not have a cellulosic cell wall, it does have a cell membrane that may be tying up protein of higher available lysine, which is being liberated with drying. Although some of these are rather severe heat treatments, the absence of any appreciable quantity of reducing carbohydrates "protects" the lysine from the expected Maillard reaction losses (Adrian, 1975).

While the available protein lysine was increased in the heat-treated samples, no clear correlations were noted between in vitro protein digestibility and heating. Protein in all dried samples was more digestible than proteins extracted from wet algae. The results for the proteins of freeze-dried, solar-dried, and drum-dried cells are comparable to those of Bouges (1972) and Durand-Chastel (1972) as reported by Lipinsky and Litchfield (1974). These reports provide evidence for 84% digestibility for dried *Spirulina maxima* cells but contradict those reported by Anusuya-Devi et al. (1981). They found that proteins from freeze-dried and sun-dried *Spirulina platensis* had a digestibility of ca. 70%, while fresh cell proteins were roughly 83% digestible. While essentially the same in vitro assay was used here as in their study (24-h incubation vs. 27 h, respectively), they started with *n*-hexane-extracted cells. Also, they measured nondigested proteins by micro-Kjeldahl assay of TCA-precipitated proteins, while in this study the precipitable proteins were dissolved in base and analyzed by using the Lowry method.

Even with the differences noted for results from other studies, the work herein shows that *Spirulina* cells may be dried in any number of ways, with little, if any, loss in protein digestibility or available lysine.

Cabinet-dried *Spirulina* was used as a dietary protein source in two separate nutrition trials. For both poultry and marine shrimp, growth and survival were equivalent to control animals that were fed diets based on more traditional sources of protein (Magarelli, 1984).

Registry No. Lysine, 56-87-1.

LITERATURE CITED

Adrian, J. *Ann. Nutr. Aliment.* **1975**, *29*, 603.
Akeson, W. R.; Stahmann, M. A. *J. Nutr.* **1964**, *83*, 257.
Ako, H., Department of Agricultural Biochemistry, University of Hawaii, Honolulu, HI, personal communication, 1983.
Anonymous. Hawaii Department of Land and Natural Resources, Aquaculture Development Program, Oct 1982.
Anusuya Devi, M.; Subbulakshmi, G.; Madhavi Devi, K.; Venkataraman, L. V. *J. Agric. Food Chem.* **1981**, *29*, 522.
AOAC. "Official Methods of Analysis", 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.
Baron, C. *Ann. Nutr. Aliment.* **1975**, *29*, 615.
Clement, G.; Giddey, C.; Menzi, R. *J. Sci. Food Agric.* **1967**, *18*, 497.
Goodno, C. C.; Swaisgood, H. E.; Catignani, G. L. *Anal. Biochem.* **1981**, 115–203.
Lipinsky, E. S.; Litchfield, J. H. *Food Technol. (Chicago)* **1974**, *28* (5), 16.
Magarelli, P. C. Aquaculture and Livestock Feeds Production Program in Hawaii, Final Report, Department of Land and Natural Resources, Hawaii, 1984.
Moy, J. H.; Bachman, W.; Tsai, W. J. *Trans. ASAE* **1980**, 242.
Skowronski, L.; United Nations Industrial Development Organization, 1980, UNIDO/IO.403.
Subbulakshmi, G.; Ganesh Kumar, K.; Venkataraman, L. V. *Nutr. Rep. Int.* **1976**, *13* (1), 19.
Wolf, J. C.; Thompson, D. R.; Warthesen, J. J.; Reineccius, G. A. *J. Food Sci.* **1981**, *46*, 1074.

Received for review May 21, 1984. Accepted September 4, 1984. This is Journal Series 2830 of the Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, HI 96822.

Protein Polymerization in Red Cell Membranes

Jorge O. Bouzas and Marcus Karel*

Human red cell membranes were used as a model of an organized lipid protein system to investigate protein damage during storage under dry conditions and controlled temperature, under air and nitrogen, and after various physical and chemical treatments. It was found that these treatments affected protein polymerization at time 0 and during storage. Enzyme damage depended not only on treatment and storage condition but also on location within the membrane. Acetylcholinesterase, an externally oriented enzyme, lost all its activity when treated with chloroform while retaining most of it when membranes were only freeze-dried or sonicated and freeze-dried. During storage no further changes were observed. G3PD, an enzyme bound to the inner membrane surface and oriented toward the crytosol, showed an entirely different behavior. After treatment it retained most of its activity, and upon storage there was a gradual decrease in activity.

Numerous reactions on proteins can take place when processed by physical or chemical means. These reactions induce undesirable changes in living cells and may decrease the nutritional value and organoleptic quality of a protein-containing food. They occur often through the formation of covalent cross-links between polypeptide chains (Cheftel, 1977). Red cell membranes have been proposed as a model for protein damage in tissues where proteins and lipids are organized in a well-defined manner.

