Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins

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The organic matrix of bone is made up mostly (~90%) of the common protein, type I collagen, and therefore most markers of bone biochemistry will generally rely on the presence of noncollagenous proteins (NCP). Over the last 15 years several laboratories around the world have been characterizing all of the major species of NCP which can be found entrapped within the mineralized matrices of a variety of vertebrate species. Virtually all of these sources of young bone have been found to contain essentially the same spectrum of NCP, although the relative levels of each protein may vary from one species to another. The gene products so far described in the literature probably represent greater than two-thirds of the total mass of soluble proteins found entrapped within the mineralized matrix. The final one-third of the extracted NCP are comprised of a large number of low abundance proteins that, in general, have not been described.

We can characterize each NCP as belonging to one of three general categories. 1) Serum-derived proteins which, because of their affinity for apatite or collagen, accumulate in the matrix. Examples of this class of NCP would include albumin and alpha 2HS glycoprotein. Alpha 2HS glycoprotein is frequently one of the most abundant NCP observed in bone matrices. 2) Products of the osteoblastic lineage, some of which are relatively specific to bone while others are made by many cell types. 3) Unknown. This last category represents a large number of proteins that may be most exciting to bone cell biology but due to their relatively low abundance in the final matrix, they have not yet been characterized.

The propeptides of collagen can serve as good reminder of critically important proteins that, due to their low affinity for the matrix and the mineral, do not accumulate in the final matrix to a level in any way reflecting either their importance to the final matrix assembly and function or to their original abundance. If either the amino-propeptide or the carboxy-propeptide were stoichiometrically retained in the final mineralized matrix, the mass of that propeptide would be equal to all of the other NCP together. Yet both the amino-propeptide (Fisher 1987b) and the carboxy-propeptide (Fisher unpublished data) are present in the mineralized matrix at levels less than 5% of that which must have been present during the assembly of the collagen matrix. Thus we can see that critically important proteins and perhaps even those very abundant when the matrix is first synthesized may not be found in the final matrix in significant amounts. Indeed, those proteins that do not have an affinity for the matrix may diffuse into the bloodstream and ultimately be the future ideal markers of bone synthesis.

Materials and methods

Antisera production: Purified proteins were isolated as previously described (Fisher 1983, 1987, Mintz 1993). Synthetic peptides were either made at facilities at NIDR, NIH, as described (Fisher 1989a) or were purchased from a variety of commercial sources. For most commercially produced custom peptides an additional cysteine was added to either the amino-terminus (for peptides meant to mimic carboxyterminal sequences) or the carboxy-terminus (for amino-terminal sequences). The peptides were conjugated to either horseshoe crab hemocyanin (LPH, US Biochemical Corp.), keyhole limpet hemocyanin (KLH, Sigma), or chicken serum albumin (CSA, US Biochemical Corp.) via sulfo-MBS (Pierce). Recombinant proteins were made in *E. coli* BL21

Table 1. cDNA probes

Gene product	Locus	Plasmid name (accession #)	Vector	Insert size (in kbp)	Comments	Reference
Human biglycan	BGN	P16 (J04599)	pBluescript	1.7	full length (of coding region)	Fisher et al. 1989b
Mouse biglycan	Bgn	clone-3 (L20276)	Shlox	2.4	full length	
Human decorin	DCN	P2	pBluescript	1.6	missing 5' flanking & first 4 codons	Fisher et al. 1989b
Human decorin	DCN	P13	pBluescript	1.7	contains exon 1a	Fisher 1993
Human decorin	DCN	P22	pBluescript	1.7	contains exon 1b	Fisher 1993
Bovine decorin		Pg28 (Y00712)	pBR322	1.3		Day et al. 1987
Bovine decorin		BDCN-BS	pBluescript	1.3	same as Pg28 but in riboprobe vector	
Mouse decorin	Dcn	mDCN-5	pBluescript	1.3	full length	
Human osteopontin	SPP1 or OPN	OP-10 (J04765)	pBluescript	1.5	alternate splice missing 42 bp	Young et al. 1990a
Bovine osteopontin		OP-12 (M66236)	pBluescript	1.4	full length	Kerr et al. 1991
Mouse osteopontin	Spp1	mop3	pCR II	1.0	full length	
Human bone sialoprotein	IBSP	B6-5g (J05213)	pBluescript	1.2	full length	Fisher et al. 1990
Mouse bone sialoprotein	Ibsp	mBSP1 (L20232)	pCR II	1.0	full length	Young et al. 1994
Human osteonectin	SPARC	hon-2	pBluescript	2.5	full length, also known as SPARC	Young et al. 1990b
Bovine osteonectin		bon-1.5	pUC19	1.5	C-term. & 3' flanking	Bolander et al. 1988 and Young et al. 1986
		bon-0.3	pUC19	0.3	middle coding	
		bon-0.2	pUC19	0.2	N-term. & 5' flanking	
Bovine osteonectin		bON-BS2	pBluescript	1.5	same as bon-1.5 but in riboprobe vector	
Mouse tetranectin	Tna	ptet (U08595)	pBluescript	1.0	full length	Ibaraki et al. 1995

