BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **219,** 36–39 (1996)
ARTICLE NO. 0177

Partial Amino Acid Sequence of a Novel 40-kDa Human Aortic Protein, with Vitronectin-Like, Fibrinogen-Like, and Calcium Binding Domains: Aortic Aneurysm-Associated Protein-40 (AAAP-40) [Human MAGP-3, Proposed]

Shichao Xia, Kathleen Ozsvath, Hitoshi Hirose, and M. David Tilson[1]

Department of Surgery, St. Luke's/Roosevelt Hospital Center, Columbia University, 1000 Tenth Avenue, New York, New York 10583

Received December 12, 1995

A microfibrillar protein (40 kDa) purified from the adventitia of the human abdominal aorta is immunoreactive with IgG harvested from the wall of abdominal aortic aneurysms. We have partially sequenced this protein and found that it has fibrinogen alpha-, beta-, and gamma-like domains, a vitronectin-like domain, and a possible site for binding calcium. Because of homologies with other microfibril-associated glycoproteins and because it is the third member of the family to be characterized in man, we suggest the name MAGP-3.

Nomenclature of the microfibrillar proteins associated with the elastin fiber is confusing. A principal component of the microfibril is fibrillin (fib-15), discovered by Sakai et al,[1] and Marfan's syndrome has been traced to mutations in the gene for fibrillin on chromosome 15.[2 3 4] A bovine microfibrillar protein (Mr $\sim$ 31 kDa) was discovered in 1986 by Gibson et al,[5 6] who coined the term 'microfibril-associated glycoprotein' (MAGP). Bashir et al have also cloned the gene for this protein.[7] Kobayashi et al reported a 36 kDa calcium-binding protein, also in cow, with tissue distribution uniquely limited to the aorta (MAGP-36).[8] The second human MAGP (deduced MW 21 kDa) was recently reported to have an open reading frame of 255 amino acids and to be linked to Smith Magenis syndrome.[9] The authors of the paper describing the Smith Magenis protein prefer the abbreviation 'MFAP', to avoid confusion with abbreviations for microfilamentous proteins.

We have recently reported that IgG from the aortic wall of patients with abdominal aortic aneurysms (AAA) is immunoreactive with a human aortic protein (MW $\sim$ 80 kDa) that has features of the bovine aortic protein of Kobayshi et al (MAGP-36).[10] MAGP-36 occurs in nature as a disulfide-bonded dimer, so we undertook further tissue extractions under reducing conditions as described by Prosser et al.[11] This approach has led to the partial characterization of a protein of $\sim$40 kDa that is immunoreactive with AAA IgG. We call this protein Aortic-Aneurysm-Associated Protein-40 (AAAP-40). The present communication is to report its partial sequence and to suggest that, since it is the third human microfibrillar protein to be described, it be called MAGP-3.

METHODS

Human aortic tissue was extracted for microfibrillar proteins according to the method of Prosser et al.[11] In brief, the tissue was first extracted in a phosphate buffer containing potassium chloride 0.6 M. The insoluble pellet was treated with bacterial collagenase in Tris buffer. The final tissue extraction utilized guanidinium chloride 6 M in buffer containing dithiothreitol 50 mM and EDTA 2 mM. Gel slices containing the protein of interest were digested with trypsin or Lys-C and amino acid sequences were determined in the Protein Chemistry Core Facility, Howard Hughes Medical Institute, Columbia University (New York, NY).

[1] Corresponding author. Fax: 212-523-6495.

0006-291X/96 $18.00

RESULTS

A 59 residue sequence of AAAP-40 as experimentally determined is shown in Table I. Alignment on MFAP-4 and MAGP-36 (bovine) is shown, along with homologous sequences from the alpha and beta chains of human fibrinogen. Five and 11 residue sequences of AAA-40 are shown in Table II, in alignment with sequences from vitronectin, MFAP-4, MAGP-36, fibrinogen-beta (from its calcium binding domain), and two other calcium binding proteins (myeloid calcium binding protein and bovine aggrecan).

DISCUSSION

Fibrinogen-like domains are well-known in the MAGP's.[8] The sequence of AAA-40 shown in Table I has regions of substantial homology with sequences in the alpha and beta chains of fibrinogen. Another sequence that we have determined (data not shown) matches residues 283–292 in the gamma chain. Since the three fibrinogen chains are believed to have a single ancestral gene, it would appear likely that AAAP-40 is related to the common ancestor since it has motifs that are used in all three fibrinogen subunits.