Several different biochemical mechanisms have been implicated in the polymerization of red cell membrane proteins. Lorand et al. (1976) described a calcium-activated transglutaminase that catalyzed the cross-linking through the formation of γ-glutamyl-ϵ-lysine bridges. A second mechanism is the oxidation of sulfhydryls to form intermolecular disulfide bridges. Another explanation for the membrane protein cross-linking observed is Schiff base formation by the reaction of amino groups with malonaldehyde, an end product of polyunsaturated fatty acid peroxidation present in the membrane as various phospholipids (Tappel, 1973), although other reactions are possible, e.g., direct radical attack on the proteins (Hochstein and Jain, 1981).

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

0021-8561/84/1432-1387$01.50/0 © 1984 American Chemical Society

In this study freeze-dried human red cell membranes were used as a model of an organized lipid protein system to investigate protein damage during storage under controlled temperature and humidity conditions after various physical and chemical treatments.

MATERIALS AND METHODS

Erythrocyte membranes were prepared from human, outdated blood preserved in citrate–phosphate–dextrose anticoagulant solution without regard to blood type, provided by the American Red Cross (Blood Services, Northeast Region), following the procedure of Steck and Kant (1974). All procedures were performed at 0–4 °C. Red cells were washed 3 times with phosphate-buffered saline (PBS) (150 mM sodium chloride, 5 mM sodium phosphate, pH 8.0) and hemoylzed in hypotonic solution, 5 mM phosphate, pH 8.0. The ghosts were pelleted by contrifugation at 22000*g* maximum for 10 min in an angle head rotor and resuspended in 5 mM sodium phosphate, pH 8.0. Sodium azide (0.02%) was added to the ghost preparation to avoid microbial growth. These membranes retain most of the functional and structural characteristics and afford the advantages of reproducible composition and ready accessibility. Membranes were then treated as follows: (a) Red cell membrane were cooled at −4 °C, frozen in liquid nitrogen, and lyophilized in a Virtis laboratory freeze-drier with no external heating at 100 μHg and at a condenser temperature of −25 °C for 24 h. (b) In an attempt to affect membrane organization, red cell membranes were treated with chloroform. A mixture (1:1 v/v) was agitated for 5 min in a shaker at room temperature. Chloroform was removed in a flash evaporator. Samples were freeze-dried as in (a). (c) A probe-type sonicator (Sonifier Cell Disruptor W-350, Heat System Ultrasonics, Plainview, NY) was used to disrupt red cell membranes. Samples were sonicated for 10 min (10% duty cycle; output control 5, 0 °C) under nitrogen. Sonicated samples were immediately cooled at −4 °C, frozen in liquid nitrogen, and lyophilized as in (a).

The effects of these procedures were evaluated by transmission electron microscopy. Time 0 samples were treated with polylysine and then fixed by immersion in 2.5% glutaraldehyde (4 °C, 30 min) followed by 1% OsO_4 in 0.1 M sodium cacodylate buffer (5–10 min, room temperature). Fixed membranes were dehydrated in ethanol series, dried through the CO_2 critical point, and examined in a JEM 100B transmission electron microscope.

Samples were stored under dried air in desiccators containing anhydrous calcium sulfate (drierite), at 37 °C, in the dark. Samples incubated under nitrogen were included as controls. After storage, samples were rehydrated to the initial concentration and the following studies were performed.

Protein Analysis. Protein cross-linking was assessed by agarose–SDS–PAGE (Steck, 1972; Girotti, 1976). Prior to electrophoresis, samples were treated as follows (Fairbanks et al., 1971): 100 μL of reconsituted red cell membranes was added to 100 μL of a solution containing 2.0% SDS, 20% sucrose, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 80 mM DTT. The mixture was incubated for 1 h at 37 °C. Aliquots of these solutions were placed at the top of the gels (50 μg of protein/mL) by using pyronin G (1 mg/mL) as the tracking dye. The 3% polyacrylamide–0.4% agarose gels were used in this part of the study. Spectrophotometric scanning at 550 nm after staining the gels with Coomassie brilliant blue was used for quantitative determination of each fraction in an Hitachi Perkin-Elmer UV–visual gel scanner with a 3390A Hewlett-Packard integrator. Total protein was evaluated by using the modification of the Lowry method described by Markwell et al. (1978).