(DE3) cells using Novagen's pET 15b or 22b expression vectors. In these cases the in-frame cDNA insert was made by PCR, gel purified, restriction digested and ligated into the vector. The resulting His-Tag fusion proteins were purified on a Ni^{2+} His-Bind Resin column (Novagen) and used directly for antiserum production without removal of the small fusion peptides. In general, three series of ten intradermal injections (1 mg each for free peptides or peptide conjugates, or 100–250 µg purified proteins or recombinant proteins) were performed under contract at an AAALAC-approved facility (Hazelton Research Products). The first contained Freund's complete adjuvant and the other two series were with incomplete adjuvant. Bleeds were taken at two week intervals. Antisera are generally checked for activity at 1:1000 or 1:2000 dilution against Western blots with each lane containing 50-100 µg of mineral compartment extract proteins from the bones of a variety of animal species.

cDNA probes: Most cDNA probes have been previously described (Table 1). Other probes have not been specifically described in publications and have been cloned either by RT-PCR or direct PCR of a cDNA library using published sequences and synthetic oligonucleotides. The PCR products were either directly cloned into the TA cloning vector (pCRII from Invitrogen) or were designed to introduce restriction sites in the oligonucleotide domains for subsequent cloning into pBluescript (Stratagene).

Results and Discussion

The following tables show the antisera and cDNA probes that are available to interested scientists. Polyclonal antisera are, of course, limited in amounts, so we require that any requests for antisera be made only for immediate use. All of these reagents are for research purposes only. Any requests for use in human studies of any type must be accompanied by signed statements guaranteeing that the study will be conducted following all local and NIH Guidelines for Patient Care and Confidentiality. Co-authorships for use of these reagents are not required unless significant scientific work or discussions are given by the provider of the reagent. Requesters will be asked to report the specific name of the antiserum or plasmid and to generously reference, where appropriate, work published by our laboratories.

