linking reagents.

In some embodiments, the multimeric MS-BLOCK Peptides are covalently linked by residues in the X, Y, or J components.

In some embodiments, the covalent linkage is a disulfide bond.

In some embodiments, the method is for *in vivo* or *ex vivo* filtering of the blood of a human or animal subject.

A further aspect of the invention is directed to a composition of matter comprising an MS-BLOCK Peptide or a derivative thereof or a combination thereof.

Optionally, the composition according has a general formula X-SBNFBBOUK-Z, where:

"S" is either the amino acid SER or ASN;

"B" is either the amino acid ASP or GLU;

"N" is the amino acid ASN;

"F" is the amino acid PHE;

"O" is either the amino acid TYR or the amino acid LEU;

"U" is either the amino acid LEU or the amino acid MET;

"K" is the amino acid LYS;

"X" are N-terminal amino acids preceding the amino acids SBNFBBOUK-Z, and

"Z" are any C-terminal amino acids following X-SBNFBBOUK.

Optionally, the composition according has a general formula X-RKLGJK-Z, where:

"R" is the amino acid ARG;

"K" is the amino acid LYS;

"L" is the amino acid LEU, ILE, or VAL;

"G" is the amino acid GLY or ALA;

"J" is three, four or five amino acids;

"X" are any N-terminal amino acids preceding the amino acids RKLGJK-Z, and
"Z" are any C-terminal amino acids following X-RKLGJK.

Optionally, the composition is combined with or bound to a natural or synthetic material that is useable as a scaffold, a filter, a bioengineered material or a particle.

Optionally, the natural or synthetic material comprises at least one material selected from the group consisting of: hydrogels, collagens, hyaluronic acids, polymers, tissue bulking agents, and protein particles.

Optionally, the composition is expressed as the coding DNA or RNA for the MS-BLOCK Peptide.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate embodiments of the invention, and together with the general description given above and the detailed description of the preferred embodiments given below, serve to explain the principles of the invention.

FIG. 1 shows a ribbon diagram of the common myelin/retinoic acid binding protein structure and the placement of MS-BLOCK Peptide components of myelin and retinoic acid binding protein structures.

FIG. 2 shows a ribbon diagram of MS-BLOCK Peptide components in myelin and retinoic acid binding protein structures.

DETAILED DESCRIPTION OF THE INVENTION

Human myelin protein P2 (PDB: 2WUT), equine myelin protein P2 from spinal cord (PDB: 1YIV), human cellular retinol-binding protein IV (PDB: 1LPJ), and cellular retinoic-acid-binding proteins I and II (PDB: 1CAB) all have two sets of highly conserves sequences in the N-terminal region. These sequences present sets of highly charged residues to the surface of the protein. Table 1 shows the positions of the two sets of residues. Table 2 shows the consensus residues for two structures identified that present highly charged residues to the same surface. Figure 1 shows the spatial relationship between the residues in Table 1 in the crystal structures by showing the secondary structure ribbon positions in which these residues reside. Figure 1 also shows the relationship between the residues in the secondary structures containing MS-BLOCK Peptides and the rest of the protein. Figure 2 shows MS-BLOCK Peptides in the same spatial relationship as in myelins and retinoic acid binding proteins. Compounds based on this presentation of common residues that present to a surface are used to bind to proteins that bind to myelin in order to provide a means of detection and treatment of MS.

Aspects of the invention are directed to MS-BLOCK Peptides derivatives thereof and combinations therof, including pharmaceutically acceptable salts, hydrates, multimers, cyclic forms, linear forms, drug-conjugates, pro-drugs and their derivatives.

Selected sequences found in myelins and retinoic acid binding proteins present the same charged residues to form a common protein surface. Selected sequences of selected myelin and retinoic acid binding proteins are shown in Table I. The first and third column of residues in Table I for each protein form helices that are presented to the protein surface for each protein. Figure 1 shows a ribbon diagram **[1]** representing the common structure of the myelin and retinoic acid binding protein structures. Figure 1 also shows the position of two helices on the surfaces of the proteins **[2 and 3]**.