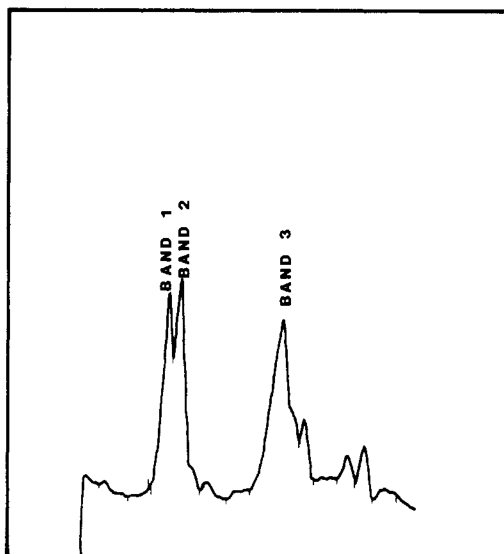
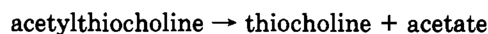


Figure 1. Scanning of the protein electrophoretic profile of untreated red cell membranes (before freeze-drying). (Gel scanning at 550 nm; Hitachi Perkin-Elmer gel scanner, Model H 200-0662; Hewlett-Packard integrator, Model 3390A.)

Enzyme Assays. Enzyme activity reactions were followed at a controlled temperature of 25 °C.

G3PD Activity. Enzyme activity was followed by measuring the increase in absorbance at 340 nm due to NADH formation between the first and second minutes of reaction as recommended by Steck and Kant (1974). Equal volumes (100 μL) of 10-fold diluted red cell membranes and Triton X-100 (0.2% v/v) were added to a 1-cm semimicrocuvette and preincubated for 1 min (25 °C). Sodium pyrophosphate, 30 mM, adjusted to pH 8.4 with HCl and made 4 mM in cysteine just before use, was added to bring the volume to 0.82 mL, followed by 0.03 mL of sodium arsenate, 0.04 M, and 0.05 mL of β-NAD, 20 mM. The 0.1 mL of glyceraldehyde 3-phosphate, 15 mM, pH 7, was added, the solution mixed, and the reaction followed spectrophotometrically.

AChE Activity. The procedure was based on the methods of Ellman et al. (1961), Steck and Kant (1974), and Moore et al. (1981). The enzyme activity was measured by following the yellow color produced from thiocholine when it reacts with dithiobis(nitrobenzoate) ion. It is based on the coupling of these reactions:


acetylthiocholine → thiocholine + acetate

thiocholine + dithiobis(nitrobenzoate) → yellow color

Aliquots of 100 μL of red cell membranes were preincubated for 5 min at 25 °C in 1-cm cuvettes with an equal volume of Triton X-100 (0.2% v/v). Sodium phosphate buffer (pH 7.4) was added to make 2.8 mL, 0.2 mL of dithiobis(nitrobenzoic acid) (DTNB), 10 mM, in 10 mM sodium phosphate buffer (pH 7.0) containing 3 mg of sodium bicarbonate per 8 mg of DTNB and 0.2 mL of acetylthiocholine chloride, 12.5 mM, were then mixed in, and the reaction was followed spectrophotometrically at 412 nm.

RESULTS

Protein Analysis. Figure 1 shows an electrophoretogram of untreated red cell membranes, before freeze-drying. Numerical designation for major polypeptides follows that from Fairbanks et al. (1971). As shown in Figure 2, protein polymerization was estimated as the sum of high

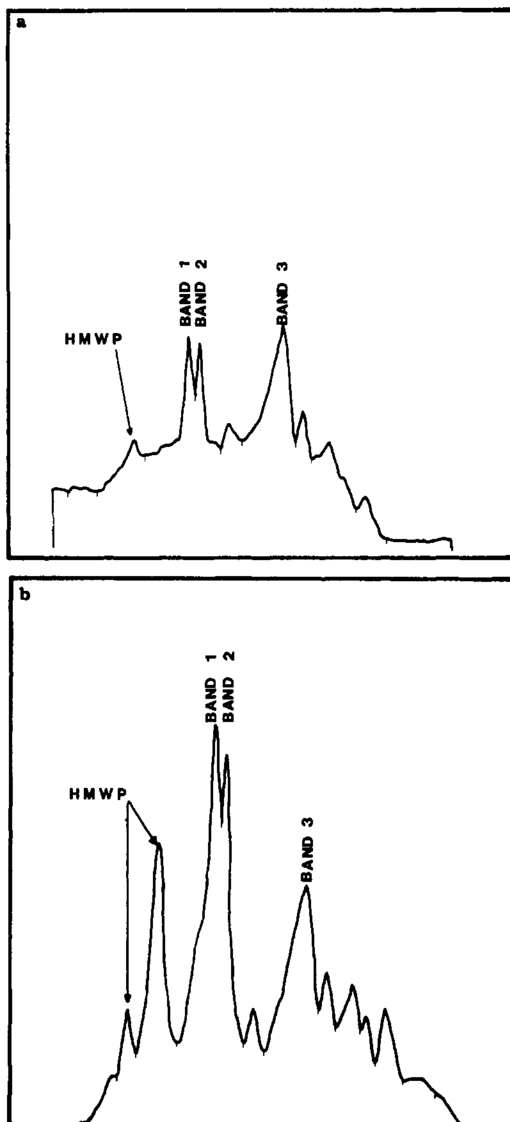


Figure 2. Scanning of the protein electrophoretic profile of freeze-dried red cell membranes showing high molecular weight protein (HMWP) formation after storage (37 °C, no light, under dry air) for (a) 1 and (b) 5 days. (Gel scanning at 550 nm; Hitachi Perkin-Elmer gel scanner, Model H 200-0662; Hewlett-Packard Integrator, Model 3390A.)

molecular weight protein fractions (HMWP) appearing before band 1 and represented in Figure 3 as a function of storage time.

