Mammoth Hair: Stability of α-Keratin Structure and Constituent Proteins

Abstract. *Hair from a woolly mammoth (*Mammuthus primigenius, *age about 32,000 years) retains the ordered structure characteristic of α-keratins, but the proteins of this hair differ in composition from and are smaller than similar proteins isolated from other keratins, for example, elephant hair. It is suggested that these changes have been caused by limited proteolysis.*

Next to bone and tooth, hair appears to be one of the most resistant of the biological structures (*1*). Hair of ground sloths has survived from the Pleistocene and there are many reports of the survival of apparently intact hair of the woolly mammoth and the woolly rhinoceros for periods of perhaps 30,000 years (*2*). Even with much less favorable conditions of storage, samples of wool fabric of the Danish bronze age (about 3200 B.P.) have been recovered in sufficiently good condition to be used by Ryder in his studies on the lines of development of the domestic sheep (*3*). Although these fibers may appear to be intact and undamaged, no modern investigation has been made of them, particularly one having reference to the survival of their internal structural arrangements and of their constituent proteins. An opportunity to make such an examination was provided by the gift of mammoth hair from Professor Troy L. Pewé (*4*). This hair, obtained in 1951 from the remains of a mammoth (*Mammuthus primigenius*) uncovered at Dome Creek, Alaska (*5*), was dated by the radiocarbon method at 32,700 ± 980 years B.P. (*6*).

All mammalian keratinous fibers appear to be constructed of aligned filaments (microfibrils) about 70 Å in diameter which are largely composed of proteins of low sulfur content and comparatively high α-helix content (low-sulfur proteins). These microfibrils are surrounded by an unaligned matrix composed of nonhelical proteins which are of extremely high sulfur content (high-sulfur proteins). The presence in mammoth hair of an intact microfibril-matrix complex has been sought by x-ray diffraction studies and by isolation and characterization of the constituent proteins. For comparative purposes the hair of the closely related Indian elephant, *Elephas maximus*, has been used (*2, 7*).

Mammoth hair gave a sharp x-ray diffraction pattern (*8*) comprising a 5.1-Å meridional reflection and the same set of low-angle meridional periodicities and equatorial reflections given by human and other animal hairs. Mammoth hair had a sulfur content of 3.8 percent and an ash content of 4 percent, while the elephant hair (*9*) was somewhat richer in sulfur (4.1 percent), with a negligible ash content. These hairs had a similar amino acid composition (Table 1) except for lower values in mammoth hair for threonine, serine, proline, and glycine and higher values for aspartic and glutamic acids, lysine, alanine, and leucine. In addition mammoth hair contained small amounts of the abnormal amino acids, lanthionine and cysteic acid.

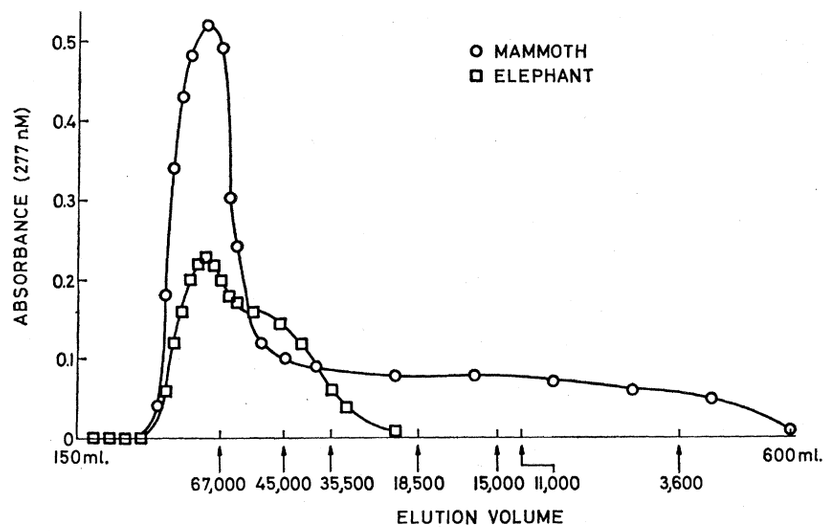

Fig. 1. A comparison of the distribution of molecular sizes in the low-sulfur proteins of elephant and mammoth hairs by gel filtration in Sephadex G200, in a system containing: urea, 8*M*, and KCl, 1*M*. Calibration points are indicated on the abscissa.

