COLLAGEN PRESERVATION IN SOFT TISSUE FROM THE MAGADAN MAMMOTH

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1. Introduction

The woolly mammoth calf (*Mammuthus primigenius*) discovered in the Magadan district of Siberia [1,2] has provided a unique opportunity for histological and biochemical studies of an extinct mammal. After the calf's entombment in permafrost for about 40 000 years [3], tissue from its dorsal abdominal wall still contains some remarkably well preserved structures as judged by scanning and transmission electron microscopy [4,5]. On the basis of our biochemical studies we can report that while the most abundant protein material is degraded collagen, undegraded apparently native type I collagen chains are also present.

Although previous biochemical studies on remains of mammoths and other extinct species identified degradation products of α-keratins in mammoth hair [6] and collagen in fossil bone and teeth by amino acid composition analysis, no purified chains of any protein had ever been isolated [7]. Our success in identifying undegraded type I collagen from a small sample of the Magadan calf's soft tissue supports the possibility that, eventually, the amino acid sequence of some mammoth polypeptides will be determined. Comparison of such genetic information from *Mammuthus* to the corresponding information from present day proteins of *Elephas* and *Loxodonta* (Asian and African elephants) could then help decipher the evolutionary history of these animals.

We carried out our biochemical study on an air-dried piece of the Magadan mammoth tissue mailed from Leningrad to Detroit [4]. Electron microscope examination [4,5] revealed besides abundant connective tissue, some skeletal muscle vestiges (see fig.1).

2. Methods and results

The mammoth tissue (440 mg) was subjected to two successive 48 h extractions with 12 ml of 1 M KCl, 25 mM Tris pH 7.5, and the residual material separated by centrifugation at 5000 × *g*, for 30 min. The supernatants were combined, brought to 55% saturation with solid ammonium sulphate. A very minute amount of material precipitated and was removed by centrifugation at 10 000 × *g*, for 30 min. The supernatant was dialyzed against water, concentrated in an Amicon ultrafiltration unit (PM-10 membrane) and lyophilized. A total of 4.6 mg was recovered. An aliquot of this material was submitted to acid hydrolysis for 24, 48, and 72 h. The amino acid compositions of the resulting hydrolysates were determined in an automatic analyser (Beckman 119CL). Peak area and molar ratio were computed by an attached 126 Beckman data system. This analysis indicated a similarity to extant mammalian collagen as shown in table 1. Of particular significance was the characteristic presence of hydroxyproline, hydroxylysine and large quantities of both proline and glycine.

The material left after the KCl extraction was minced and 50 ml of 5% trichloroacetic acid (TCA) added, heated for 30 min at 70°C and centrifuged at 10 000 × *g*, for 20 min [9]. The supernatant was extracted 3 times with diethyl ether and after lyophilization a total of 211 mg of protein was recovered. An aliquot of this material was submitted to acid hydrolysis and automatic amino acid analysis. For purposes of comparison native collagen from African elephant tendon was isolated and purified in our laboratory. The amino acid analyses from both the elephant collagen and from the mammoth TCA extracted collagen were remarkably similar (see fig.2).