Helix one **[2]** represents the protein sequences found in Table I in the first column for each protein:

1CBS: SER-GLU-ASN-PHE-GLU-GLU-LEU-LEU-LYS
2WUT: SER-GLU-ASN-PHE-ASP-ASP-TYR-MET-LYS
3NR3: SER-GLU-ASN-PHE-ASP-ASP-TYR-MET-LYS
1LPJ: SER-ASP-ASN-PHE-GLU-GLY-TYR-MET-LEU

The structure of the presentation of the consensus sequence for residues in helix one **[2]** is shown in detail in Figure 2 and is:

SER [12A-12B] -
ASP/GLU [11A-11B] -
ASN [10] -
PHE [9] -
ASP/GLU [8A-8B] -
ASP/GLU/GLY [7] -
TYR/LEU [6A-6B] -
MET/LEU [5] -
LYS/LEU [4A-4B]

The second helix **[3]** represents the protein sequences found in Table I in the third column for each protein (where "( )" stands for a missing residue):

1CBS: ARG-LYS-ILE-ALA-VAL-ALA-ALA-ALA-SER-LYS
2WUT: ARG-LYS-LEU-GLY-ASN-LEU-ALA-( )-( )-LYS
3NR3: ARG-LYS-LEU-GLY-ASN-LEU-ALA-( )-( )-LYS
1LPJ: ARG-LYS-ILE-ALA-LYS-LEU-LEU-( )-( )-LYS

The structure of the presentation of the consensus sequence for residues in helix two **[3]** is shown in detail in Figure 2 and is:

ARG **[13]** -
LYS **[14]** -
ILE/LEU **[15]** -
ALA/GLY **[16A-16B]** -
VAL/ASN/LYS **[17]** -
ALA/LEU **[18A]** -
ALA/LEU **[18B]** -
ALA/( ) **[18C]** -
SER/( ) **[18C]** -
LYS **[19]**

The claimed MS-Block Peptides incorporate sequences found in helices one **[2,3A-12B]** and two **[3, 13-19]**:

MS-Block Peptide 1:

MS-BLOCK Peptide 1 or derivative thereof has the following general formula: X-SBNFBBOUK-Z, where: “S” is either the amino acid SER or ASN, “B” is either the amino acid ASP or GLU, “N” is the amino acid ASN, “F” is the amino acid PHE, “O” is either the amino acid TYR or LEU, “U” is either the amino acid LEU or MET, “K” is the amino acid LYS, “X” are N-terminal amino acids preceding the amino acids SBNFBBOUK-Z and “Z” are any C-terminal amino acids following X-SBNFBBOUK.

MS-Block Peptide 2:

MS-BLOCK Peptide 2 or derivative thereof has the following general formula: X-RKLGJK-Z, where: “R” is the amino acid ARG, “K” is the amino acid LYS, where “L” is the amino acid LEU, ILE, or VAL, “G” is the amino acid GLY or ALA, “J” is three, four or five amino acids, “X” are any N-terminal amino acids preceding the amino acids RKLGJK-Z, and “Z” are any C-terminal amino acids following X-RKLGJK.

MS-BLOCK Peptides can be made by conventional means including expression in bacteria and cell free synthesis. Nucleic acids, including DNA and RNA. encoding MS-BLOCK Peptides can be made by conventional means including synthesis in bacteria or on DNA and RNA automated chemical synthesizers.

An Embodiment

Any construct which presents helix one [2] and helix two [3] in a similar structural configuration to that of retinoic acid binding protein can be used as an embodiment of the invention. As an example, the following peptide presents both helices in the desirable configuration:

MET-CYS-LEU-VAL-SER- (X peptide of helix one with disulfide bridging CYS residue)
SER-GLU-ASN-PHE-ASP-ASP-TYR-MET-LYS-ALA- (helix one **[2]**)
LEU-GLY-VAL-GLY-LEU-ALA-THR- (Z peptide of helix one/X peptide of helix two)
ARG-LYS-LEU-GLY-ASN-LEU-ALA-LYS- (helix two **[3]**)
PRO-CYS-GLY-LYS (Z peptide of helix two with disulfide bridging CYS residue)

In this case the construct provides for a disulfide bridge stabilizing the helix one **[2]** and two **[3]** structures. Table 2 shows the coordinates for the structure of the protein incorporating MS-BLOCK Peptide One and MS-BLOCK Peptide Two as an example embodiment.

Additional advantages and modifications will readily occur to those skilled in the art. Therefore, the invention in its broader aspects is not limited to the specific details and representative embodiments shown and described herein. Accordingly, various modifications may be made without departing from the spirit or scope of the general inventive concept as defined by the appended claims and their equivalents.

The foregoing descriptions of specific embodiments of the present invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application, to thereby enable others skilled in the art to best use the invention and various embodiments with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto and their equivalents.