Determination of total protein by the modified Lowry assay gave constant values for all samples during incubation time, except for the 4.5-day chloroform-treated samples, which showed an average 20% loss. This amount, assumed to be formation of insoluble polymers, was used to correct determinations by gel scanning as indicated in Figure 3. It is important to note, however, that it was not possible to correct for a protein fraction observed on top of the gel, which has to be assumed to correpond to high molecular weight protein fractions.

Disappearance of bands 1 and 2 was also estimated and represented in Figure 4, with the insoluble protein loss correction included in Figure 4b. No significant differences were found in the amount of band 3. Experiments under nitrogen showed negligible polymerization with no significant disapperance of bands 1 and 2.

Enzyme Activity. Using the activity of the fresh membrane preparation (not freeze-dried), as a reference value, we plotted the remaining activity of acetylcholinesterase (AChE) (Figure 5) and glyceraldehyde-3-phosphate dehydrogenase (G3PD) (Figure 6) as a function of storage time and treatment conditions. Both enzymes showed clearly the effect of treatment, but only G3PD showed a storage effect.

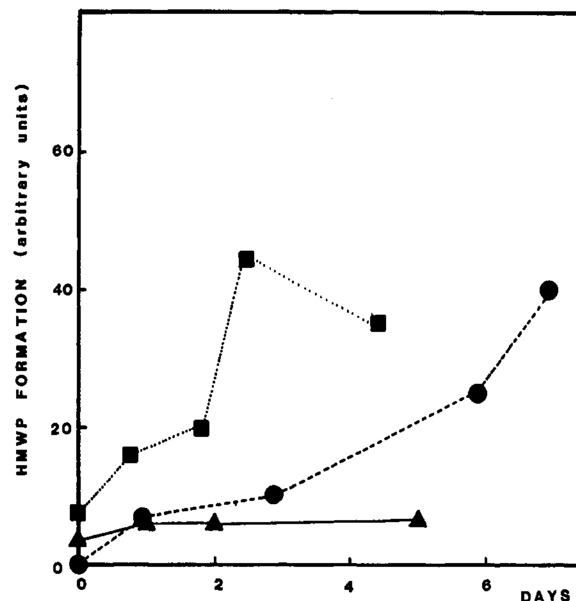


Figure 3. High molecular weight proteins (HMWP) formation during storage of red cell membranes (same conditions as in Figure 2) after (●) freeze-drying, (■) Cl_3CH treatment and freeze-drying, and (▲) sonication and freeze-drying. Arbitrary units represent the relative proportion of each fraction obtained by gel scanning and calculated as a percentage of the total integrated area. Corrections for the chloroform-treated samples were based on the following data: total protein before incubation = 2.11 mg/mL; total protein, day 4.5 = 1.72 mg/mL; percent HMWP detected = 20.

DISCUSSION

As shown in Figure 7, untreated red cell membranes maintain the structural integrity, characteristic of erythrocytes, but when they were freeze-dried (Figure 8), sonicated (Figure 9), or chloroform treated (Figure 10), noticeable changes were observed.

Freeze-drying caused disruption into large fragments ($\simeq$2.3 μm), which seem to be highly folded, while sonication resulted in the formation of very small vesicles ($\simeq$0.5–0.25 μm). The effect of chloroform was totally different, membranes shrunk from about 4 to 2 μm in diameter.

Protein polymerization was affected by these morphological changes. First, HMWP were detected in time 0 freeze-dried samples that had been either sonicated or chloroform treated (Figure 3) but not in only freeze-dried samples. Second, HMWP formation rate was also affected by the initial treatment. Chloroform-treated freeze-dried samples resulted in significantly faster HMWP formation, followed by only freeze-dried samples. It is surprising that sonication resulted in negligible protein polymerization. The formation of HMWP correlated well with the decrease in bands 1 and 2 (Figure 4). We should note that in the present work using freeze-dried red cell membranes no losses were detected in band 3, in contrast to the work of Funes and Karel (1984), who studied protein polymerization in red cell membrane aqueous solutions.

HMWP's were not dissociated by SDS, reflecting the formation of covalent linkages, since the detergent breaks all classes of noncovalent bonds. The treatment with DTT (dithiothreitol) before agarose–SDS–PAGE reflects also that the cross-links are not disulfide bonds.