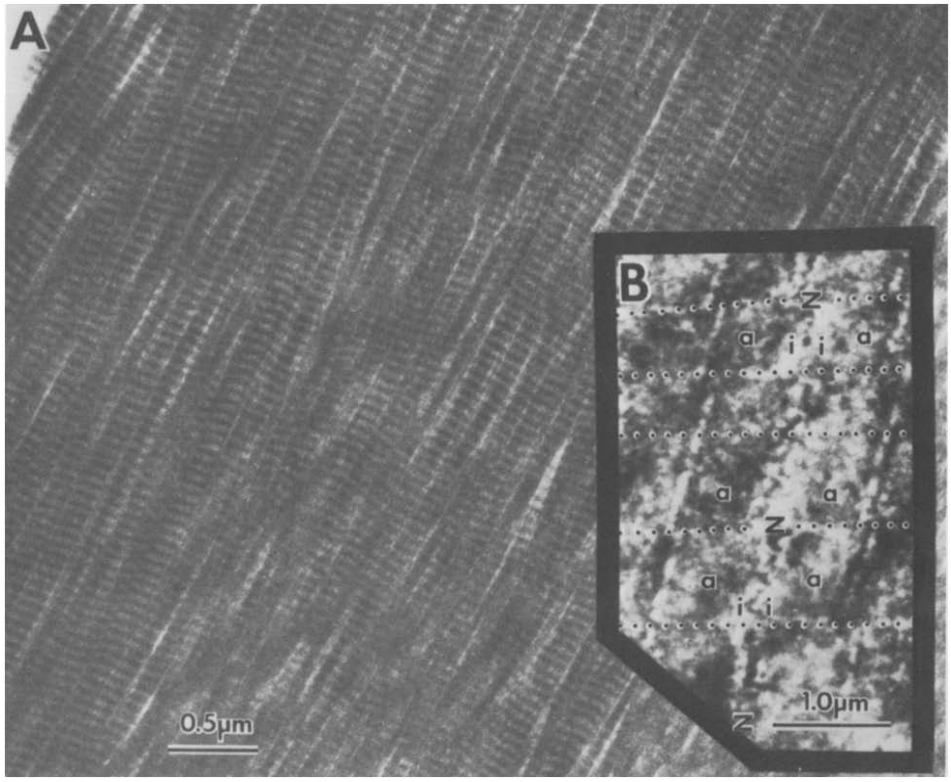

Fig.1. (A) Electron micrograph of *Mammuthus primigenius* collagen showing the characteristic periodicity of about 64 nm. (B) Remnants of skeletal muscle are suggested by myofibril preservation with edges denoted by (*). Sarcomere vestiges of A (a) and I (i) bands appear with Z bands most prominent.

Table 1
Amino acid composition of mammoth, elephant and rat collagens[a]

	Mammoth		Elephant tendon	Rat tail tendon [8]
	A	B		
Aspartic acid	48	46	52	45
Hydroxyproline	107	69	71	94
Threonine	20	20	21	20
Serine	32	32	47	43
Glutamic acid	82	90	79	71
Proline	108	108	113	122
Glycine	370	355	313	331
Alanine	96	111	110	107
Valine	21	28	25	23
Methionine	5	7	5	8
Isoleucine	8	10	12	10
Leucine	22	26	33	23
Tyrosine	3	2	6	3
Phenylalanine	9	13	15	12
Hydroxylysine	10	12	8	7
Histidine	3	4	11	4
Lysine	22	19	28	27
Arginine	34	48	51	50

[a] Residues per 1000. (A) KCl-extracted material. (B) TCA-extracted material

Sample of both the KCl and TCA-extracted material from the mammoth tissue were analysed by 5% polyacrylamide gel electrophoresis [10] in the presence of 0.1% sodium dodecyl sulfate (SDS). The former only contained relatively low MW polypeptides (<68 000 daltons) whereas the latter had, in addition to these polypeptides, two faint bands corresponding to the positions of native α1 (I) and α2 (I) collagen chains (standards obtained from Sigma Chemical Co.).