TABLE 1

Database Accession PDB 1CBS: Cellular Retinoic-Acid-Binding Protein I & II										
RES NUM	12	13	14	15	16	17	18	19	20	
RES NAM	SER	GLU	ASN	PHE	GLU	GLU	LEU	LEU	LYS	
RES NUM	21	22	23	24	25	26	27	28		
RES NAM	VAL	LEU	GLY	VAL	ASN	VAL	MET	LEU		
RES NUM	29	30	31	32	33	34	35	36	37	38
RES NAM	ARG	LYS	ILE	ALA	VAL	ALA	ALA	ALA	SER	LYS
Database Accession PDB 2WUT: Human Myelin Protein P2										
RES NUM	13	14	15	16	17	18	19	20	21	
RES NAM	SER	GLU	ASN	PHE	ASP	ASP	TYR	MET	LYS	
RES NUM	22	23	24	25	26	27	28	29		
RES NAM	ALA	LEU	GLY	VAL	GLY	LEU	ALA	THR		
RES NUM	30	31	32	33	34	35	36	37		
RES NAM	ARG	LYS	LEU	GLY	ASN	LEU	ALA	LYS		
Database Accession PDB 3NR3: Human Peripheral Myelin Protein P2										
RES NUM	14	15	16	17	18	19	20	21	22	
RES NAM	SER	GLU	ASN	PHE	ASP	ASP	TYR	MET	LYS	
RES NUM	23	24	25	26	27	28	29	30		
RES NAM	ALA	LEU	GLY	VAL	GLY	LEU	ALA	THR		
RES NUM	31	32	33	34	35	36	37	38		
RES NAM	ARG	LYS	LEU	GLY	ASN	LEU	ALA	LYS		
Database Accession PDB 1LPJ: Cellular Retinol-Binding Protein IV										
RES NUM	13	14	15	16	17	18	19	20	21	
PDB 1LPJ	SER	ASP	ASN	PHE	GLU	GLY	TYR	MET	LEU	
RES NUM	22	23	24	25	26	27	28	29		
PDB 1LPJ	ALA	LEU	GLY	ILE	ASP	PHE	ALA	THR		
RES NUM	30	31	32	33	34	35	36	37		
PDB 1LPJ	ARG	LYS	ILE	ALA	LYS	LEU	LEU	LYS		

Table 1: Shows the residues of helix one **[2,3A-12B]**, residues of helix two **[3, 13-19]**, and residues between helix one and two from myelin and retinoic acid binding protein structures.

