CHEMISTRY LETTERS, pp. 1635-1638, 1989.

Stabilization of Unstable Components in Langmuir-Blodgett Film System by Introduction of Water-Soluble Antioxidants

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Easily oxidizable components simply arranged and/or highly oriented in a monolayer on an aqueous subphase as well as in a Langmuir-Blodgett film were found to be chemically stabilized by the addition of biological water-soluble antioxidants such as glutathione, uric acid, and ascorbic acid.

During the course of our Langmuir-Blodgett (LB) film studies,[1-3] we have recognized that some of the LB-film components such as chlorophylls, merocyanine dyes with a long hydrocarbon chain, and egg yolk lecithin tend to decompose for forming their oxygen-mediated degradation products. Stabilization of these unstable components seems to be essentially important for the future application of LB-film systems into various industrial areas. The chemical degradation of the film components seems to inhibit any practical use of the LB-film system. Fortunately, since it has generally been recognized[3,4] that there exists the intracellular defense system against oxygen radical-mediated tissue damage, the biological antioxidant system shown in Fig. 1 may provide some useful informations for stabilization of the film components. Recently, we have developed a method for the determination of the water-soluble antioxidants such as ascorbic acid (AA), glutathione (GSH), and uric acid (UA) by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD).[5,6]

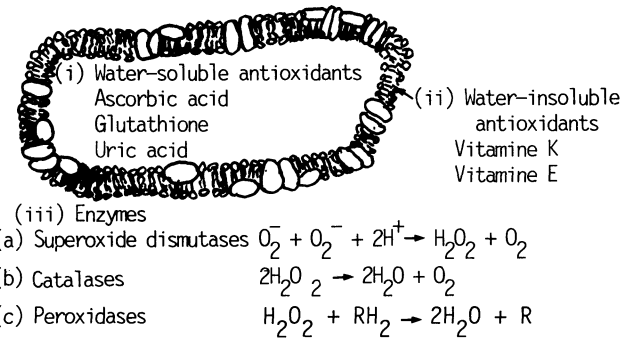

Fig. 1. Biological antioxidant system. Water-soluble antioxidants are distributed in the intra- and extracellular fluids, whereas the water-insoluble (lipid-soluble) antioxidants are distributed in the cellular membrane.

As pointed out by Kamel et al.,[7] it has been well established that cholesterol (Ch) will undego air-oxidation, and that the rate and extent of oxidation are dependent on the experimental conditions. Kamel et al.[7] found that the addition of AA in an aqueous subphase, on which a Ch monolayer was prepared, stabilized the film component. We also have confirmed the effect found by Kamel et al.[7] However, AA has generally been recognized as the chemically very unstable agent.[8] We have found that the use of UA and GSH in place of AA tends to stabilize the film compo-

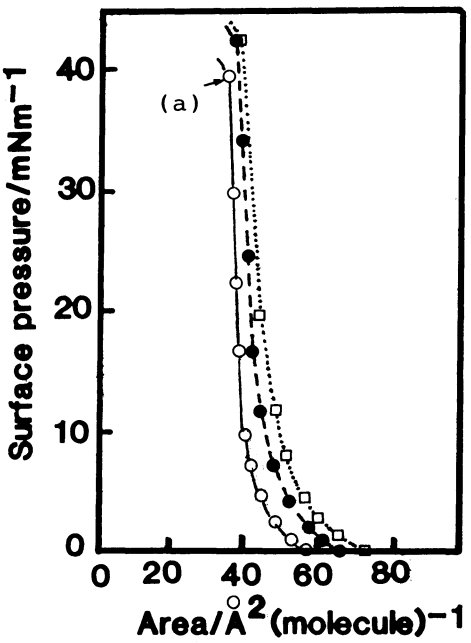

Fig. 2. Surface pressure-area curves of cholesterol monolayers spread on the aqueous subphase. Compression initiated immediately after spreading (○); 45 min after spreading (●); and 90 min after spreading (□).

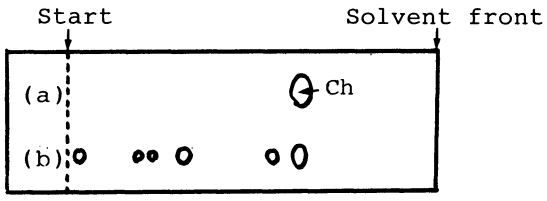

Fig. 3. Thin-layer chromatograms of components in a cholesterol monolayer for (a) 0 min and (b) 90 min after spreading.

nent for a long period compared with that of AA.