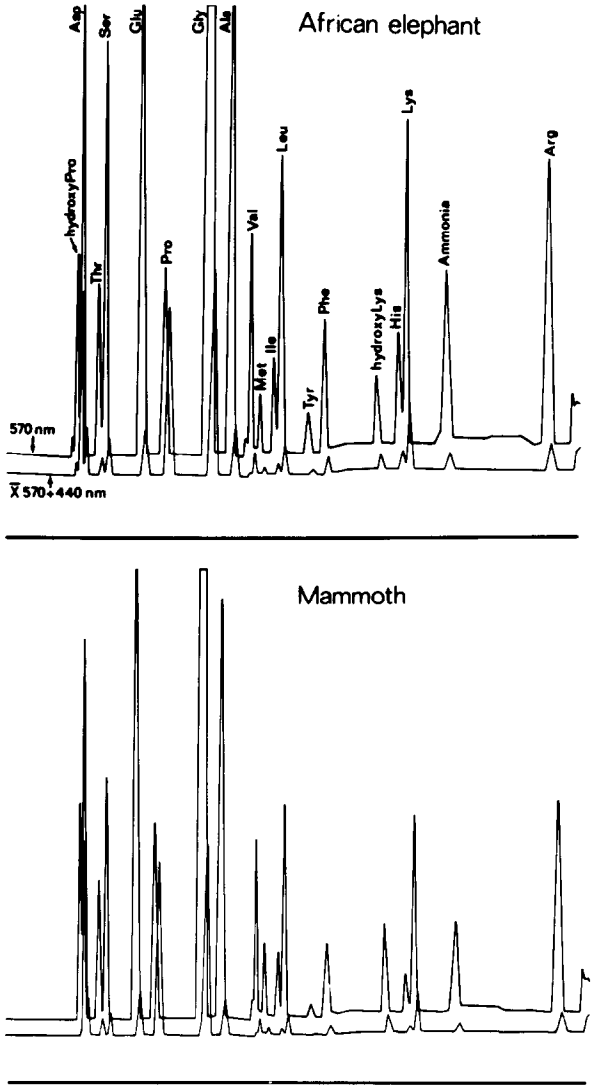

Fig.2. Amino acid analyses recordings of African elephant tendon and mammoth soft tissue collagens. Both patterns are remarkably similar, reflecting the excellent preservation of collagen at the biochemical level in the Magadan mammoth in spite of the many millennia since the calf died. Analysis conditions: single column, pH 3.10 (37 min), pH 3.95 (23 min), and pH 6.20 (51 min); temperature: 55°C (28 min) and 65°C (83 min).

In order to better characterize these high MW proteins the mammoth TCA extracted material was reduced with 1% 2-mercaptoethanol in the presence of 1% SDS at 60°C for 30 min, passed twice through a glass filter and concentrated in an Amicon ultrafiltration unit using a UM-50 membrane. Acrylamide gel electrophoresis of this material revealed well defined α1 and α2 collagen chains and also included the dimeric (β) and trimeric (γ) components of these molecules. This electropherogram and its scanning densitometry are shown in fig.3. The undegraded fractions represented ~10% of the filtered sample and about 1% of the starting material. Besides the native collagen chains there were substantial quantities of polypeptides with smaller MW, mainly concentrated at around 68 000, 53 000 and 44 000 daltons.

Both KCl and TCA-extracted mammoth material were treated with a specific bacterial collagenase (form III, Advanced Biofacture) utilizing the method of Peterkofsky and Diegelman [11]. None of the previously described polypeptide bands native or undegraded appeared in the electropherograms of the treated samples (see fig.3), providing further proof of the collagenous nature of all these components.

3. Discussion

The present study has demonstrated that a high yield of collagen polypeptides can be isolated from well preserved mammoth soft tissue. About 50% of the 440 mg of air dried initial material consisted of polypeptides which closely resemble in amino acid composition purified collagen prepared from modern African elephant tendon. In the electron microscope examination of this Magadan mammoth tissue (fig.1), collagen fibrils were present in abundance and showed, in spite of an age of around 40 000 years, their characteristic banding and diameter. No doubt, the coiling of the three subunits of tropocollagen with their tight packing and multiple hydrogen bonding, together with the staggering and cross-linkage between tropocollagen molecules, played an important role in maintaining the morphological integrity of these fibrils, even though some of the peptide bonds were hydrolized at random.