TABLE 2

ATOM	57	N	MET	A	8	-4.227	4.007	19.529	1.00	0.00	N
ATOM	58	CA	MET	A	8	-3.950	5.317	20.110	1.00	0.00	C
ATOM	59	C	MET	A	8	-5.223	6.106	20.300	1.00	0.00	C
ATOM	60	O	MET	A	8	-6.024	6.193	19.367	1.00	0.00	O
ATOM	61	CB	MET	A	8	-2.946	6.090	19.212	1.00	0.00	C
ATOM	62	CG	MET	A	8	-2.509	7.478	19.729	1.00	0.00	C
ATOM	63	SD	MET	A	8	-1.330	8.212	18.584	1.00	0.00	S
ATOM	64	CE	MET	A	8	0.042	7.083	18.854	1.00	0.00	C
ATOM	65	N	CYS	A	9	-5.448	6.574	21.474	1.00	0.00	N
ATOM	66	CA	CYS	A	9	-6.667	7.296	21.827	1.00	0.00	C
ATOM	67	C	CYS	A	9	-6.402	8.778	21.949	1.00	0.00	C
ATOM	68	O	CYS	A	9	-5.393	9.107	22.563	1.00	0.00	O
ATOM	69	CB	CYS	A	9	-7.237	6.690	23.124	1.00	0.00	C
ATOM	70	SG	CYS	A	9	-7.680	4.955	22.879	1.00	0.00	S
ATOM	71	N	LEU	A	10	-7.238	9.650	21.391	1.00	45.45	N
ATOM	72	CA	LEU	A	10	-7.051	11.118	21.522	1.00	45.82	C
ATOM	73	C	LEU	A	10	-7.195	11.520	22.979	1.00	45.20	C
ATOM	74	O	LEU	A	10	-8.208	11.216	23.603	1.00	45.09	O
ATOM	75	CB	LEU	A	10	-8.136	11.928	20.750	1.00	46.56	C
ATOM	76	CG	LEU	A	10	-7.858	13.444	20.659	1.00	45.72	C
ATOM	77	CD1	LEU	A	10	-6.709	13.715	19.717	1.00	44.82	C
ATOM	78	CD2	LEU	A	10	-9.095	14.245	20.267	1.00	46.66	C
ATOM	79	N	VAL	A	11	-6.201	12.224	23.505	1.00	44.90	N
ATOM	80	CA	VAL	A	11	-6.298	12.765	24.859	1.00	44.61	C
ATOM	81	C	VAL	A	11	-6.224	14.295	24.879	1.00	44.50	C
ATOM	82	O	VAL	A	11	-6.521	14.865	25.886	1.00	42.84	O
ATOM	83	CB	VAL	A	11	-5.272	12.144	25.801	1.00	44.16	C
ATOM	84	CG1	VAL	A	11	-5.524	10.673	25.955	1.00	46.22	C
ATOM	85	CG2	VAL	A	11	-3.844	12.277	25.259	1.00	46.00	C
ATOM	86	N	SER	A	12	-5.838	14.936	23.769	1.00	44.75	N
ATOM	87	CA	SER	A	12	-5.698	16.384	23.674	1.00	45.20	C
ATOM	88	C	SER	A	12	-5.820	16.882	22.268	1.00	44.84	C
ATOM	89	O	SER	A	12	-5.340	16.234	21.341	1.00	44.56	O
ATOM	90	CB	SER	A	12	-4.291	16.772	24.133	1.00	46.08	C
ATOM	91	OG	SER	A	12	-4.310	16.854	25.534	1.00	50.03	O
ATOM	92	N	SER	A	13	-6.353	18.088	22.110	1.00	44.95	N
ATOM	93	CA	SER	A	13	-6.401	18.755	20.814	1.00	45.75	C
ATOM	94	C	SER	A	13	-6.220	20.271	20.997	1.00	46.52	C
ATOM	95	O	SER	A	13	-6.796	20.860	21.911	1.00	46.84	O
ATOM	96	CB	SER	A	13	-7.730	18.435	20.133	1.00	46.27	C
ATOM	97	OG	SER	A	13	-7.841	19.027	18.838	1.00	46.11	O
ATOM	98	N	GLU	A	14	-5.400	20.895	20.161	1.00	47.10	N
ATOM	99	CA	GLU	A	14	-5.273	22.348	20.145	1.00	47.08	C
ATOM	100	C	GLU	A	14	-5.473	22.850	18.711	1.00	46.74	C
ATOM	101	O	GLU	A	14	-4.799	22.391	17.797	1.00	47.29	O
ATOM	102	CB	GLU	A	14	-3.885	22.769	20.662	1.00	48.03	C
ATOM	103	CG	GLU	A	14	-3.768	24.314	20.715	1.00	48.33	C
ATOM	104	CD	GLU	A	14	-2.392	24.840	21.099	1.00	48.75	C
ATOM	105	OE1	GLU	A	14	-2.316	25.702	21.998	1.00	48.32	O
ATOM	106	OE2	GLU	A	14	-1.398	24.394	20.500	1.00	56.00	O
ATOM	107	N	ASN	A	15	-6.398	23.777	18.487	1.00	47.18	N
ATOM	108	CA	ASN	A	15	-6.560	24.453	17.181	1.00	46.95	C
ATOM	109	C	ASN	A	15	-7.186	23.666	16.024	1.00	45.95	C
ATOM	110	O	ASN	A	15	-6.890	23.941	14.858	1.00	45.51	O
ATOM	111	CB	ASN	A	15	-5.211	25.015	16.711	1.00	48.42	C
ATOM	112	CG	ASN	A	15	-4.914	26.354	17.287	1.00	51.93	C
ATOM	113	ND2	ASN	A	15	-3.628	26.760	17.245	1.00	53.47	N
ATOM	114	OD1	ASN	A	15	-5.820	27.040	17.767	1.00	57.59	O
ATOM	115	N	PHE	A	16	-8.036	22.692	16.332	1.00	45.04	N
ATOM	116	CA	PHE	A	16	-8.583	21.784	15.317	1.00	45.01	C
ATOM	117	C	PHE	A	16	-9.701	22.473	14.544	1.00	44.20	C
ATOM	118	O	PHE	A	16	-9.809	22.315	13.332	1.00	43.37	O
ATOM	119	CB	PHE	A	16	-9.083	20.469	15.977	1.00	44.41	C
ATOM	120	CG	PHE	A	16	-9.509	19.394	15.010	1.00	44.19	C
ATOM	121	CD1	PHE	A	16	-8.665	18.971	14.001	1.00	44.73	C
ATOM	122	CD2	PHE	A	16	-10.717	18.738	15.162	1.00	43.80	C