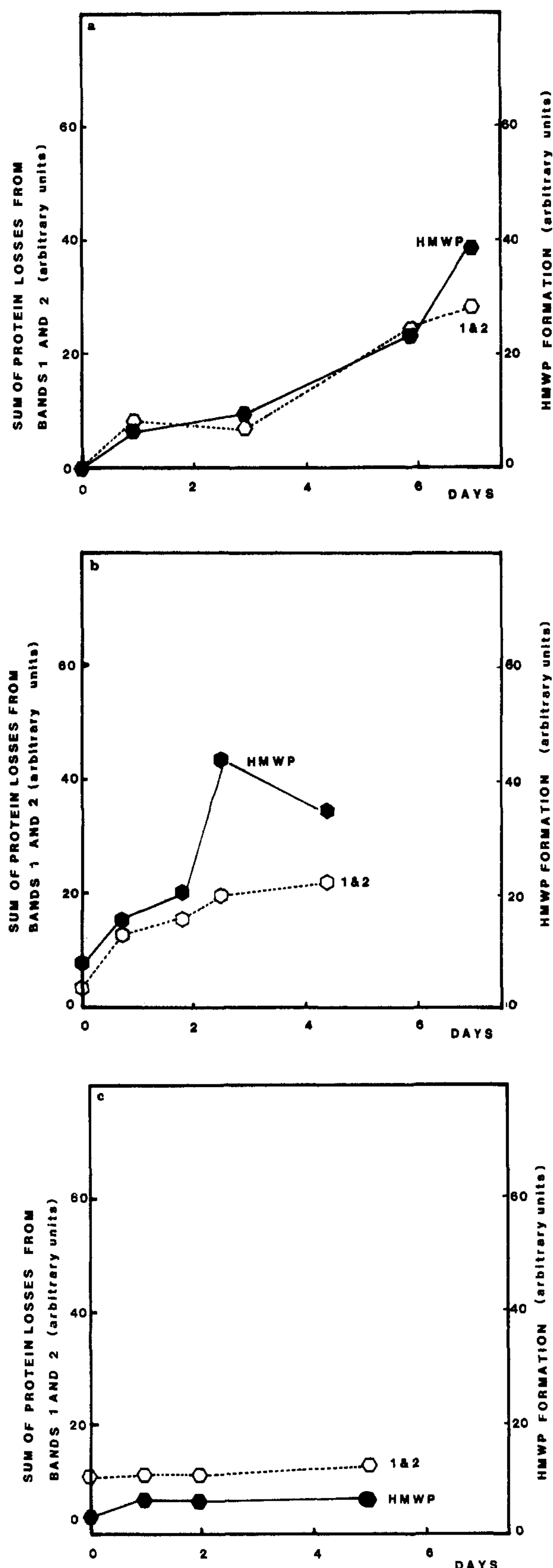


Figure 4. Protein losses from bands 1 and 2 (○) and high molecular weight protein (HMWP) (●) formation during storage (same condition as in Figure 2) after (a) freezing-drying, (b) Cl_3CH treatment and freeze-drying, and (c) sonication and freeze-drying. Arbitrary units are as in Figure 3. Corrections for chloroform treated samples were based on the following data for bands 1 plus 2: total protein before incubation = 2.11 mg/mL; total protein, day 4.5 = 1.72 mg/mL; percent bands 1 plus 2 = 31. The amount of HMWP detected was corrected as indicated in Figure 3.

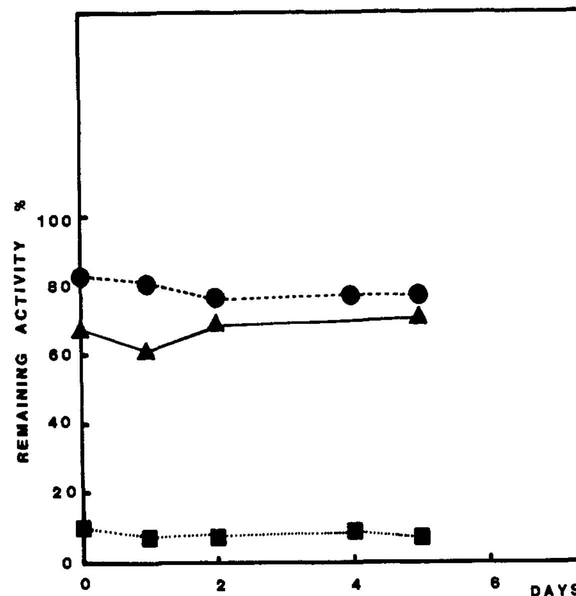


Figure 5. Acetylcholinesterase (AChE) remaining activity during storage (same conditions as in Figure 2) after (●) freeze-drying, (■) Cl_3CH treatment and freeze-drying, and (▲) sonication and freeze-drying.