Table 2. Proteoglycan polyclonal antisera

Gene product	Antiserum name	Antigen source	Known species cross-reactivity	Comments	References
Human decorin	LF-30	GIGPEVPDDRDF-[KLH]	Hum & Mon only	increased signal strength with pretreatment with ABC'ase	Fisher et al. 1989b Bianco et al. 1990
Human decorin propeptide	LF-110 LF-111	QVSWAGPFQQRGLFDC-[LPH] QVSWAGPFQQRGLFDC-[CSA] (amino acids 5-17)	Hum, others not determined but seq is highly conserved	no cross to BGN propeptide	
Human decorin	LF-122	recombinant protein including propeptide	Hum, Mon, Pig, She, Chi, cow(?)		
Human biglycan	LF-15 & LF-51 & LF-121	GVLDPDSVTPTYSA-[BSA] (amino acids 11-24) same conjugate for all 3 antisera	Hum & Mon only	increased signal strength with pretreatment with ABC'ase	Fisher et al. 1989b Bianco et al. 1990
Human biglycan propeptide	LF-104 LF-105	LPFEQRGFWDFTLDDC-[LPH] LPFEQRGFWDFTLDDC-[CSA]	Hum, Mon, Rat, Mou others not determined		
Human versican	LF-99 LF-98	LHKVKVGKSPPVRC-[LPH] LHKVKVGKSPPVRC-[CSA]	only human tested		Bernstein et al. 1995b
Human biglycan	LF-121	recombinant protein including propeptide	Hum and Mon only		
Bovine decorin	LF-94 LF-95	IGPEEHFPEVPEC-[LPH] IGPEEHFPEVPEC-[CSA]	only cow tested for these 2 antisera	Cow domain analogous to Human LF-30	
Bovine biglycan	LF-96 LF-97	LPDLDSLPPTYSC-[LPH] LPDLDSLPPTYSC-[CSA]	only cow tested for these 2 antisera	Cow domain analogous to Human LF-15 series	
Murine decorin	LF-113 LF-114	IIPYDPDNPLISMC-[LPH] IIPYDPDNPLISMC-[CSA]	only tested for Mou and Rat	Mouse domain analogous to Human LF-30	
Murine biglycan	LF-106 LF-107	VPDLDSVTPTFSAMC-[LPH] VPDLDSVTPTFSAMC-[CSA]	only tested for Mou and Rat	Mouse domain analogous to Human LF-15 series	

The following abbreviations are used in the tables: Hum human; Mon monkey; She sheep; Chi chicken; Mou mouse; Rab rabbit. Amino acids are standard single letter codes.

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Table 3. Sialoprotein polyclonal antisera

Gene product	Antiserum name	Antigen source	Known species cross-reactivity	Comments	References
Human osteopontin					
	LF-7	human bone OPN	Hum & Mon only	also known as SPP1, BSP-I	Fisher et al. 1987a
	LF-19	IPVKQADSGE homopolymer	Hum & Mon only		Fisher et al. 1989b
	LF-85	TVDTYDGRGDSVVYGLR	Hum, Mon	human osteopontin RGD domain	
	LF-86	SLS-[LPH] (both)			
	LF-77	GGGRGDS-(LPH)	only Hum tested	may detect many	
	LF-78	(both)	not well characterized	RGD-containing proteins	
	LF-123	recomb. carboxyl half starts at thrombin site	Hum, Mon, Pig, Cow, She, Rat, Mou	does not contain the RGD domain	based on OP-10 plasmid (Young et al. 1990a)
	LF-124	recombinant amino half ending at thrombin site	Hum, Mon, Cow, She, Rat, Mou	contains the RGD domain	based on OP-10 plasmid (Young et al. 1990a)
Human bone sialoprotein					
	LF-6	human bone BSP	Hum, Mon, Dog, Rat, Mou		Fisher et al. 1987a Bianco et al. 1991, 1993
	LF-83	YESENGEPRGDNYRAYE	Hum, Mon, Dog,	epitope appears	Mintz et al. 1993,
	LF-84	D-[LPH] (both)	Rat, Mou, Chi	to be RAYED	Bianco et al. 1993
	LF-100	AIQLPKKAGDIC-[LPH]	Hum, Mon, Dog, Rat	mid-protein antigen	Mintz et al. 1993, Bianco et al. 1993
	LF-101	AIQLPKKAGDIC-[CSA]	Hum, Mon, Dog, Rat, Pig, Cow	similar to LF-100	
	LF-119	recombinant RGD domain (AA257-317)	Hum, Mon, Dog, Chi		based on B6-5g plasmid (Fisher et al. 1990)
	LF-120	recombinant Frag 1 (AA 129-281)	Hum, Mon, Pig, Dog, Rat		based on B6-5g plasmid (Fisher et al. 1990)
	LF-125	recombinant AA 36-61	Hum, Mon, Pig, She, Rat	first Tyr-rich domain	based on B6-5g plasmid (Fisher et al. 1990)
Bovine bone sialoprotein					
	Bovine BSP	bovine bone BSP	Hum, Cow	limited amounts	Fisher et al. 1983
Rat (UMR) bone sialoprotein					
	LF-87	rat BSP isolated from UMR-106-BSP media	Rat, Mou, Hum	fully sulfated form of rat BSP	Bianco et al. 1993
	LF-90	rat UMR-106 BSP growin in chlorate	Rat	low (<5%) sulfate groups on BSP	

Abbreviations, see Table 2.

