

Studies on a novel protein, cutaneous fatty acid-binding protein, involved in the action of bioactive lipids in the skin

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Cutaneous fatty acid-binding protein (C-FABP) has been isolated from rat skin and its cDNA has been identified. As C-FABP contains five cysteines, which is rare in other FABPs so far isolated, we examined whether or not these cysteine residues are involved in the FABP function. Determination of the locations of intramolecular disulfide bonds of native C-FABP revealed that two are present between Cys-67 and Cys-87, and between Cys-120 and Cys-127. Native C-FABP purified from rat skin exhibited the ability to bind to stearic acid whereas both native C-FABP reduced with dithiothreitol and bacterially expressed C-FABP showed little binding activity. The reduced C-FABP after renaturation showed the partial recovery of the binding activity. Ion-Spray mass spectrometry of native, recombinant, or renatured recombinant C-FABP revealed that two exact disulfide bonds (between Cys-67 and Cys-87, and between Cys-120 and Cys-127), five free sulfhydryl groups, or partial exact but not non-native disulfide bonds are present in each molecule respectively. Hypothetical three dimensional structure of C-FABP showed that atomic distances between the sulfur atoms of Cys-67 and Cys-87 and of Cys-120 and Cys-127 enable to form two intramolecular disulfide bonds. These results suggest that two exact intramolecular disulfide bonds are required for its high binding activity toward fatty acids. In situ hybridization and immunohistochemical analyses showed that C-FABP is expressed in the epidermis and sebaceous glands of normal rat skin. Northern blot analysis revealed that C-FABP mRNA is highly expressed in skin, tongue and eye. Interestingly, the C-FABP message was most abundant in eye, and was present at lower levels in brain, testis and fat. These results indicate that C-FABP functions in various tissues containing epithelial cells.