All the experiments were conducted at 20 ± 1 °C. An aliquot of chloroform soluiton of Ch (1.0×10^{-4} mol·dm^{-3}) was delivered on to an aqueous subphase (freshly distilled water, pH 5.9 ± 0.1), to which 1.0×10^{-4} mol·dm^{-3} AA, UA and/or GSH were added when necessary. Figure 2 shows the change of surface pressure-area (F-A) curves of Ch monolayers prepared on the aqueous subphase without addition of any agents as a function of time. The tendency of the change shown in Fig. 2 seems to be consistent with that by Kamel et al.[7] Immediately after each monolayer was conventionally removed onto a glass plate according to the LB-film technique after the measurement of its F-A curve, the film components were qualitatively estimated by the thin-layer chromatographic (TLC) method employed by Kamel et al.[7] The TLC examinations revealed that Ch tended to decompose showing at least five spots of its unidentified degradation products on a chromatogram (see Fig. 3 (b)). The addition of AA into the aqueous subphase inhibited the remarkable change of F-A curves up to 90 min after the monolayer preparation keeping the original F-A curve shown in Fig. 2 (a). The HPLC-ECD examinations revealed that AA tended to decompose, but the rate for its degradation was not quantitative. For an example, about 20% of the AA amount added into the aqueous subphase was decomposed for 10 h after the subphase preparation (see Fig. 4).

Figure 5 (a) shows a typical liquid chromatogram of the freshly prepared aqueous subphase containing AA and UA. As shown in Fig. 5, the HPLC-ECD method[5,8] allows the determination of easily oxidizable components such as UA and AA. This fact means that UA can be easily oxidized[5] and is thus a potent candidate of the water-soluble antioxidants.[3,9] UA is

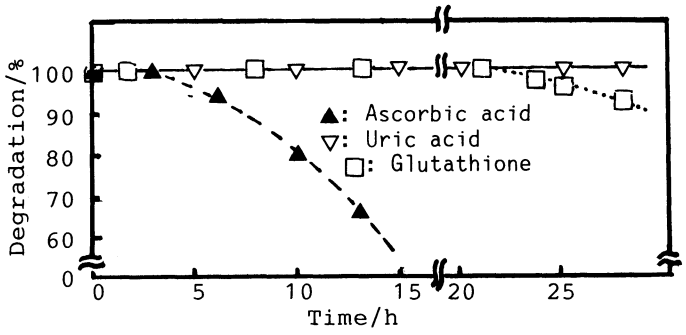

Fig. 4. Decay of the water-soluble antioxidants in the aqueous subphase, on which a Ch monolayer was prepared, as a funciton of time.

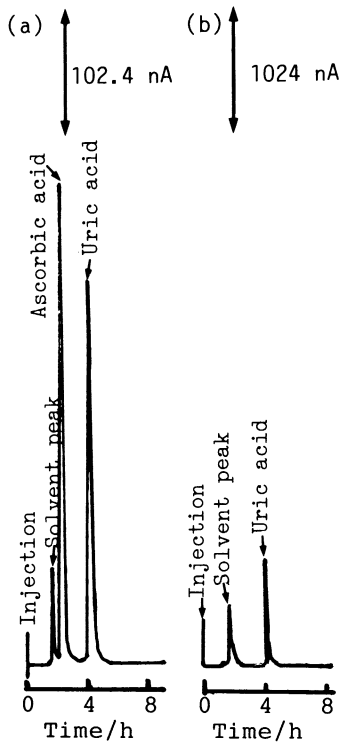

Fig. 5. The typical liquid chromatograms. For explanations, see the text.

chemically stable compared with AA as shown in Fig. 4. The examinations by the HPLC-ECD technique[5,6] revealed that the chemical stability of the water soluble antioxidants in the aqueous subphase was in the following order: UA>GSH≫AA (see Fig. 4).

On the basis of the above described information, the chemical stabilization of Ch in a monolayer was tentatively examined by addition of UA into the aqueous subphase, on which a Ch monolayer was prepared. The examinations by the HPLC-ECD[5,8] and TLC[7] methods revealed that the monolayer- and subphase-components were not decomposed at least for one day after the monolayer preparation on the aqueous subphase with UA. Degradation products of Ch were formed in a monolayer for two days after its preparation (see Fig. 6), whereas UA in the aqueous subphase was found to maintain its initial concentration (see Fig. 4).

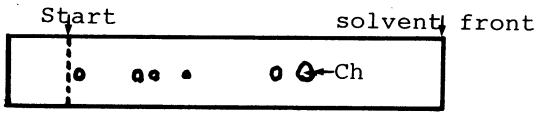

Fig. 6. A typical thin-layer chromatogram of components in a Ch monolayer for 2 days after its preparation.