The low-sulfur proteins prepared from elephant and mammoth hairs by reduction and alkylation (*10*) were qualitatively similar in amino acid composition (Table 1) and in general resembled the low-sulfur proteins of other keratins (*11*). However, they differed quantitatively in essentially the same way as did the parent keratins. By measuring the optical rotatory dispersion and calculating the parameter b_0, I made an estimate of the proportion of α-helix in the low-sulfur proteins of mammoth and elephant hairs (*11*). Accurate measurements were difficult to make, particularly in the case of the mammoth protein, because of the presence of black pigment, but each preparation contained α-helix, mammoth having about 23 percent and elephant about 28 percent.

The size distribution of the low-sulfur proteins of mammoth and elephant hairs was compared by gel filtration through a column of Sephadex G200 calibrated by measuring the elution volumes of the *S*-carboxymethyl derivatives of seven pure proteins (Fig. 1). The molecular weights of the mammoth low-sulfur proteins cover a very wide range down to as low as perhaps 3500, whereas there is no major constituent in the elephant low-sulfur protein with a molecular weight below 20,000. The large peak in the elution pattern of the mammoth low-sulfur protein coincides with the elution of black melanin pigment. On electrophoresis of the low-sulfur proteins of many, if not all, α-keratins in starch gels at *p*H 8.5 two major bands, numbered 7 and 8, can be seen (*12*). While the pattern of elephant low-sulfur protein conforms to this generalization (Fig. 2A), that of mammoth low-sulfur protein shows no bands but only an ill-defined smear of higher mobility.

The high content of *S*-carboxymethylcysteine, serine, and proline in the high-sulfur proteins of mammoth and elephant hairs (Table 1) showed them to be typical members of this group of keratin proteins (*10*); however, they differed from each other in much the same way as did the parent keratins. In starch gel electrophoretograms the high-sulfur proteins of wool and elephant hair (Fig. 2B) gave well-resolved patterns, each composed of a

set of bands, of mobilities characteristic for the particular keratin, whereas mammoth high-sulfur protein spread as a diffuse smear largely concentrated toward the front.

The x-ray diffraction pattern given by mammoth hair is consistent with the presence of a native α-keratin structure, in which α-helical proteins are coiled and arranged in the form of microfibrils (about 70 Å in diameter), precisely spaced and embedded in a nonfilamentous matrix. Compatible with this conclusion, on solubilization, two distinct types of proteins were isolated, one of high sulfur content, the other partly α-helical and low in sulfur content.

However, there is contrary evidence which suggests that mammoth hair cannot be regarded as a native keratin. First, the presence in mammoth hair of cysteic acid showed that cystine residues had been partly oxidized, and the presence of lanthionine (0.16 residue in every 100 amino acid residues) suggested that part of the cystine had been subjected to a β-elimination reaction at alkaline *p*H values. Second, electrophoresis in starch gels showed that components of smaller size than normal were present in both high- and low-sulfur protein fractions, and this was supported in the case of the low-sulfur proteins by measurements of molecular size, by using gel filtration. Furthermore, the amino acid analyses of mammoth hair and its constituent proteins showed that they differed from those of elephant hair and its proteins in a way suggestive of a loss of some amino acid residues from mammoth hair. The most likely cause of the changes in size and composition seems to be limited enzymatic proteolysis.

Table 1. The amino acid composition of elephant and mammoth hairs and their constituent proteins. Values are presented as the number of residues of each amino acid in 100 residues in the protein. The methods used for preparing the reduced and *S*-carboxymethylated proteins and for their subsequent acid hydrolysis and amino acid analysis are given in reference (*10*).

Amino acid	Keratin		Low-sulfur protein		High-sulfur protein	
	Elephant	Mammoth	Elephant	Mammoth	Elephant	Mammoth
Lysine	2.74	3.15	3.64	3.95	0.82	1.33
Histidine	0.84	0.72	0.72	0.65	0.69	0.73
Arginine	6.85	7.19	7.29	7.42	5.17	6.38
Cysteic acid	0.00	0.41	0.00	0.19	0.00	0.60
CMcys*	0.00	0.00	6.18	5.17	18.60	19.00
Aspartic acid	7.08	8.51	9.07	10.20	3.12	4.36
Threonine	7.24	6.27	5.47	4.96	9.92	8.96
Serine	11.00	7.02	8.94	5.90	14.10	8.40
Glutamic acid	14.20	17.50	16.60	20.20	9.25	10.40
Proline	7.60	5.59	3.90	3.00	13.20	12.10
Glycine	6.50	4.37	5.67	3.58	7.38	5.40
Alanine	6.06	6.94	7.38	8.07	3.23	4.47
Half-cystine	6.80†	7.42†	0.00	0.00	0.00	0.00
Valine	6.03	6.45	6.16	6.30	5.25	6.92
Methionine	0.47	0.56	0.59	0.57	0.00	Traces
Isoleucine	3.68	4.17	3.62	4.05	2.64	3.00
Leucine	7.64	9.35	9.71	11.20	3.53	4.68
Tyrosine	2.77	2.46	2.89	2.75	1.83	1.87
Phenylalanine	2.10	1.83	1.98	1.88	1.49	1.27
Tryptophane			0.46	0.25		

* *S*-carboxymethylcysteine. † The half-cystine analyses are much lower than would be expected from the sulfur content of the fibers. The reason for this discrepancy is not known.