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ATOM    123  CE1 PHE A  16      -9.033  17.992  13.136  1.00 42.22           C
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ATOM    137  O   ASP A  18      -8.551  26.229  10.644  1.00 44.78           O
ATOM    138  CB  ASP A  18      -8.332  27.456  13.625  1.00 47.03           C
ATOM    139  CG  ASP A  18      -8.986  28.705  14.230  1.00 49.56           C
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ATOM    141  OD2 ASP A  18      -8.232  29.469  14.859  1.00 56.04           O
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ATOM    159  CG  MET A  20     -11.678  20.337  10.900  1.00 47.17           C
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ATOM    168  CD  LYS A  21     -12.541  28.567  11.459  1.00 49.28           C
ATOM    169  CE  LYS A  21     -13.315  29.802  11.672  1.00 50.32           C
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ATOM    173  C   ALA A  22      -8.528  25.325   5.570  1.00 48.95           C
ATOM    174  O   ALA A  22      -8.128  25.601   4.447  1.00 49.94           O
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ATOM    177  CA  LEU A  23      -9.231  23.136   4.828  1.00 47.63           C
ATOM    178  C   LEU A  23     -10.533  23.307   4.079  1.00 47.46           C
ATOM    179  O   LEU A  23     -10.817  22.522   3.184  1.00 47.86           O
ATOM    180  CB  LEU A  23      -9.272  21.746   5.421  1.00 46.82           C
ATOM    181  CG  LEU A  23      -8.061  21.211   6.120  1.00 46.09           C
ATOM    182  CD1 LEU A  23      -8.478  19.958   6.866  1.00 45.88           C
ATOM    183  CD2 LEU A  23      -6.961  20.933   5.148  1.00 47.72           C
ATOM    184  N   GLY A  24     -11.347  24.287   4.480  1.00 47.67           N
ATOM    185  CA  GLY A  24     -12.649  24.544   3.850  1.00 47.74           C
ATOM    186  C   GLY A  24     -13.790  23.620   4.297  1.00 47.79           C
ATOM    187  O   GLY A  24     -14.792  23.507   3.589  1.00 49.70           O
ATOM    188  N   VAL A  25     -13.690  23.010   5.478  1.00 46.88           N
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ATOM    204  CG  LEU A  27     -21.291  26.170  11.675  1.00 47.38           C
ATOM    205  CD1 LEU A  27     -20.125  26.144  12.659  1.00 48.87           C
ATOM    206  CD2 LEU A  27     -21.963  27.530  11.769  1.00 48.11           C
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ATOM    222  O   ARG A  30     -16.728  20.394  14.207  1.00 47.06           O
ATOM    223  CB  ARG A  30     -16.544  22.602  11.747  1.00 45.96           C
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ATOM    225  CD  ARG A  30     -14.894  24.105  10.491  1.00 47.89           C
ATOM    226  NE  ARG A  30     -15.350  25.158  11.358  1.00 50.41           N
ATOM    227  CZ  ARG A  30     -16.133  26.181  10.992  1.00 53.04           C
ATOM    228  NH1 ARG A  30     -16.544  26.364   9.728  1.00 52.93           N
ATOM    229  NH2 ARG A  30     -16.486  27.064  11.909  1.00 52.55           N
ATOM    230  N   LYS A  31     -18.788  21.091  13.587  1.00 46.68           N
ATOM    231  CA  LYS A  31     -19.378  20.819  14.918  1.00 47.02           C
ATOM    232  C   LYS A  31     -19.216  19.309  15.332  1.00 47.61           C
ATOM    233  O   LYS A  31     -18.968  18.982  16.500  1.00 48.23           O
ATOM    234  CB  LYS A  31     -20.841  21.291  14.992  1.00 47.04           C
ATOM    235  CG  LYS A  31     -21.039  22.792  15.053  0.50 47.29           C
ATOM    236  CD  LYS A  31     -22.526  23.152  14.993  0.50 47.31           C
ATOM    237  CE  LYS A  31     -22.763  24.618  15.269  0.50 46.59           C
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ATOM    243  CB  LEU A  32     -19.595  16.160  13.481  1.00 46.06           C
ATOM    244  CG  LEU A  32     -21.072  16.364  13.181  1.00 47.20           C
ATOM    245  CD1 LEU A  32     -21.465  15.399  12.050  1.00 49.02           C
ATOM    246  CD2 LEU A  32     -21.881  16.135  14.445  1.00 44.16           C
ATOM    247  N   GLY A  33     -16.780  17.312  14.039  1.00 47.55           N
ATOM    248  CA  GLY A  33     -15.343  17.078  14.100  1.00 47.63           C
ATOM    249  C   GLY A  33     -14.770  17.524  15.426  1.00 47.99           C