Kobayashi et al noted that MAGP-36 has the property of calcium-binding, although a candidate site for the calcium-binding domain has not been proposed. Kielty and Shuttleworth have observed that incubation of intact microfibrils with EDTA rapidly results in gross disruption of microfibrillar organization, which can be reversed by replacing calcium.[12] Since fibrillin has 43-EGF-like motifs with calcium binding consensus sequences, and calcium has been proposed to orchestrate the assembly of tropoelastin to the microfibril and hold it in register for crosslinking,[13] we hypothesize that the calcium-binding domain of AAAP-40 may play a role in calcium-dependent microfibril assembly in the aorta. When we searched GenBank for homologies of AAAP-40 and MAGP-36, we found sequences in calcium-binding myeloid-related protein (pir|A44111: #144–154), the calcium-binding domain of human fibrinogen-beta, and bovine aggrecan (pir|A39808: #59–66) that have similarities to MFAP-4, MAGP-36, and AAAP-40. Bold type is used in Table II to highlight residues that appear to be conserved, with possible significance for the calcium-binding function.

Another matrix protein detected in human embryonic tissue (sulfated protein 30 kDa = SP-30) has been reported to be immunoreactive with monoclonal antibodies against human vitronectin,[14] and it co-distributes in tissue with the protein that is immunoreactive with antibody against MAGP-31.[14] A sequence of AAAP-40 that matches residues # 230–240 in human vitronectin is also shown in Table II. Tomasini-Johansson et al proposed that SP30 is the human homolog of MAGP-31, but

TABLE I

					$	**(Y)**	**F**	**P**	**(F)**	**V**	**D**	**L**	**M**	**V**	**M**	**A**	**N**	**Q**	**P**	**M**			**AAAP-40**
122	T	L	K	Q	K	Y	E	L	R	V	D	L	E	D	F	E	N	N	T	A			MFAP-4
	T	L		L	K	Y	E	L	R	V	D	L	E	D	F	E	X	N	T	A			MAGP-36
								L	R	V	E	L	E	D	.	A	.	N	.	A			Hum Fib-A
	G	**E**	**!**	**Y**	**Y**	**D**	**F**	**F**	**Q**	**Y**	**T**	**X**	**G**	**M**	**A**	**K**	**E**	**Y**	**D**	**G**	**F**	**Q**	**AAAP-40**
142	Y	A	K	Y	A	D	F	S	I	S	P	N	A	V	S	A	E	E	D	G			MFAP-4
	F	A	K	Y	A	D	F	S	I	S	P	N	A	V	S	A	E	E	D	G			MAGP-36
	Y	**T**	**X**	**G**	**M**	**A**	**K**	**(I** | **Y)**	**A**	**G**	**N**	**A**	**L**	**M**	**D**	**G**	**A**	**S**	**G**	**L**	**M**	**AAAP-40**
162	Y	T	L	F	V	A	G	F	E	D	G	G	A	G	D	S	L	S	Y	H			MFAP-4
	Y	T	L	Y	V	S	G	F	E	D	G	G	A	G	D	S	L	T	Y	H			MAGP-36
	Y	.	I	.	V	.	K		T	A	G	N	A	L									Hum Fib-b

This table shows sequence of AAAP-40, as determined experimentally. The sequence of AAAP-40 is aligned along a continuous sequence of MFAP-4, beginning at residue 140. Homologous regions of MAGP-36 bovine and fibrinogen alpha (residues 120-132) and beta residues 338-353 (human) are also shown. ''( )'' is used to designate an ambiguous residue; ''.'' denotes a non-conserved residue; and ''!'' denotes a tryptic cleavage site.

TABLE II

Q	**E**	L	E	K										$	F	E	**D**	G	V	L	D	P	D	Y	P	AAAP-40
														R	F	E	**D**	G	V	L	D	P	D	Y	P	VN
F	C	**L**	Q	Q	**P**	L	D	C	D	D	I	Y	A	Q	G	**Y**	Q	S	**D**	G	VYL	I	Y	P	S		MFAP-4
S	**E**	L	Q	L	**P**	L	D	**E**	D	D	**I**	Y	A	Q	G	**Y**	Q	A	**D**	G	VYL	I		P	S		MAGP-36
T	**E**	L	.	.	.	**L**	.	**E**	.	D	**V**	Y	.	.	.	**Y**	.	.	**D**								CaBP-M
					P	.	D	**E**	.	D	**V**	Y															Aggr
S	**E**	L	E	K	H	Q	L	.	.	D	.	**T**															Fib-b

Alignment of experimentally determined sequences of AAAP-40 on sequences from human vitronectin (VN, residues 230-240) and MFAP-4 (beginning at residue #34). Alignments with other calcium-binding proteins are shown, with the most highly conserved residues highlighted in bold type: calcium-binding myeloid-related protein[15], CBP-M; aggrecan (bovine)[16], Aggr; human fibrinogen beta (residues 144-157 from calcium-binding domain), Fib-b.

since MAGP-31 does not have a vitronectin-like domain, we believe that SP-30 is more likely to be closely related to AAAP-40.

