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THE FIBROUS PROTEINS IN VARIOUS TYPES OF ICHTHYOSIS

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The stratum corneum of individuals with ichthyosis vulgaris, sex-linked ichthyosis, lamellar ichthyosis, and epidermolytic hyperkeratosis has been studied. An α x-ray diffraction pattern has been observed in all specimens and the solubility of the α fibrous proteins appears to be the same as in normal stratum corneum. Sodium dodecyl sulfate (SDS)–polyacrylamide electrophoresis of the fibrous proteins showed variable patterns within the different types of ichthyosis, while amino acid analyses of the proteins were quite similar to those from normal stratum corneum. These data suggest that the fibrous proteins in the ichthyosis are not abnormal, but further studies on the individual polypeptide chains are necessary to rule out more subtle differences.

Ichthyosis refers to a group of heterogeneous diseases which have in common a markedly thickened stratum corneum. A genetic basis has been demonstrated in most patients [1], but acquired ichthyosis has been reported in association with drug therapy [2] and malignancy [3]. Since structural proteins are the principal constituents of the stratum corneum, an abnormality in their structure could be an important factor in the pathogenesis of these diseases. As a result of recent advances in our knowledge of the macromolecular chemistry of keratinization it is now possible to carry out definitive studies on human stratum corneum. The purpose of this paper is to describe our observations on the physicochemical properties of the principal structural protein of stratum corneum, the α protein, in several types of ichthyosis.

MATERIALS AND METHODS

Materials. All chemicals used were of reagent grade except iodoacetic acid which was crystallized from anhydrous ether and petroleum ether. Stratum corneum was scraped from the skin with a scalpel blade and stored at −20°C in a desiccator. Samples from 4 patients with ichthyosis vulgaris, 3 with sex-linked ichthyosis, 2 with epidermolytic hyperkeratosis, 7 with lamellar ichthyosis, and membranes from 2 collodion babies were studied.

Extraction procedures. A 20% homogenate of the various tissues was prepared in 6 M urea in 0.1 M Tris, pH 9.0, (Tris–urea) and stirred at room temperature for 24 hr. Following centrifugation the extraction was repeated a second time. The undissolved pellet was then extracted in Tris–urea with 0.1 M merceptoethanol under nitrogen at room temperature overnight. The suspension was centrifuged and an aliquot of the supernatant treated with iodoacetic acid to give the *S*-carboxymethyl (SCM) derivative [4]; the alkylated and remaining untreated extracts were dialyzed against distilled water and lyophilized.

X-ray diffraction. X-ray diffraction analysis was done using nickel-filtered copper Kα radiation (λ = 1.54 Å) at 40 kv at a specimen to film distance of 1.50 cm. Regenerated filaments for x-ray diffraction analysis were prepared by dissolving the protein in 80% formic acid, picking up the solution on the tip of forceps, and stretching while drying at room temperature.

Amino acid analysis. Samples for amino acid analysis were hydrolyzed in 6 N HCl for 24 hr under vacuum at 110°C and run on a Beckman 116 amino acid analyzer. The analyses were done in duplicate and the data expressed as residues per 100 residues not including cystine.

Electrophoresis. Disc electrophoresis in urea was done by the method of Davis [5] with the addition of urea at a concentration of 6 M to the gels and buffer. Sodium dodecyl sulfate (SDS) electrophoresis employed the same running gel and buffer but with the addition of 0.1% SDS instead of urea. The samples for SDS electrophoresis were heated at 50°C in 1% SDS and 1% mercaptoethanol for 30 min just prior to being used, which resulted in complete equilibration.

Sulfur content. Total sulfur content was determined gravimetrically following its oxidation to sulfate and the addition of barium (Belmont Analytical Lab) as previously described [6].

RESULTS

X-ray diffraction. X-ray diffraction analyses of scales from patients with various types of ichthyosis show α patterns similar to those from normal patients, with 5.14 Å meridional and 9.8 Å equatorial reflections. There is an additional meridional reflection at 4.15 Å which can be eliminated by prior extraction of the tissue with a chloroform–methanol mixture (3/1) [7].

Extraction of tissue. The solubility of the stratum corneum proteins was studied by first extracting the tissue with Tris–urea and then with the same buffer with the addition of 0.1 M mercaptoethanol.* The yield with the Tris–urea buffer for

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* The extracted proteins were dialyzed, lyophilized, and weighed.

normal stratum corneum is 20 ± 6 gm/100 gm and with the Tris–urea–mercaptoethanol buffer is 45 ± 7. The values obtained for the ichthyotic stratum corneum samples are within 1 standard deviation of the mean normal value. X-ray diffraction patterns of filaments regenerated from the Tris–urea-soluble proteins show no evidence of an α pattern but an α pattern is observed in the Tris–urea–mercaptoethanol-soluble proteins.

Electrophoretic patterns. Disc electrophoresis of the Tris–urea–merceptoethanol-extracted proteins in urea gives patterns with rather poor resolution. Polyacrylamide electrophoresis in 0.1% SDS gives three quite clear patterns as shown in the Figure. A number of components are present but one (*A*) predominates. Variable amounts of aggregated protein can be noted by the staining at the origin. Within the various types of ichthyosis, examples of each of these patterns have been observed. Pattern 3 was seen with normal stratum corneum.