ATOM    250  O   GLY A  33     -13.807  16.959  15.909  1.00 48.01           O
ATOM    251  N   ASN A  34     -15.350  18.556  16.011  1.00 47.90           N
ATOM    252  CA  ASN A  34     -14.889  19.011  17.311  1.00 47.86           C
ATOM    253  C   ASN A  34     -15.420  18.132  18.463  1.00 48.08           C
ATOM    254  O   ASN A  34     -14.748  18.003  19.470  1.00 48.72           O
ATOM    255  CB  ASN A  34     -15.220  20.489  17.508  1.00 47.81           C
ATOM    256  CG  ASN A  34     -14.450  21.390  16.566  1.00 47.63           C
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ATOM    261  C   LEU A  35     -16.385  15.123  19.224  1.00 47.63           C
ATOM    262  O   LEU A  35     -16.529  14.346  20.143  1.00 46.79           O
ATOM    263  CB  LEU A  35     -18.544  16.288  19.280  1.00 48.40           C
ATOM    264  CG  LEU A  35     -19.460  17.492  19.446  1.00 49.48           C
ATOM    265  CD1 LEU A  35     -20.888  17.100  19.131  1.00 48.65           C
ATOM    266  CD2 LEU A  35     -19.345  18.071  20.849  1.00 51.35           C
ATOM    267  N   ALA A  36     -15.717  14.800  18.117  1.00 47.57           N
ATOM    268  CA  ALA A  36     -15.065  13.481  17.966  1.00 47.78           C
ATOM    269  C   ALA A  36     -13.908  13.280  18.947  1.00 48.46           C
ATOM    270  O   ALA A  36     -13.089  14.209  19.185  1.00 48.62           O
ATOM    271  CB  ALA A  36     -14.531  13.276  16.507  1.00 46.97           C
ATOM    272  N   LYS A  37     -13.818  12.062  19.487  1.00 48.71           N
ATOM    273  CA  LYS A  37     -12.646  11.651  20.255  1.00 49.32           C
ATOM    274  C   LYS A  37     -12.060  10.358  19.659  1.00 49.91           C
ATOM    275  O   LYS A  37     -12.272   9.248  20.147  1.00 49.34           O
ATOM    276  CB  LYS A  37     -13.038  11.521  21.738  1.00 50.46           C
ATOM    277  CG  LYS A  37     -13.135  12.872  22.439  0.50 48.81           C
ATOM    278  CD  LYS A  37     -13.278  12.678  23.912  0.50 49.04           C
ATOM    279  CE  LYS A  37     -13.691  13.973  24.606  0.50 48.57           C
ATOM    280  NZ  LYS A  37     -13.911  13.743  26.066  0.50 48.07           N
ATOM    281  N   PRO A  38     -11.336  10.497  18.553  1.00 49.71           N
ATOM    282  CA  PRO A  38     -11.103   9.279  17.759  1.00 49.53           C
ATOM    283  C   PRO A  38     -10.065   8.321  18.318  1.00 49.19           C
ATOM    284  O   PRO A  38      -9.215   8.678  19.157  1.00 49.77           O
ATOM    285  CB  PRO A  38     -10.606   9.830  16.412  1.00 50.17           C
ATOM    286  CG  PRO A  38     -10.456  11.296  16.598  1.00 50.18           C
ATOM    287  CD  PRO A  38     -10.686  11.683  17.990  1.00 49.89           C
ATOM    288  N   CYS A  39     -10.121   7.095  17.839  1.00  0.00           N
ATOM    289  CA  CYS A  39      -9.088   6.080  18.026  1.00  0.00           C
ATOM    290  C   CYS A  39      -8.364   5.798  16.731  1.00  0.00           C
ATOM    291  O   CYS A  39      -9.099   5.674  15.728  1.00  0.00           O
ATOM    292  CB  CYS A  39      -9.743   4.818  18.621  1.00  0.00           C
ATOM    293  SG  CYS A  39     -10.460   5.174  20.242  1.00  0.00           S
ATOM    294  N   GLY A  40      -7.131   5.720  16.798  1.00  0.00           N
ATOM    295  CA  GLY A  40      -6.334   5.431  15.609  1.00  0.00           C
ATOM    296  C   GLY A  40      -5.588   4.128  15.761  1.00  0.00           C
ATOM    297  O   GLY A  40      -4.885   4.021  16.758  1.00  0.00           O
ATOM    298  N   VAL A  41      -5.887   3.226  14.919  1.00  0.00           N
ATOM    299  CA  VAL A  41      -5.265   1.905  14.914  1.00  0.00           C
ATOM    300  C   VAL A  41      -4.278   1.777  13.778  1.00  0.00           C
ATOM    301  O   VAL A  41      -4.735   1.883  12.636  1.00  0.00           O
ATOM    302  CB  VAL A  41      -6.379   0.788  14.820  1.00  0.00           C
ATOM    303  CG1 VAL A  41      -5.879  -0.682  14.767  1.00  0.00           C
ATOM    304  CG2 VAL A  41      -7.406   0.828  15.977  1.00  0.00           C
END
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Table 2: Shows the residues of helix one **[2,3A-12B]**, residues of helix two **[3, 13-19]**, and residues between helix one and twowo from myelin and retanoic acid binding protein structures