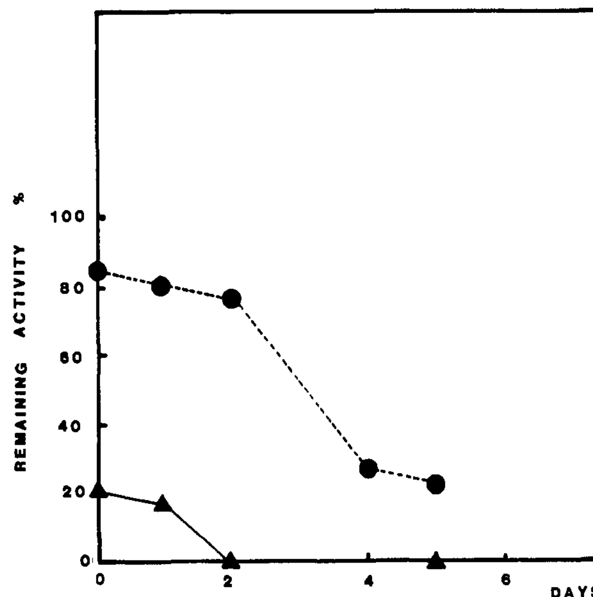


Figure 6. Glyceraldehyde-3-phosphate dehydrogenase remaining activity during storage (same conditions as in Figure 2) after (●) freeze-drying and (▲) sonication and freeze-drying.

The increase in HMWP was affected by the atmosphere under which samples were stored. Increases were observed only when samples were exposed to air: no polymers were found in samples stored under nitrogen, suggesting a correlation between HMWP and lipid oxidation reported by several authors, e.g., studies on rat liver microsomes and intact heptocytes by Koster and Slee (1980) and Koster et al. (1982) and again in red cell membranes exposed to hydroperoxides (Koster and Slee, 1983) and to lipid vesicles (Alloisio et al., 1983). The formation of HMWP has been shown to be closely associated with the lipid oxidation process, since lipid oxidation inhibition results in lack of formation of HMWP's (Koster and Slee, 1983). Moreover, Hochstein and co-workers (Jain and Hochstein, 1979, 1980; Corry et al., 1980; Rice-Evans and Hochstein, 1981) have stated that the formation of HMWP's is subsequent to peroxidation of membrane lipids, although they suggest that other polymerization reactions are also possible.

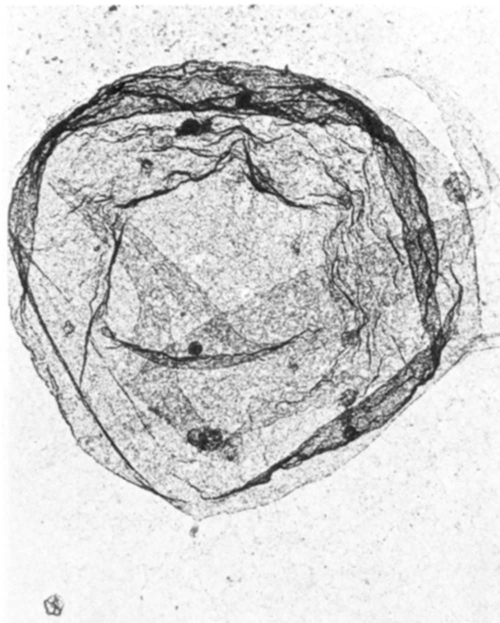


Figure 7. Transmission electron micrograph whole mount of untreated red cell membranes (magnification 17 000×).

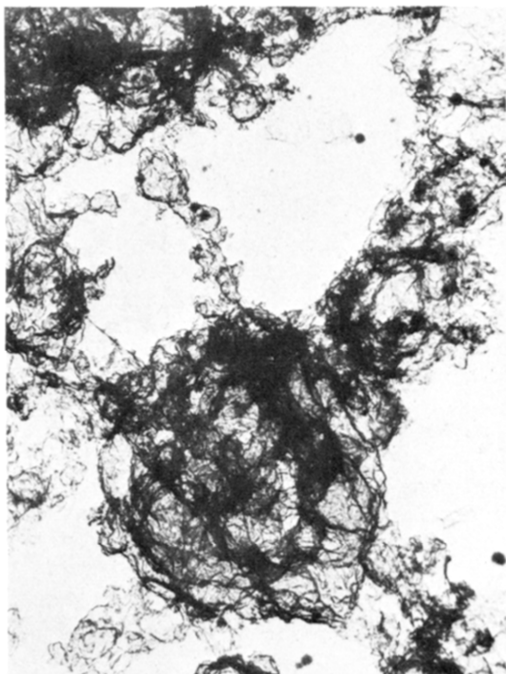


Figure 8. Transmission electron micrograph whole mount of red cell membranes after freeze-drying (magnification 17 000×).