In addition, we found that Ch in an LB-film, which was prepared from a monolayer on the aqueous subphase with UA at a surface pressure of 30 mN/m, was chemically stabilized. We assume that UA might be incorporated into the LB-film system. Figure 7 shows a typical replica image of a glass plate covered with a single monolayer of Ch. The replica image was obtained according to the same procedure as employed in our previous report.[10] The deposition of the monolayer onto a glass plate was performed with a deposition ratio of unity. As pointed out by the arrows in Fig. 7, the surface of a glass plate without Ch molecules was visualized as the pond-like micro-pores. The areas pointed out by the arrows should be covered with the aqueous ultra-thin layer containing UA derived from the aqueous subphase, on which the mother monolayer was prepared. The surface of a glass plate is hydrophilic. After the evaporation of the water molecules from the micro-pores, UA molecules might be left over in the numerous ponds. The subsequent deposition of a Ch monolayer onto the monolayer-coated glass plate also might allow the same event as described above. The examinations by the HPLC-ECD method[5] revealed that UA was apparently present in the LB-film system of Ch prepared from the mother monolayer showing a peak of UA in a chromatogram (see Fig. 5 (b)).

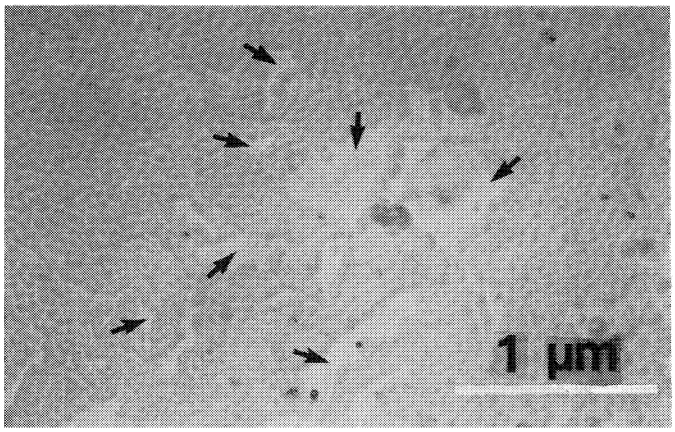

Fig. 7. Transmission electron micrograph of the replica film. For explanations, see the text.

We have found that GSH also plays its role as an antioxidant similar to UA. The thin-layer chromatogram of components in a Ch monolayer placed on the auqeous subphase with GSH for two days after its preparation was found to be close to that shown in Fig. 6. However, GSH in the aqueous subpahse was gradually decomposed (see Fig. 4).

Introduction of these antioxidants into an LB-film system might have induced the irregular structure into the LB-film system. However, the electron microscopic examinations according the procedure employed in our previous report[10] revealed that there was any significant difference between the images of the LB-films with and without UA, probably due to the fact since nobody has been able to prepare any LB-film systems having their perfect film structure, the irregular structure at molecular level is always present in each LB-film system.

As demonstrated in this paper, the biological antioxidant system seems to provide some significant informations on the chemical stabilization of the LB-film components. Some other effects of the biological antioxidant system for stabilization of unstable film components are described below without showing any quantitative data. A tentative examination has suggested[11] that the addition of L-α-tocopherol (a component of Vitamine E) into the LB-film system also tends to stabilize the film component, although its addition tends to increase fluidity of a mother monolayer prepared on the auqeous subphase without any agent. So far as we have examined, the addition of the enzymes shown in Fig. 1 is unable to give any remarkable effect for the chemical stability of the LB-film components such as chlorophylls and Ch.[11] As a preliminary study, we have found[11] also that the introduction of UA and/or GSH into an LB-film system tends to protect against the photo-oxidation of the film components such as chlorophylls and merocyanine-dyes with a long alkyl chain.

References

1) K. Iriyama, J. Memb. Biol., 278, 115 (1980), and references cited therein.
2) K. Iriyama, M. Yoshiura, Y. Ozaki, T. Ishii, and S. Yasui, Thin Soild Films, 132, 229 (1985), and references cited therein.
3) K. Iriyama and S. Suga, Jikeikai Med. J., 34, 169 (1987).
4) K. Iriyama, M. Yoshiura, T. Iwamoto, Y. Kamada, H. Nozawa, A. Mizuno, and H. Matsuzaki, Cur. Eye Res., 5, 479 (1986), and references cited therein.
5) M. Yoshiura, T. Iwamoto, and K. Iriyama, Innov. Tech. Biol. Med., 8, 679 (1987).
6) M. Yoshiura, T. Iwamoto, and K. Iriyama, Innov. Tech. Biol. Med., 9, 413 (1988).
7) A.M. Kamel, M.D. Weiner, and A. Felmeister, J. Colloid Interface Sci., 35, 163 (1971).
8) K. Iriyama, M. Yoshiura, T. Iwamoto, and Y. Ozaki, Anal. Biochem., 141, 238 (1984).
9) E. Niki, Y. Yamamoto, and Y. Kamiya, Chem. Lett., 1985, 1267.
10) A. Tanaka, M. Yamaguchi, T. Iwasaki, and K. Iriyama, Chem. Lett., 1989, 1219.
11) M. Yoshiura and K. Iriyama, to be published elsewhere with some other related data.

(Received May 24, 1989)