I. 6. THE DECOMPOSITION OF KERATIN BY MICROORGANISMS

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Numerous reports have claimed that keratin is digested by microorganisms. All of these studies either used keratin that was denatured (by autoclaving or other mistreatments), or they presented inadequate evidence that keratin actually was digested by the microorganism. Consequently, no microorganism has been definitely proven capable of digesting native keratin.

It was found that wool and other keratinaceous substances can be sterilized by vapors of ethylene oxide without detectable alteration in the chemical composition or enzymatic resistance of the keratin. Keratin that was sterilized in this manner was considered to be undenatured and was employed in a study of keratin digestion by microorganisms.

Strains of *Streptomyces fradiae* were found to be unique in their ability to digest keratin; on a dry weight basis, 80 to 90% of ethylene-oxide-sterilized keratins (wool and feathers) were solubilized when present as the sole carbon and nitrogen source in the medium. Extractions with chloroform and water, and exposure to vapors of ethylene oxide (each performed at room temperature) were the only treatments that these keratins had received. *S. fradiae* 3739 was also able to solubilize (almost totally) wool that had been sterilized with chloroform. Under optimal conditions, complete solubilization occurred within four days of incubation.

The following conditions of incubation were employed: agitation at 37°, in a medium with an initial pH of 7.7, were found to be most favorable for the decomposition of wool by *S. fradiae* 3739. Calcium and magnesium ions were found to stimulate the attack of *S. fradiae* on wool.

Soluble sulfhydryl compounds were found to be present in cultures of *S. fradiae* 3739, during wool digestion, in an amount equivalent to two-thirds of the cystine originally present in the wool that was digested. Paper chromatography of the *N*-ethylmaleimide (NEM) derivatives of these sulfhydryl compounds revealed (Fig. 1) at least three distinct sulfhydryl compounds, none of which was cysteine or glutathione. After acid hydrolysis of the culture broth, cysteine-NEM was detected on chromatograms, indicating that cysteine peptides were present in the culture broth. Am-

monia accumulated in the cultures of *S. fradiae*, during the digestion of wool, and accounted for 75% of the nitrogen in the digested wool.

Cell-free broths obtained from cultures of *S. fradiae* were found to digest keratins and casein rapidly. Heating (85°, five minutes) destroyed this activity, whereas dialysis did not. The action of these culture broths on wool and casein occurred optimally at pH 8.5 to 10. The effect of various common enzyme-inhibitors indicated that sulfhydryl groups were not essential, but that a metal was required for the digestion of both keratin

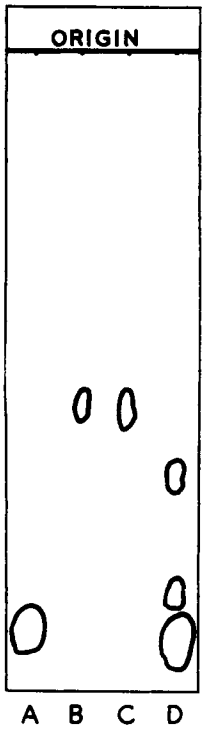

FIG. 1. Descending chromatogram of sulfhydryl-*N*-ethylmaleimide compounds. A, broth from culture of *S. fradiae* decomposing wool at 35°; acid hydrolyzed, and *N*-ethylmaleimide added; B, acid hydrolyzate of wool, reduced with zinc and *N*-ethylmaleimide added; C, cysteine-*N*-ethylmaleimide; D, broth from culture of wool-decomposing *S. fradiae* at 35°; *N*-ethylmaleimide added.

and casein. Magnesium stimulated the digestion of wool by the culture broth, under certain conditions.

The maximum extent of digestion (in several successive treatments of the keratin with the enzyme solution) of various keratins, by the culture broths of *S. fradiae* was 10 to 20% (by weight); in contrast, papain and trypsin could digest, at the most, only one-half as much of each keratin.

It was concluded that growing cultures of *S. fradiae* could rapidly and completely digest native keratin. This is the first convincing demonstration that any microorganism has such ability. The mechanism by which *S. fradiae* digests wool is apparently similar to that proposed for the di-

gestion of wool by insects; that is, by the combined attack of reducing and proteolytic agents, neither of which alone can account for the extent of keratin decomposition that the organism accomplishes.

Discussion

LINDLEY: Have you any ideas on the composition of those peptides at all?

NICKERSON: No.

BENESCH: Doesn't your work show that you have a mixture of a proteolytic enzyme and a disulfide reductase?

NICKERSON: I think the enzyme itself is strictly a proteolytic enzyme. When we have the organism present, we can obtain complete digestion of wool or feathers. When we remove the organism and now work with the enzyme (which we have highly purified) we can never get complete digestion. We have not yet detected the presence of a hydrogen-donating system in the cell-free enzyme preparations that can cooperate with the proteolytic system. I think the organism operates a disulfide-splitting system that attacks wool, but we do not know anything about this yet.

Our big problem is that we get about 35 different peptides in the digests from wool. Now we are trying, with the hanging curtain electrophoresis apparatus, to collect sufficient amounts of the peptides for study. Initially we will go after the three —SH containing peptides.