Attempts to obtain direct evidence of lipid peroxidation gave inconclusive results, because of unreliability of MDA (malonaldehyde) and peroxide value tests in freeze-dried systems (Chipault and Hawkins, 1971; Melton, 1982).

Red cell ghosts treatments also affected remaining enzyme activity at time 0. AChE, an externally oriented enzyme, lost all its activity when treated with chloroform while retaining most of it when membranes were only freeze-dried or sonicated and then freeze-dried. During storage, no further changes were observed. Apparently, the inactivation of this enzyme requires the presence of a liquid medium.

G3PD, an enzyme bound to the inner membrane surface and oriented toward the cytosol, showed an entirely different behavior. Upon storage there was a gradual decrease in activity (Figure 6). The fact that sonicated freeze-dried

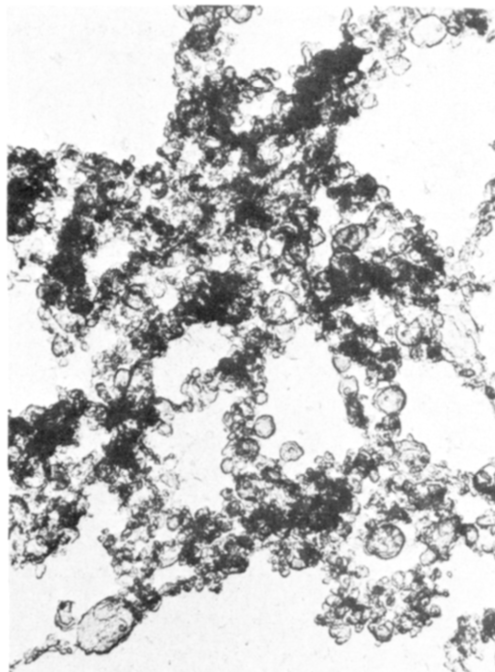


Figure 9. Transmission electron micrograph whole mount of red cell membranes after sonification and freeze-drying (magnification 17 000×).

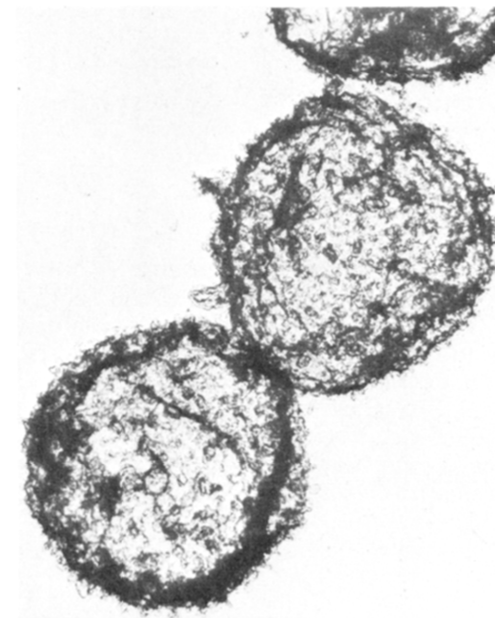


Figure 10. Transmission electron micrograph whole mount of red cell membranes after Cl_3CH treatment and freeze-drying (magnification 17 000×).

and freeze-dried only samples showed similar behaviors in spite of the fact that protein polymerization showed entirely different patterns suggest that the stability of this particular enzyme was affected by a mechanism not related with HMWP formation.

Finally, we should emphasize the role of organization in the protein damage indicators presented in this study. Enzyme location within the membrane affected the pattern of enzyme activity damage. During polymerization we observed losses only of bands 1 and 2. The specificity of the damage highlights the role of membrane organization. Lastly, the effect of membrane treatments studied here as analogues to common laboratory and industrial procedures suggests the need for further studies. It is not clear

what organizational changes occurred at the molecular level that explain the different protein polymerization patterns observed in this study.

Abbreviations Used: MDA, malonaldehyde; TEM, transmission electron microscopy; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; G3PD, glyceraldehyde-3-phosphate dehydrogenase; AChE, acetylcholinesterase; HMWP, high molecular weight protein; DTT, dithiothreitol; DTNB, dithiobis(nitrobenzoic acid).

Registry No. AChE, 9000-81-1; G3PD, 9001-50-7; chloroform, 67-66-3.