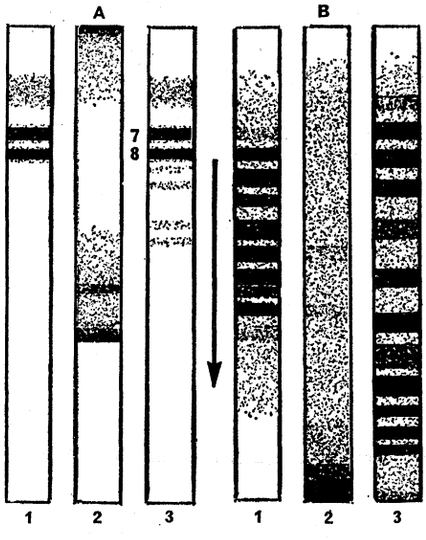

Fig. 2. A comparison, by starch-gel electrophoresis, of the proteins of elephant hair, mammoth hair, and wool. (A) Low-sulfur proteins run in a system containing: urea, 8.3*M*; citric acid, 7.5 m*M*; tris, 50 m*M*; sodium borate, 25 m*M*; at *p*H 8.5 to 8.6. (B) High-sulfur proteins run in a system containing: urea, 2.75*M*, and acetic acid, 4.8*M*, at *p*H 2.4. (1) elephant, (2) mammoth, (3) sheep.

Little is known about the proteolysis of native keratins, but studies on the limited proteolysis of the low-sulfur proteins of wool (*13*) have shown that as fragments having low molecular weight are split from a resistant core, the amino acid composition of the residual protein changes progressively, with increases in aspartic and glutamic acids, lysine, and leucine, together with decreases in *S*-carboxymethylcysteine, glycine, proline, serine, and threonine. With the important exception of *S*-carboxymethylcysteine (present as cystine in a native keratin) these changes are very similar to the differences observed between mammoth and elephant hairs.

It is therefore suggested that proteolysis has split the protein chains at a number of points, resulting in the formation of a set of peptides of varying sizes. Small peptides may be leached from the fiber, whereas larger peptides, including cross-linked cystine peptides, may be unable to penetrate cell membranes or may be held firmly by molecular interactions. In this way a major change in composition could take place with only minimum changes in the physical structure of the fiber and in its cystine content.

A possible degradative agent is not hard to find, for, as Farrand (*2*) has pointed out, most mammoths have been decomposed more or less extensively before being frozen, and it appears that this Dome Creek mammoth had been very extensively damaged (*5*). The putrid odor of many mammoth carcasses and the surrounding burial ground is sufficient proof of the existence of proteolytic enzymes in the environment of the hair (*2*). It would be of considerable interest to see whether hair from a more recent mammoth or from one of the better preserved Siberian specimens shows less damage (*14*).

The hypothesis presented above is currently being tested by treating wool with proteolytic enzymes for comparatively short periods and then isolating and characterizing the constituent proteins. Substantial decreases in molecular size have been found in low-sulfur proteins isolated from wool treated with pronase or chymotrypsin.

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9. The gift of Mr. R. Strahan, director of the Sydney Zoological Gardens.
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14. I would appreciate the gift of such fibers and also of samples of α-keratins from the Pleistocene.

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Antigen-Binding Cells in Normal Mouse Thymus

Abstract. *The thymus of a normal adult mouse contains lymphocytic cells with a large number of antigen-binding receptors. Binding of the antigen by these cells is specific and can be inhibited by cross-reactive materials. It is possible that the interaction of thymocytes with antibody-forming precursor cells, required in the primary immune response to certain antigens, is mediated by these specific antigen-binding cells of the thymus.*

