Molecular and Ultrastructural Studies of a Fibrillar Collagen from Octocoral (Cnidaria)

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Supplementary information

Amino acid sequences of fibrillar and non-fibrillar collagens

Over 25 types of fibrillar and non-fibrillar collagens have been reported in humans. However, only fibrillar (ex. type I and II) and network forming (type IV) types of collagen have been reported in the earliest branches of multicellular organisms such as Cnidarians (Exposito et al., 2008). The amino acid composition of type IV collagen from humans has been under evaluation for decades. The usual G-X-Y repeat found in fibrillar collagens is often found to be disrupted in human type IV collagen (Glanville, 1987). There are considerable differences in macromolecular structure of these two collagen types. These differences are a result of the amino acid composition and positioning of the X and Y amino acids within the G-X-Y repeat itself, between the two types of collagen (Ulla et al., 1986, Brazel et al., 1987). As demonstrated in the table, there is a higher concentration of glycine in the *S. ehrenbergi* collagen than in non-fibrillar collagen (type IV), further validating that the former is a fibrillar form of collagen.

Table S1 expands on the amino acid differences presented in Table 1. The differences in the amino acid composition between the various types of collagen lends further validates the interpretations made on the structure of the fibrillar collagen described here.

Table S1: Comparison of amino acid contents between type I (fibrillar) and type IV (network forming) collagens from *S. ehrenbergi* with other species. The accession numbers of the sequences are indicated respectively.

	S. ehrenbergi	Rat Type I Collagen	Human-Type II,Collagen	Human- Col4α1	Cnidarian- Col 4α1*
		(3HR2_A, 3HR2_B)	(P02458)	(AAI51221)	(KXJ15387)
G	24.89	33.22	31.87	28.64	17.80
P	5.08	12.00	21.27	19.47	12.20
Hyp†	4.15	9.96	0.00	0.00	0.00
A	6.49	10.53	9.96	3.48	4.27
R	5.35	4.95	5.02	2.70	5.00
E	9.52	4.56	5.18	4.19	3.54
S	4.35	4.18	3.27	4.31	6.71
D	8.34	2.84	3.27	3.48	4.88
K	2.96	2.97	3.67	5.57	7.56
Q	n/a	2.71	2.95	4.31	2.93
T	n/a	2.04	2.31	2.58	4.88
L	3.29	2.39	2.63	5.51	6.22
V	3.97	2.36	1.43	3.06	5.12
F	1.69	1.21	1.04	2.76	1.83
N	n/a	1.53	1.67	0.96	3.29
M	1.96	0.67	0.56	1.86	2.56
I	2.47	1.12	1.59	3.48	4.76
Y	1.52	0.35	0.64	1.08	2.44
H	n/a	0.41	0.56	1.02	1.34
C	n/a	0.00	0.64	1.20	1.46
W	n/a	0.00	0.48	0.36	1.22

*Partial sequence available.

† Hyp = Hydroxyproline.

Structural comparison of fibrillar and network forming collagens

Evident differences can be seen in the X-ray diffraction (XRD) patterns obtained from type IV collagen extracted from the wall of dogfish egg case when compared to those from fibrillar collagens, such as type I and II and the mesenterial collagen from *S. ehrenbergi*. A detailed XRD analysis on type IV collagen can be found in Gathercole et al., 1993 and Knupp and Squire, 1998. Electron microscopy and XRD information from these publications indicates a marked difference between these datasets from type IV and fibrillar (type I and II) collagen. For instance, TEM images from type IV collagen from the dogfish egg shell show an alternating banding pattern every 32 nm (Knupp and Squire, 1988). This banding pattern is considerably different form fibrillar collagens, in that there are more discreet striations within the D-perioidic repeat (67 nm) in fibrillar collagens.

Similar differences in XRD patterns can be observed. For example, the XRD patterns type IV collagen show a principle repeat of 81.2 nm (Knupp and Squire, 1998), whereas those from the collagen fibers from *S. ehrenbergi* show a principle repeat of 66 nm. This further validates the use of phase information from mammalian fibrilar collagen (type I and II) to derive 1-D electron density maps for the collagen fibers from *S. ehrenbergi*.