THE ENZYMIC DERIVATION OF CITRULLINE RESIDUES FROM ARGININE RESIDUES IN SITU DURING THE BIOSYNTHESIS OF HAIR PROTEINS THAT ARE CROSS-LINKED BY ISOPEPTIDE BONDS

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I. ABSTRACT

An enzymic activity present in hair follicles is described that can convert arginine residues to citrulline residues in proteins in situ. The Ca²⁺ dependent enzyme activity has been detected in hair follicle extracts but not in similar extracts of serum, liver or brain. The enzyme appears to act on proteins other than hair proteins and the citrulline produced can be quantitated in acid hydrolysates by a colorimetric procedure. The formation of citrulline has been confirmed by amino acid analysis and does not appear to be related to the formation of isopeptide linkages which is catalysed by the transamidase present in hair follicles.

II. INTRODUCTION

The proteins of the medulla and inner root sheath cells of hair follicles (Figure 1a) were shown to be distinct from keratin (Rogers, 1958, 1962). One of the major distinctions is that they contain citrulline, not previously described as a constituent of proteins. In addition they contain relatively large amounts of glutamic acid and are cross-linked with isopeptide bonds instead of cystine. Ever since these findings were reported (Rogers, 1962; Steinert, Harding and Rogers, 1969; Harding and Rogers, 1971, 1976) attempts have been made to elucidate the origin of the citrulline residues to give some explanation for the apparently
Figure 1. (a) Diagrammatic representation of a hair follicle in cross-section showing the arrangements of the different cell layers at a level at which all of the cell contents are hardened (keratinized in the case of the hair cortex). It is to be noted that the cells of the inner root sheath are separated from those of the medulla by the hair cortex. (b) Similar to (a) but at a lower level (lower third) of the follicle at which the cells of the inner root sheath and medulla are not hardened but contain granules of trichohyalin.

unique occurrence of this amino acid in peptide linkage.

Early experiments showed that citrulline was derived directly by modification of arginine at some stage in protein synthesis (Allen, Lindley and Rogers, 1964). Recent evidence suggests that this modification occurs in a protein precursor of the medulla and inner root sheath (Rogers and Harding, 1976) the material called trichohyalin (Vörner, 1903) which is abundant in the cells in the basal regions of the hair follicle (Figure 1b). A protein fraction has been isolated from hair follicles which has characteristics consistent with it having originated from trichohyalin. Its amino acid composition is distinct from that of α-keratin, but it is similar to that of the mature protein of the medulla and inner root sheath cells except that the arginine content is abnormally high, citrulline is absent and no isopeptide cross-links are detectable (Table 1). The protein fraction appears by polyacrylamide gel electrophoresis to consist of at least three major molecular weight classes ranging from 50,000 to