Table 4. Collagen polyclonal antisera

Gene product	Antiserum name	Antigen source	Known species cross-reactivity	Comments	References
Bovine αI(1) amino-propeptide	LF-9	bovine bone N-propeptide	Hum, Mon, Pig, She		Fisher et al. 1987b
Human αI(1) amino-propeptide	LF-39 LF-40	EEGQVEGQDEDIPPITC] VQ-[KLHJ (both) (AA 2-20)	Hum, Cow, She		Fisher et al. 1989a
Human αI(1) carboxy-propept.	LF-41 LF-42	LFCVPGVDFGFEQDPAGVD LP-[KLH] (both) (last 21 AA)	All species except, perhaps, Rab	detects proαI(1) andC-propeptide	Fisher et al. 1989a
Human αI(1) carboxy-telopept.	LF-67 LF-68	SAGFDFSFLPQPPQEKAHD GGRYYRA (both)	All species except, perhaps, Rab	detects proαI(1), pCαI(1) , αI(1) +/- reduction	Bernstein et al. 1995b
Human αI(2) amino- telopeptide	LF-116	QYDGKGVGLGPGPC-[LPH]	Hum, not mouse, others not tested		
Human type III collagen C-propeptide	LF-69 LF-70	DIGGPDQEFGVDVGPVCFL (C-terminal 19 AA) (both)	Hum, Mon, others not tested	seq. homology to type II but not tested. Reduction increases reaction	Bernstein et al. 1995b
Human type III collagen N-telopeptide	LF-71 LF-72	EYDSYDVKSGVAC-[KLH] (N-terminal 12 AA) (both)	Hum, Mon, others not tested		Bernstein et al. 1995b

Abbreviations, see Table 2.

Table 5. Miscellaneous polyclonal antisera

Gene product	Antiserum name	Antigen source	Known species cross-reactivity	Comments	References
Human osteonectin	HON	human bone osteonectin with final purification on SDS PAGE	Hum, Mon tried	Also known as SPARC	Fisher et al. 1987a
Human osteonectin	LF-37	EALPDETEVVEETVAEVT EVP-[KLH] (AA 4-25)	Hum, Mon		
Bovine osteonectin	BON-I BON-II	bovine bone osteonectin (both)	All species tested		Ingram et al. 1993
Bovine osteonectin	LF-55 LF-56	first 56 AA of mature Cow sequence (both)	only Cow and Hum tested		
Bovine osteonectin	LF-53 LF-54	AA 27-56 of mature Cow sequence (both)	only Cow and Hum tested		
Chicken osteonectin	LF-8	chicken bone osteonectin	Chi, Quail		
Chicken osteonectin	LF-45	APQEALPDETEVIED-[KLH]	Chi, Quail	AA 1-15 of mature	
Mouse osteonectin	LF-23 LF-24	TEVAEEIVE EET-[KLH] (both) (AA 5-16 of mature)	Mou, Rat, She, Chi		Wewer et al. 1988
Bovine osteocalcin	LF-32	bovine bone osteocalcin	Cow, Hum, Mon	also known as bone gla protein (BGP)	Ingram et al. 1993
Bovine alkaline phosphatase	LF-52	from bovine kidney affinity purified by Sigma	unknown	bone-liver-kidney form of the enzyme	
Human alkaline phosphatase	LF-47 LF-48	LVPEKEKDPKYWRDQAQ ETC-[KLH] (both) (AA 1-19)	Hum (weak), Cow, Pig, Rab	human sequence but weak activity	Tulli et al. 1992
Human osteo-inductive factor (OIF)	LF-102 LF-103	CLKRLPIGSYF-(LPH) CLKRLPIGSYF-(CSA)	both not tested	C-term of OIF, both uncharacterized	

Abbreviations, see Table 2.

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