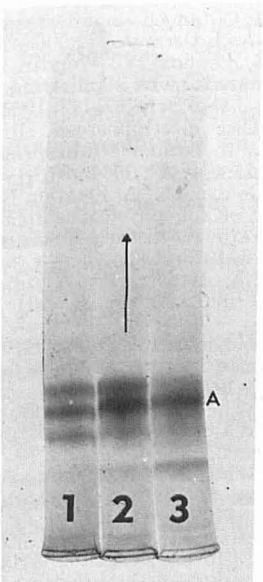

FIG. Sodium dodecyl sulfate (SDS) polyacrylamide electrophoretic patterns of the α fibrous protein isolated from stratum corneum. Pattern 3 was seen in normal stratum corneum while, within the various types of ichthyosis, examples of each of the three patterns have been observed.

Amino acid analysis. Amino acid analysis of the Tris–urea–mercaptoethanol-soluble proteins from the various major types of ichthyosis are shown in the Table. Although there are minor variations in some of the residues, the results tend to be rather consistent. Also included are analyses of the solubilized proteins from the collodion membranes of two children who later developed ichthyosis vulgaris. No results on cystine content are given as we have found such data on human scales to be rather inconsistent. Sulfur content on some samples is shown as this analysis was very consistent.

DISCUSSION

The chemical basis of the ichthyotic disorders is not known, except for the harlequin fetus [8], where it has been shown that a cross-β fibrous protein is present instead of the usual α one. In a previous study, minor differences were reported in peptide maps of sodium hydroxide-solubilized proteins from the stratum corneum of patients with ichthyosis [9]. The type of ichthyosis was not identified and no conclusion could be reached as to the significance of the changes. In the major forms of ichthyosis described in this report, no consistent differences have been revealed in the x-ray diffraction pattern and solubility of the stratum corneum

TABLE. *Amino acid analysis of the α fibrous proteins isolated from the stratum corneum of various types of ichthyosis*[a]

	Vulgaris	Sex-linked	Epidermolytic	Lamellar	Collodion	Normal
Lysine	4.7 ± 0.7	4.0 ± 0.3	5.0 ± 0.5	4.6 ± 0.2	4.7 ± 0.3	4.3 ± 0.3
Histidine	1.1 ± 0.3	1.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.2	1.1 ± 0.3	1.0 ± 0.2
Arginine	4.5 ± 0.7	4.0 ± 0.1	4.6 ± 0.3	4.3 ± 0.4	4.4 ± 0.4	4.7 ± 0.2
Aspartic acid	9.2 ± 0.3	9.0 ± 0.4	9.5 ± 0.6	9.1 ± 0.3	9.3 ± 0.3	9.4 ± 0.5
Threonine	3.4 ± 0.2	3.2 ± 0.3	3.4 ± 0.3	3.7 ± 0.2	4.2 ± 0.1	3.9 ± 0.1
Serine	11.4 ± 1.0	12.2 ± 0.8	11.3 ± 0.3	11.7 ± 0.8	12.1 ± 0.1	11.5 ± 0.6
Glutamic acid	13.1 ± 0.7	13.2 ± 0.4	13.6 ± 0.2	13.8 ± 0.4	13.7 ± 0.1	13.7 ± 0.4
Proline	1.8 ± 0.4	1.6 ± 0.3	1.9 ± 0.6	2.2 ± 0.4	3.1 ± 0.1	2.3 ± 0.2
Glycine	21.6 ± 1.4	23.3 ± 0.3	21.6 ± 2.2	21.0 ± 0.8	19.0 ± 0.6	18.4 ± 0.5
Alanine	5.9 ± 1.2	5.0 ± 0.6	4.9 ± 0.4	5.2 ± 0.3	5.8 ± 0.1	5.5 ± 0.2
Valine	3.3 ± 0.3	3.6 ± 0.4	3.6 ± 0.1	3.7 ± 0.4	3.8 ± 0.2	3.7 ± 0.2
Methionine	1.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.5 ± 0.1
Isoleucine	3.5 ± 0.3	3.7 ± 0.3	3.4 ± 0.3	3.6 ± 0.3	3.0 ± 0.1	3.2 ± 0.1
Leucine	8.9 ± 0.7	8.7 ± 0.3	8.3 ± 0.1	8.4 ± 0.3	8.1 ± 0.1	8.4 ± 0.3
Tyrosine	3.4 ± 0.2	3.4 ± 0.4	3.2 ± 0.1	3.3 ± 0.3	3.0 ± 0.3	3.2 ± 0.2
Phenylalanine	3.2 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.4	3.4 ± 0.1	3.4 ± 0.1
Sulfur %	0.95 ± 0.03	0.96 ± 0.02	0.91 ± 0.03	0.93 ± 0.01	0.97 ± 0.02	0.94 ± 0.03

[a] Residues per 100 residues ± standard deviation.

or the amino acid composition and electrophoretic pattern of the isolated fibrous proteins. That the extracted fibrous protein is entirely normal must be viewed with some reservation in view of the recent observation that different polypeptide components can be detected in the α protein of epidermis [10].

The several polypeptides which have been observed in the α fibrous protein of cow snout epidermis have been shown to be different but they do have similar amino acid analyses. Human fibrous protein may also consist of several components. It would be possible to miss several such polypeptide chains and alterations in them, however, since SDS–polyacrylamide electrophoresis only detects differences in molecular weight.

A further problem to be considered is the variability observed in the SDS–acrylamide electrophoretic patterns. In the case of cow snout epidermal α polypeptides, it has been shown that there is a differential susceptibility to enzymatic hydrolysis. It is not unlikely that in the final stages of keratinization some modification occurs in the fibrous proteins secondary to the release of hydrolytic enzymes. In the case of the α proteins of wool it has been found that such changes may not be detectable until the proteins are treated with denaturing solvents which then allows fragments to be released [11]. Such changes in the polypeptide chains could be responsible for the variability in the relative intensity of the electrophoretic components which have been described, considering the abnormal keratinization which occurs in the various forms of ichthyosis.

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