In the mouse, precursors of antibody-forming cells interact with thymus-derived, antigen-reactive cells (ARC) to proliferate and differentiate into hemolysin-producers (*1, 2*). Thymus cells respond to antigenic stimulation with immediate proliferation (*3*), but without antibody formation (*4*). Recognition of antigen by cells is believed to be mediated by antigen receptors, presumably antibody molecules (*5*). Hence ARC of thymus, if specific, should be capable of binding the antigen. Naor and Sulitzeanu (*6*) have demonstrated the presence of specific, antigen-binding cells in the spleen and lymph nodes of normal mice by their sensitive autoradiographic technique. However, antibody-forming cells to many antigens have been demonstrated in the spleen (*7*), but not in the thymus (*8*) of normal animals. We examined the thymus of normal mice for presence of antigen-binding cells (presumably ARC and not background antibody-forming cells) by immunofluorescence and A-H assay. The A-H assay (an acronym for antigen binding to cells determinated by enzymatic fluorogenic group hydrolysis) (*9*) utilizes β-galactosidase of *Escherichia coli* (βGz) as antigen and is based on the measurement of the enzymatic activity of cell-bound βGz by Rotman's method (*10*) which allows the detection of the activity of single molecules of this enzyme. Accordingly, about 10^8 washed cells from A/Jax mice (8 to 10 weeks old) were incubated with 15 to 50 μg of βGz at 37°C for 1 hour or at 4°C overnight. As a control, a portion of each cell suspension was incubated without enzyme. After the incubation, the cells were washed four times each with 20 ml of Hanks balanced salt solution (HBS) at 4°C to remove unbound enzyme. These cells will be referred to as "prepared" cells. A sample was removed, and nucleated cells were counted in a hemacytometer. Cell suspensions were then diluted in HBS to obtain the desired number of cells (i) for determination of total antigen-binding activity; (ii) for enumeration of active cells and measurement of their activity; and (iii) for morphological studies by the immunofluorescence technique.

To determine total activity, 0.2 ml of a cell suspension containing approximately 1×10^7 to 2×10^7 cells was mixed with 0.2 ml of $4.8 \times 10^{-5}M$ fluorescein di-β-galactopyranoside (FDβG), and the intensity of the fluorescence produced during 6 hours at room temperature was measured by a Turner fluorometer (model 111, equipped with 47B and 2A-12 filters). The results (Table 1) show that 10^7 thymus cells from normal mice bind considerably more βGz than do the same number of spleen cells. To determine the specificity of βGz binding, the cell suspensions were incubated with 5.0 mg of heat-inactivated βGz at 37°C for 1 hour before the active enzyme was added. Heated enzyme (56°C for 10 minutes) is antigenically cross-reactive but enzymatically inactive. In the presence of Mn^{2+}, Mg^{2+}, and reducing agents, the heated enzyme may regain up to 30 percent of its original enzymatic activity after 10 to 12 hours. Hence, in experiment 3, the overnight incubation was omitted. Some binding of the enzyme by thymus and spleen cells is specific and can be inhibited by excess heated enzyme. Presence of 5 percent fetal calf serum or other unrelated proteins has little or no effect.

Table 1. Antigen-binding activity of normal mouse thymus and spleen cells. Results are expressed as the increase in fluorometer units per 10^7 nucleated cells. In experiment 3, the cells were first incubated for 1 hour at 37°C with 5.0 mg of heated βGz (inactive enzyme) and then for an additional hour with 50 μg of active βGz. They were washed after the second incubation period and assayed together with the test samples, which were not exposed to inactive enzyme.

Cell	Experiment 1	Experiment 2	Experiment 3	
			Test sample	Inactive enzyme + βGz
Thymus	10.7	11.0	12.2	3.9
Spleen	4.0	3.5	6.8	3.1

For enumeration of active cells in a population, a modified A-H assay was used. Accordingly, "prepared" cells were diluted with HBS to contain approximately 5×10^6 cell/ml. A portion was mixed with an equal volume of $4.8 \times 10^{-5}M$ FDβG, and hundreds of 0.2-μl droplets were placed in oil chambers with a "Terasaki Dispensor" (Hamilton Co., Whittier, Calif.). Each droplet, therefore, contained approximately 500 cells. In the course of a 30- to 60-minute incubation at 37°C, fluorescent droplets with considerable activity could be visualized by the naked eye when the chamber was illuminated by a blue light. Each active droplet was then examined under a microscope, and the ones with clumps were disregarded. The activity of the droplets was measured by an Aminco photomultiplier-microphotometer attached to a Zeiss fluorescence microscope (*9*). Since the frequency of active droplets is usually less than 1×10^{-2}, the probability that each active droplet contains more than one active cell is low. Based on this reasoning, the frequency of active cells in normal mouse spleen and thymus was calculated (Table 2). The frequency of active cells in the normal thymus is greater than that of those in the spleen. This is in contrast to the findings of Byrt and Ada (*11*) in which antigen-binding cells are more frequent in spleen than thymus. This discrepancy, however, may be due to the different antigens used in the two studies and to the fact that only the cells which