LITERATURE CITED

Alloisio, N.; Giraud, F.; Boutalbi, J.; Chailley, B.; Delaunay, J. *Biochim. Biophys. Acta* **1983**, *727*, 255.
Cheftel, J. C. In "Food Proteins"; Whitaker, J. R.; Tannenbaum, S. R., Eds.; Avi Publishing Co., Inc.: Westport, CT, 1977.
Chipault, J. R.; Hawkins, J. M. *J. Agric. Food Chem.* **1971**, *19*, 495.
Corry, W. D., Meiselman, H. J.; Hochstein, P. *Biochim. Biophys. Acta* **1980**, *597*, 224.
Ellman, G. L.; Courtney, K. D.; Andres, V.; Fatherston, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
Fairbanks, G.; Steck, T.; Wallach, D. F. H. *Biochemistry* **1971**, *10*, 2606.
Funes, J. A.; Karel, M. *J. Food Biochem.* **1984**, in press.
Girotti, A. W. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 1367.
Hochstein, P.; Jain, S. K. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 183.
Jain, S. K.; Hochstein, P. *Biochim. Biophys. Acta* **1979**, *586*, 128.
Jain, S. K.; Hochstein, P. *Arch. Biochem. Biophys.* **1980**, *201*, 683.
Koster, J. F.; Slee, R. G. *Biochim. Biophys. Acta* **1980**, *620*, 489.
Koster, J. F.; Slee, R. G. *Biochim. Biophys. Acta* **1983**, *752*, 233.
Koster, J. F.; Slee, R. G.; van Berkel, T. J. C. *Biochim. Biophys. Acta* **1982**, *710*, 230.
Lorand L.; Weissmans, L. B.; Epel, D. L.; Burner-Lorand, J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4479.
Markwell, M. A.; Hass, S.; Bieber, L. L.; Tobert, N. E. *Anal. Biochem.* **1978**, *87*, 206.
Melton, S. L. *Food Technol. (Chicago)* **1982**, *37*, 105.
Moore, R. B.; Manery, J. F. *Arch Biochem. Biophys.* **1981**, *211*, 171.
Rice-Evans, C.; Hochstein, P. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1537.
Steck, T. L. *J. Mol. Biol.* **1972**, *66*, 295.
Steck, T. L.; Kant, J. A. *Methods Enzymol.* **1974**, *31*, 172.
Tappel, A. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1973**, *32*, 1870.

Received for review October 6, 1983. Revised manuscript received March 19, 1984. Accepted August 17, 1984. J.O.B. was a fellow of the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina. This work was also supported in part by Contract DAAG-29-81-K-0081 with the U.S. Army Research Office.

Lipophilization of α_{s1}-Casein. 3. Purification and Physicochemical Properties of Novel Amphipathic Fatty Acyl Peptides

Zahurul Haque[1] and Makoto Kito*

Fatty acyl peptides (A) were obtained from lipophilized α_{s1}-casein containing covalently attached caprylic (8:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. The secondary structure was essentially the same regardless of the ligand size. Monomer weight was about 3500 and aggregation numbers were 3 (A_3), 6 (A_6), 12 (A_{12}), and 24 (A_{24}); the trimer was the most predominant. The driving force for initial aggregation (A_3) was spontaneous, but further aggregation seemed to be influenced by the ligand size; longer ligands led to larger aggregates. Surface tension (dyn/cm) decreased with the increase in ligand length, being lowest for 18:0-A. Surface tension equilibrium was attained within 60 min only when the ligand length was 18 carbons. The critical micelle concentration in 5 mM phosphate buffer, pH 6.8, was 1.5, 1.3, 1.2, 2.3, 1.8, and 1.7 M for 12:0-A, 14:0-A, 16:0-A, 18:0-A, 18:1-A, and 18:2-A, respectively. Foaming activity was higher when the ligand length was greater, and the foam density seemed to decrease with an increase in ligand length and in unsaturation.

Amphipathicity and micelle forming ability of proteins and peptides are properties that are being increasingly appreciated. The importance of hydrophobic interactions for the entry of certain peptides into cells has been noted (Uchida et al., 1980). Cytochrome b_5, a mitochondrial membranal enzyme, has been well investigated (Fleming et al., 1978) and appears to contain a hydrophobic taillike region distinctly separate from the hydrophilic milieu. A number of workers have reported the in vivo posttranslational covalent attachment of fatty acids to proteins (Henderson et al., 1983). Biologically active polypeptides have also been noted to contain covalently linked fatty acids (Carr et al., 1982).

It has previously been shown that fatty acids may be attached covalently to proteins by relatively mild methods (Haque and Kito, 1982, 1984), and the chemical, functional, and conformational effects of "lipophilization" (Haque et al., 1982; Haque and Kito, 19873a,b) have also been shown. However, a clearer understanding of the effect of lipophilization would ensue if the subject of study were peptides. On the other hand, even though previous work (Haque and Kito, 1983b) has shown Lys-32 and/or -34 to be the most reactive nucleophile in α_{s1}-casein, confirmation was desirable. And finally, the bulk purification of highly amphiphilic peptides containing fatty acyl anchors has not been attempted. Most efforts have been made using preparative HPLC (Tanabe et al., 1979), and conventional methods of peptide purification, such as ion exchange or

Research Institute for Food Science, Kyoto University, Uji, Kyoto-611, Japan.
[1]Present address: Department of Food Science, Cornell University, Ithaca, NY 14853.

 © 1984 American Chemical Society