CLAIMS

What is claimed is:

1. A method for binding myelin binding proteins in any of:
   a human subject,
   an animal subject ,
   a human derived substance;
   an animal-derived substance;
   said method comprising the step of: administering to the subject or applying to the animal-derived substance an effective amount of a MS-BLOCK Peptide or derivative thereof or combination thereof.
2. The method of claim 1 wherein the MS-BLOCK Peptide or derivative thereof or combination thereof is used to detect multiple sclerosis.
3. The method of claim 2 wherein the MS-BLOCK Peptide or derivative thereof or combination thereof is labeled with a detectable compound.
4. The method according to claim 1 wherein the method is carried out to bring about at least one therapeutic or diagnostic effect selected from the group consisting of: detecting myelin binding proteins; inhibiting multiple sclerosis; treating or preventing multiple sclerosis related disease; and binding myelin binding proteins; .
5. The method according to claim 1 for treating multiple sclerosis.
6. The method according to claim 1 wherein the MS-BLOCK Peptide or derivative thereof has the following general formula: X-SBNFBBOUK-Z, where:
   “S” is either the amino acid SER or ASN,
   “B” is either the amino acid ASP or GLU,
   “N” is the amino acid ASN,
   “F” is the amino acid PHE,
   “O” is either the amino acid TYR or LEU,
   “U” is either the amino acid LEU or MET,
   “K” is the amino acid LYS,
   “X” are N-terminal amino acids preceding the amino acids SBNFBBOUK-Z, and
   “Z” are any C-terminal amino acids following X-SBNFBBOUK.
7. The method according to claim 1 wherein the MS-BLOCK Peptide or derivative thereof has the following general formula: X-RKLGJK-Z, where:
   “R” is the amino acid ARG,
   “K” is the amino acid LYS,
   “L” is an amino acid selected from the group of LEU, ILE, or VAL;
   “G” is the amino acid GLY or ALA,

"J" is three, four or five amino acids

"X" are any N-terminal amino acids preceding the amino acids RKLGJK-Z, and

"Z" are any C-terminal amino acids following X-RKLGJK.

8. The method according to claim 7 wherein J is selected from the group of peptides consisting of: ASN-LEU-ALA, ASN-LEU-LEU, VAL-ARG-LEU, LYS-LEU-LEU, GLY-MET-ALA, and VAL-ALA-ALA-ALA-SER.

9. The method according to claim 1 wherein the MS-BLOCK Peptide or derivative thereof or combination thereof comprises a multimeric MS-BLOCK Peptide.

10. The method according to claim 9 wherein the multimeric MS-BLOCK Peptide is bound to an oligomerizing substance and wherein the method is carried out for treating a multiple sclerosis.

11. The method according to claim 10 wherein the oligomerizing substance is selected from the group consisting of: peptides, small molecule, and cross-linking reagents.

12. The method according to claim 1 wherein the multimeric MS-BLOCK Peptides are covalently linked by residues in the X, Y, or J components.

13. The method according to claim 12 wherein the covalent linkage is a disulfide bond.

14. The method for *in vivo* or *ex vivo* filtering of the blood of a human or animal subject.

15. A composition of matter comprising an MS-BLOCK Peptide or derivative thereof or combination thereof.

16. The composition according to claim 15 having the following general formula: X-SBNFBBOUK-Z, where:

"S" is either the amino acid SER or ASN,

"B" is either the amino acid ASP or GLU,

"N" is the amino acid ASN,

"F" is the amino acid PHE,

"O" is either the amino acid TYR or LEU,

"U" is either the amino acid LEU or MET,

"K" is the amino acid LYS,

"X" are N-terminal amino acids preceding the amino acids SBNFBBOUK-Z, and

"Z" are any C-terminal amino acids following X-SBNFBBOUK.

17. The composition according to claim 15 having the following general formula: X-RKLGJK-Z, where:

"R" is the amino acid ARG,

"K" is the amino acid LYS,

"L" is the amino acid LEU, ILE, or VAL,

"G" is the amino acid GLY or ALA,

"J" is three to five amino acids,

"X" are any N-terminal amino acids preceding the amino acids RKLGJK-Z, and

"Z" are any C-terminal amino acids following X-RKLGJK.

18. The composition according to claim 15 combined with, or bound to, a natural or synthetic material that is useable as a scaffold, filter, or bioengineered material or particle.

19. The composition according to claim 18 wherein the natural or synthetic material comprises at least one material selected from the group consisting of: hydrogels, collagens, hyaluronic acids, polymers, tissue bulking agents, and protein particles.