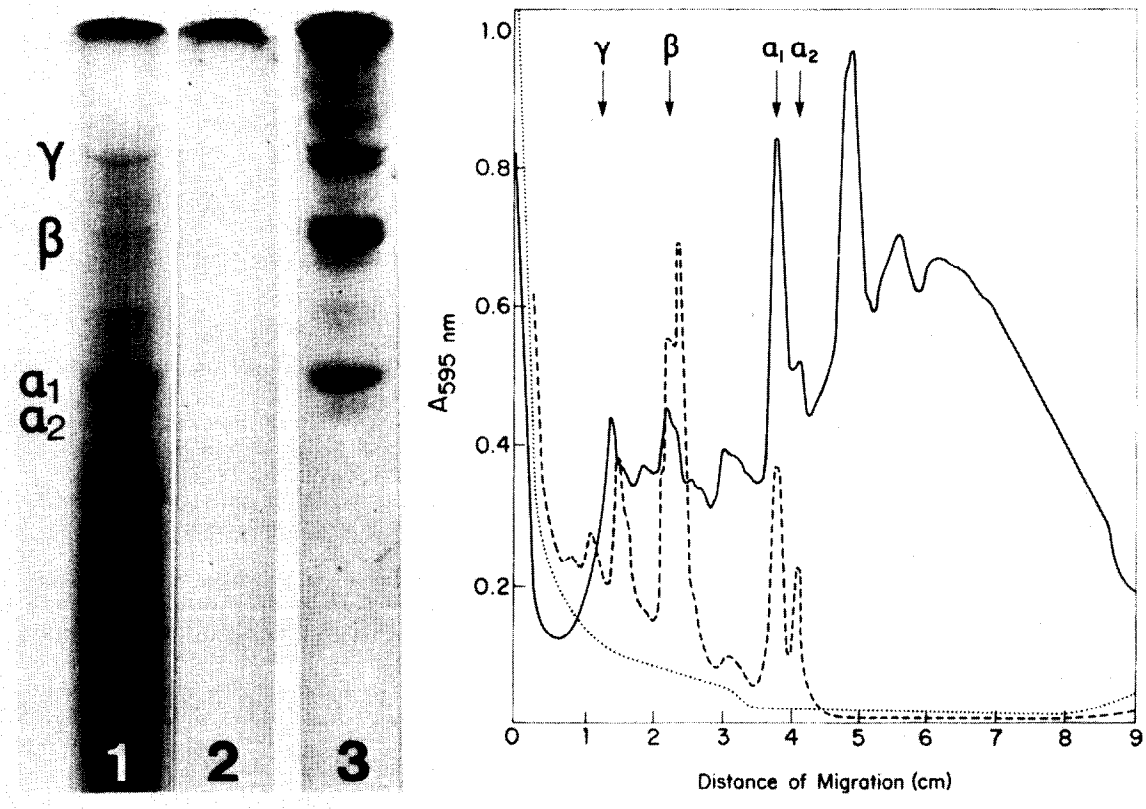

Fig.3. SDS-acrylamide gel electropherograms of (1) mammoth collagen, (2) same specimen as in 1 after treatment with collagenase, and (3) bovine skin type I collagen. The trimeric (γ), dimeric (β), and monomeric ($\alpha 1$ and $\alpha 2$) molecules of type I mammoth collagen, as well as the lower molecular weight polypeptides resulting from random hydrolysis, are evident in gel 1. The scanning densitometric traces shown on the right correspond to gel 1 (continuous line), gel 2 (dotted line), and gel 3 (dashed line).

If additional mammoth connective tissue becomes available, it should be possible to isolate and purify sufficient collagen (either native chains or randomly derived large fragments) for amino acid sequencing. Such sequence data could then serve as a phylogenetic indicator for the position of *Mammuthus* within the subfamily Elephantinae. Obtaining this data would not be easy. In fact, the collagen chains of extant animals have been little used in evolutionary studies because the repetitive nature of their amino acid sequence (approximately one third of the molecule being Gly and one quarter Pro) and their large size (~1052 residues) have precluded their ready sequencing in a wide variety of species [12]. However, the aligned partial sequences of bovine and rat $\alpha 1$ chains differ by 2.5% of their residues. A larger difference (8.7%) exists between $\alpha 2$ chains from the same species. This rate of substitution in $\alpha 2$ chains during the last 60 to 70 million years approximates low values encountered in some myoglobins [13,14]. Thus since *Mammuthus, Elephas*, and *Loxodonta* are present in the fossil record at about 5 million years ago, apparently as descendants of the older African genus *Primelephas* [15,16], the collagens of these genera might well differ by several amino acid substitutions.

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