CHAPTER I
INTRODUCTION

Today, food allergy or food hypersensitivity in children is still a big problem for many parents. Food allergy is an adverse reaction after ingestion of food or food additives. Allergens are the cause of the hypersensitive symptoms such as atopic dermatitis or eczema, asthma, gastrointestinal anaphylactic reaction and anaphylaxis. Allergens in food products are commonly found to be proteins such as those in eggs, cow's milk, wheat, nuts, fish. Common occurrences in newborn babies or childhood are allergic to cow's milk or chicken eggs or egg products which later affect their growth (www.allergyasthmatherapy.com/Conditions/Food-Allergies.htm). Allergens of eggs are predominantly found in egg whites rather than egg yolks. Egg whites compose of ovomucoid, ovalbumin, ovotransferin and lysozyme (Atkins, 2000).

Lysozyme from hen egg white is selected as a model allergen in this study. Although total protein in egg white consists of only 3.4% of lysozyme, there are a high percentage of people who are allergic specifically to lysozyme for instance, in Japan (67%), United States (61%) and France (35%) (http://www.food-allegens.de/).

One technique generally used to desensitize allergens is "specific allergen immunotherapy". With this technique, the patients are injected with specific allergens that they are allergic to for a period of time. The patients always suffer from this treatment, especially children. It is due to the fact that this treatment is painful and the time of the treatment is also highly dependent on a response of individual's immune system and other factors such as age, gender, race, disease and drug (Demoly et al, 1998). Consequently, the present study was intended to propose the mechanism and the delivery possibility of the protein allergen into epidermal layer without needles.

Some scientists are interested in the state of protein which can be translocated across the lipid bilayer of the membrane, although proteins are hydrophilic and charged (Rothman and Kornberg, 1986). It is found that the state of high interest is a non-native state called 'molten globule intermediate (MG) state'. Some researchers revealed the advantages of MG transition which are flexibility of side chains, exposure of hydrophobic residues, shielded charges and compactness (Bychkova et al, 1988; Van der Goot et al, 1991, 1992).

In vitro MG state or partially folded state of globular proteins has been widely studied and induced to by the use of thermal variations, organic solvents, salts and acids (Bychkova et al, 1988; Goto et al, 1990a, 1990b; Van der Goot et al, 1991, 1992; Privilov, 1996; Bhattacharjya et al, 1997; Bakuni, 1998; Dubey and Jagannadham, 2003). MG state or partially folded characteristic is defined as a loss in the tertiary structure, retaining the native-like secondary structure and an evidence of compactness. Moreover, MG state shows hydrophobicity more than native state (Ohgushi and Wada, 1983; Bychkova et al, 1988; Oas and Kim, 1988; Creighton, 1990; Ptitsyn et al, 1990; Peng and Kim, 1994; Ptitsyn and Uversky, 1994; Privilov, 1996). Several techniques can be used to investigate MG state such as circular dichroism (CD), fluorometry, nuclear magnetic resonance (NMR) spectroscopy and differential scanning calorimetry (DSC) (Privilov, 1996; Bhattacharjya et al, 1997; Katamari et al, 1998).

Lysozyme is a globular protein containing 129 amino acid residues and was used as a model protein in the present study. Lysozyme can be induced to generate MG state by the conditions stated above. Organic solvents such as trifluoroethanol (TFE) (Radford et al, 1992), hexafluoroethanol (Bhattacharjya and Balaram, 1997), methanol (Katamari et al, 1998) and ethanol (Goda et al, 2000) are found to be one factor that affects the conformational transformation of lysozyme

From the characteristics of the MG state, we expect that the modified protein will have the potential to penetrate into the epidermal layer by its hydrophobic property. Therefore, the present study was aimed to understand the mechanism of conformational change and to study the possibility of penetration of modified lysozyme through a model skin. It was divided into two parts. The first one was, characterization of lysozyme folding mechanism part. It was to study solvent effect on the conformational changes of lysozyme using ethanolic solution and diluted hydrochloric acid solution. The effects of ethanolic, acidic and the combined environment were determined by various techniques. The conformational reversibility of modified lysozyme to active structure was also considered. The second part was the penetration part. It was to determine the possibility of modified lysozyme which was presented as MG or partially folded characteristic to penetrate the model skin using Franz-diffusion cell technique. Pig's ear skin was selected as an in vitro model skin.

Objectives of the present study were:

1. To study solvent effects on the conformational modification of lysozyme.
2. To determine the conformational reversibility of modified lysozyme which was presented as MG or partially folded characteristic to the active conformation.
3. To evaluate the penetration of MG lysozyme using pig's ear skin as an in vitro model skin.