20. The composition according to claim 15 expressed as the coding DNA or RNA for the MS-BLOCK Peptide.

ABSTRACT

Compounds comprising peptides and derivatives thereof and combinations thereof, including pharmaceutically acceptable salts, hydrates, multimers, cyclic forms, linear forms, drug-conjugates, pro-drugs and their derivatives. Also disclosed are methods for making and using such compounds including methods for using such compounds in the diagnostic binding to, and therapeutic inhibiting of, myelin binding proteins in human and animal subjects.

1 / 2

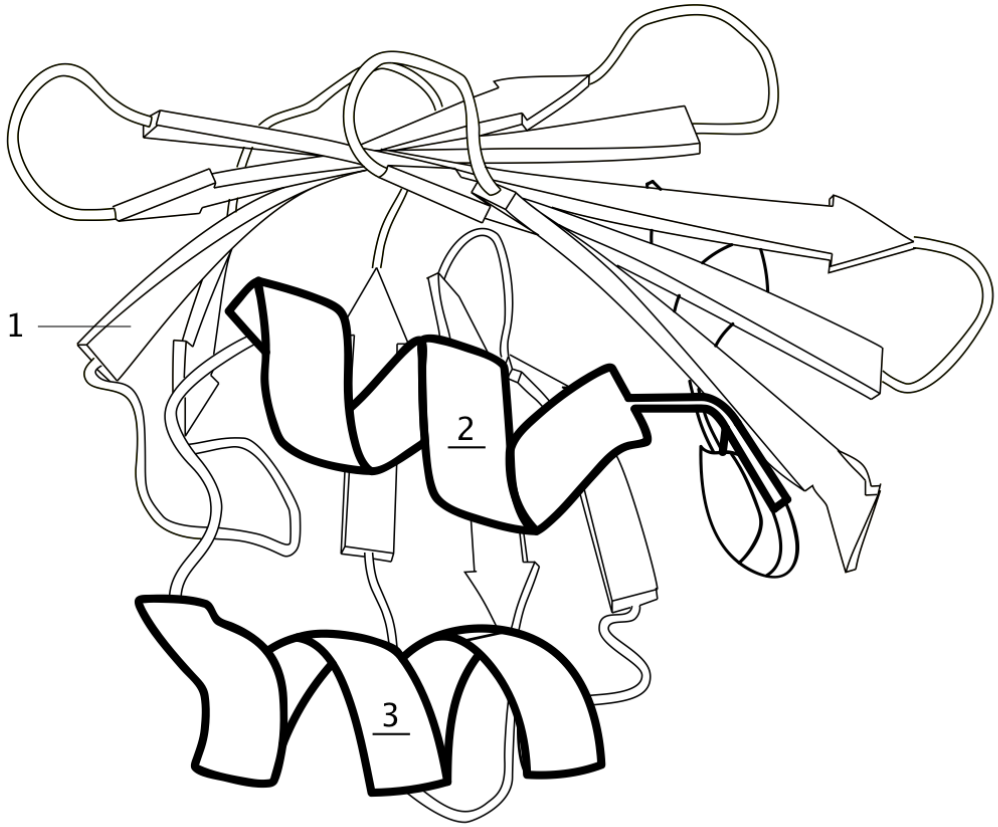

FIGURE 1

2 / 2

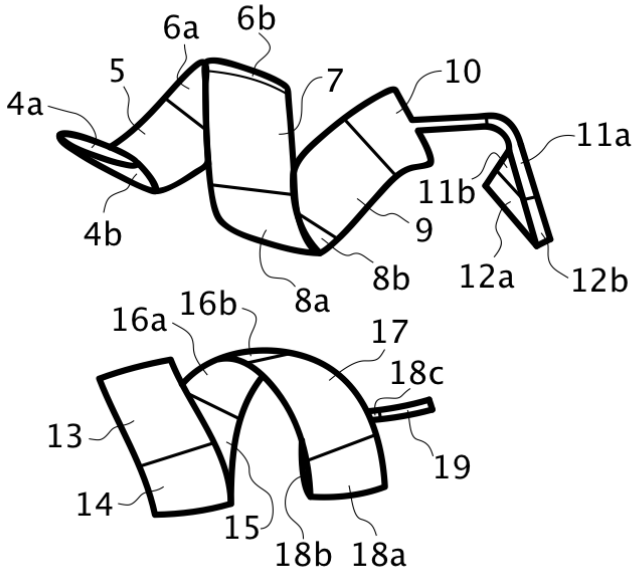
6a
6b
5
7
10
4a
11a
11b
4b
9
8b
12a
12b
8a
16a
16b
17
18c
13
19
14
15
18b
18a

FIGURE 2