Finally, a brief comment on the nomenclature problem. Perhaps the simplest approach for the present would be to assign the human MAGP's a number in the order of their discovery. Thus, the first would be the principal component of the microfibril, fibrillin.

The second would be the protein of Smith Magenis syndrome, and the third would be the protein described in the present communication. MAGP-3 is probably the human homolog of the bovine aortic protein of Kobayashi (MAGP-36), and the human homolog of Gibson's MAGP-31 has yet to be identified. Notwithstanding the proposal of Zhao et al to call this family of glycoproteins ''MFAP,''[9] we favor retention of the abbreviation MAGP, since the first bovine member of this family was so-named by Gibson et al ten years ago and the term has been widely used ever since.

REFERENCES

1. Sakai, L. Y., Keene, D. R., and Engvall, E. (1986) Fibrillin, a new 350-kD glyco-protein component of extracellular microfibrils. *J. Cell Biol.* **103,** 2499–2509.
2. Dietz, H. C., Cutting, G. R., and Pyeritz, R. E., *et al.* (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* **352,** 337–339.
3. Dietz, H. C., Saraiva, J. M., Pyeritz, R. E., Cutting, G. R., and Francomano, C. A. (1992) Clustering of fibrillin (FBN1) missense mutations in Marfan syndrome patients at cysteine residues in EGF-like domains. *Human Mutation* **1,** 366–374.
4. Kainulainen, K., Pulkkinen, L., Savolainen, A., Kaitila, I., and Peltonen, L. (1990) Location on chromosome 15 of the gene defect causing Marfan syndrome. *N. Engl. J. Med.* **323,** 935–939.
5. Gibson, M. A., Huges, J. L., Fanning, J. C., and Cleary, E. G. (1986). The major antigen of elastin-associated microfibrils is a 31 kDa glycoprotein. *J. Biol. Chem.* **261,** 11429–11436.
6. Gibson, M. A., Sandberg, L. B., Grosso, L. E., and Cleary, E. G. (1991) Complementary DNA cloning establishes microfibril-associated glycoprotein (MAGP) to be a discrete component of the elastin-associated microfibrils. *J. Biol. Chem.* **266,** 7596–601.
7. Bashir, M. M., Abrams, W. R., Rosenbloom, J., Kucich, U., Bacarra, M., Han, M.-D., Brown-Augsberger, P., Mecham, R., and Rosenbloom, J. (1994) Microfibril-associated glycoprotein: characterization of the bovine gene and of the recombinantly expressed protein. *Biochemistry* **33,** 593–600.
8. Kobayashi, R., Mizutani, A., and Hidaka, H. (1994) Isolation and characterization of a 36-kDa microfibril-associated glycoprotin by the newly synthesized isoquinolinesulfonamide affinity chromatography. *Biochem. Biophys. Res. Communic.* **198,** 1262–6.
9. Zhao, Z., Lee, C.-C., Jiralerspong, S., Juyal, R. C., Lu, F., Baldini, A., Greenberg, F., Caskey, C. T., and Patel, P. I. (1995) The gene for a human microfibril-associated glycoprotein is commonly deleted in Smith-Magenis syndrome patients. *Human Mol. Genetics* **4,** 589–597.
10. Tilson, M. D. (1995). Similarities of an autoantigen in aneurysmal disease of the human abdominal aorta to a 36-kDa microfibril-associated bovine aortic glycoprotein. *Biochem. Biophys. Res. Communications* **213,** 40–43.
11. Prosser, I. W., Gibson, M. A., and Cleary, E. G. (1984) Microfibrillar protein from elastic tissue: a critical evaluation. *Aust. J. Exp. Bio. Sci.* **62,** 485–505.

12. Kielty, C. M., and Shuttleworth, C. A. (1993). The role of calcium in the organization of the fibrillin microfibrils. *FEBS* **336,** 323–326.
13. Mecham, R. P., and Heuser, J. E. (1991) *in* Cell Biology of Extracellular Matrix (Hay, E. D., Ed.), 2nd ed., pp. 79–109, Plenum Press, New York.
14. Tomasini-Johansson, B. R., Ruoslahti, E., and Pierschbacher, M. D. (1993). A 30 kDa sulfated extracellular matrix protein immunologically crossreactive with vitronectin. *Matrix* **13,** 203–214.
15. GenBank accession number pir|A44111: residues 144–154.
16. GenBank accession number pir|rA39808: residues 59–66.