Enzymatic carboxyl methyl esterification of proteins: Studies on sickle erythrocyte membrane

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It is increasingly evident that several covalent post-translational modifications of proteins play important regulatory roles in many cellular functions (Uy and Wold, 1977; Paik and Kim, 1980). These reactions occur on preformed proteins by group transfer reactions on specific amino acid side chains and thus have the capacity to modulate protein function post-synthetically. Among these modifications, carboxyl methylations of proteins are being studied in membranes of prokaryotes and eukaryotes in relation to their effects on membrane functions: Thus, the role of membrane methylation in motile bacteria has now been established as the biochemical sensory input signal in chemotactic action (Springer et al., 1979; Springer and Koshland, 1977). In the case of eukaryotes, methylation is studied in various membrane systems involving secretion/excitation and motility/chemotaxis (Diliberto et al., 1976b; Gagnon et al., 1979; O'Dea et al., 1978).

Following our earlier finding that an enzyme which methylates free carboxy groups of proteins is abundantly present in mammalian erythrocytes (Kim, 1974), the human erythrocyte membrane became our experimental model for the past few years (Kim and Galletti, 1979). Since human erythrocyte membrane proteins have been well characterized, and erythrocytes with shape and function abnormalities can be readily obtained from patients, this membrane is particularly suited for the investigation of structure-function relationships mediated by membrane methylation-demethylation reactions. In this review, we will present studies carried out on carboxyl methylation of membrane proteins from patients with sickle cell anemia (SS) and discuss the potential involvement of membrane structure on the membrane protein methylation.

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CHARACTERISTICS OF PROTEIN CARBOXYL METHYLATION:

Carboxyl methylation of proteins is catalyzed by protein methylase II (S-adenosylmethionine:protein-carboxyl O-methyltransferase, E.C.2.1.1.24, protein carboxyl methylase) which transfers the methyl group of S-adenosyl-L-methionine (AdoMet) to free carboxyl groups of the substrate protein and yields protein-methyl ester (Scheme I).

Scheme I: Protein Carboxyl O-Methylation

The reaction is characterized by the following prominent features:

1. The reaction neutralizes one anionic charge associated with the protein substrate, and could thereby potentially induce alterations in the conformation and the function of the protein (Kim and Paik, 1970; Paik and Kim, 1980).

2. The methylated product, protein carboxyl methyl ester is abnormally alkali sensitive compared to similar methyl ester prepared chemically (Kim and Paik, 1976). The hydrolysis product is methanol.

3. The demethylated methyl donor, S-adenosyl-L-homocysteine (AdoHcy), is a potent product inhibitor for the reaction with a $K_i$ value about the same as $K_m$ for AdoMet (1~2 μM) (Kim, 1974; Jamaluddin et al., 1975). AdoHcy is also a well known inhibitor for all AdoMet-dependent methyltransferases (Usdin et al., 1979).

4. Protein methylase II reaction is essentially irreversible, and thus is energy requiring. The demethylation of the product may take place through a specific esterase (Stock and Koshland, 1978; Gagnon, 1979) and/or spontaneously by chemical hydrolysis.