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Spreadability of Ovalbumin Monolayers at Air-water Interface. Effects of Additives to Spreading Solutions

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The effect of additives (urea, potassium chloride, butanol, and thioglycol) to spreading solutions upon spreadability of ovalbumin monolayers has been studied in terms of the rate constants and the limiting areas for intermediate (fast) and final (slow) processes of uncoiling of the protein molecule. It has been found that the addition results in the disappearance of the first (fast) process, which appeared upon spreading without additives. In the second (slow) process, the rate constant is found to be the same as that for the case without additives regardless of the type of the additives. Sodium dodecyl sulfate (SDS) added to the spreading solution is showed a peculiar behavior, which is explained in relation to the interaction between the protein and SDS molecules.

It has been reported that the monolayers of proteins[1-5] and synthetic polypeptides[6-8] are markedly affected by their addition to subphase of various salts, surface-active agents, or other substances which influence the net-work formation involving hydrogen bonding. The increase or decrease thereby observed was interpreted by the interaction between protein and the additive molecules. Most of the interpretations were based on the tacit assumption that the systems were always in the equilibrated states. It has been pointed out in the preceding paper[9] that the properties of protein monolayers should preferably be analysed as a reflection of kinetic processes rather than the equilibrated state. Thus, the isoelectric point was characterized by acidity, when the spreading was achieved instantaneously to give the highest rate of uncoiling of the polypeptide chain. A similar type of instantaneous spreading has also been observed[10,11] when adequate additives are contained in aqueous subphases or in spreading solutions. The present paper deals with the effect of additives to the aqueous solution of ovalbumin on its spreadability at air-water interface, in order to make clear how the mixed solutions of the protein and the additives reveal themselves in the monolayer properties to be studied from the kinetic aspects.

Experimental

Apparatus used was the same as that in the previous work.[9] The surface pressure (F) was determined by means of a Whilhelmy plate balance of ± 0.05 dyn/cm sensitivity. A glass tray contained subphase water, on which ovalbumin monolayers were spread from aqueous solutions of various additives, such as urea, potassium chloride, butanol, thioglycol, and sodium dodecyl sulfate. The aqueous surface prior to the spreading was clean enough to observe $F<5$

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dyn/cm, when it had been compressed to 1/20 area ratio after having been kept standing for 24 hr.

The sample of ovalbumin was the same as that in the previous work.[9] The additives were purified thoroughly by appropriate procedures; recrystallization and/or distillation, followed by ethereal elimination of possible impurities in a Soxhlet extractor. They were all clean enough to recognize appreciable increment in the surface pressure for the protein-free surface, to which their solutions were applied for blank test. All experiments were carried out on subphase of initial pH 5.3±0.1 adjusted with a small amount of hydrochloric acid at 25.0±0.5°C.

Results and Discussion

The surface denaturation processes can quantitatively be expressed as[9]

$$(A_\infty^0 - A^0) = (A_\infty^0 - A_2^0)\exp(-k_2 t) + (A_2^0 - A_1^0)\exp(-k_1 t) \quad (1)$$

where A^0 is the limiting area at a given time, t, elapsed from the spreading, A_∞^0 the area at $t=\infty$, A_1^0 the area for the first process starting at $t=0$, A_2^0 that for the second, and k_1 and k_2 are the rate constants for the two processes, in which the whole denaturation processes are comprised. This is the case of protein monolayer spread from aqueous solution without any additives, appearing as the dotted and dashed lines in Fig. 1. The additives to the spreading solution caused the disappearance of the second term in Eq. (1), as seen in Fig. 1, where the six straight lines for four additives run almost in parallel with the dashed line. This means that the k_2 values are not changed by the addition of any of the four additives to the solution, with the $(A_\infty^0 - A_2^0)$ values characteristic of their effect. Such an effect of the additives has been reported for potassium chloride added to the spreading solution of myosin[10] and bovine serum albumin,[11] for which the instantaneous spreading could be understood either as $A_\infty^0 = A_2^0 = A_1^0$ or as $k_1 = k_2 = \infty$.

Differentiation of Eq. (1) gives

$$dA^0/dt = k_2(A_\infty^0 - A_2^0)\exp(-k_2 t) + k_1(A_2^0 - A_1^0)\exp(-k_1 t). \quad (2)$$

For the region,

$$t > \frac{1}{k_1 - k_2}\ln\left(\frac{k_1}{k_2}\cdot\frac{A_2^0 - A_1^0}{A^0 - A_2^0}\right), \quad (3)$$

or for

$$(A_2^0 - A_1^0) = 0, \quad (4)$$

Eq. (1) is simplified as

$$dA^0/dt = k_2(A_\infty^0 - A^0). \quad (5)$$

Eq. (3) is the case of the tailing period of the dashed line, while Eq. (4) corresponds to the disappearance of the dotted line. This means that the addition results in the verified applicability of Eq. (5) to any of the four additives. Taking into account of the conditions, dA^0/dt is plotted in Fig. 2 against A^0. It can be seen from the figure that all of the five lines give more or less the same values in their slant and intercept at the abscissa, suggesting the same k_2 and A_∞^0 values in Eq. (5). Thus, the difference in the intercept at the ordinate in Fig. 1 is an indication of the difference in A_2^0 values for individual additives. The additives seem to affect the spreading processes in such a manner as to eliminate one of the two steps involving A_1^0 and to keep the other, starting with individual A_2^0 values and ending at the same A_∞^0 value, which is 0.83 m²/mg or 16.4 Å²/residue, as in the previous report.[9]

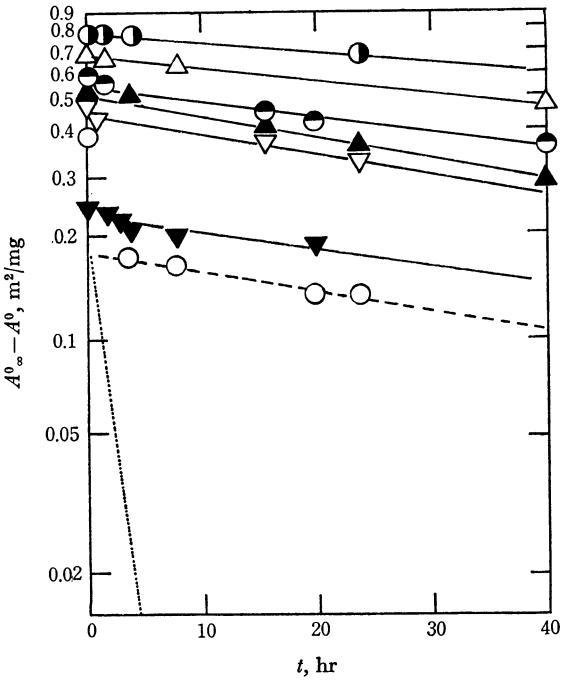

Fig. 1. Time dependences of $(A^0_\infty - A^0)$ for the monolayers of ovalbumin spread from its solutions containing urea (1M, ◑), KCl (1M, △▲), butanol (0.1%, ◓), and thioglycol (0.1%, ▽▼), or without any additives (○). The dashed and dotted lines express the first and second terms, respectively, in Eq. (1) for the case without additives. In the case of KCl and thioglycol, experiments were carried out twice independently and the results are shown as upward and downward triangles, respectively.

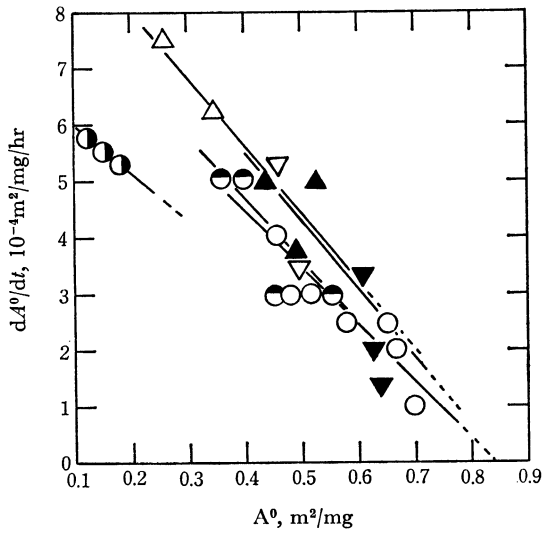

Fig. 2. Relationship of dA^0/dt and A^0 for ovalbumin monolayers spread from aqueous solutions containing urea (1M, ◑), KCl (1M, △▲), butanol (0.1%, ◓), and thioglycol (0.1%, ▽▼), or without any additives (○).

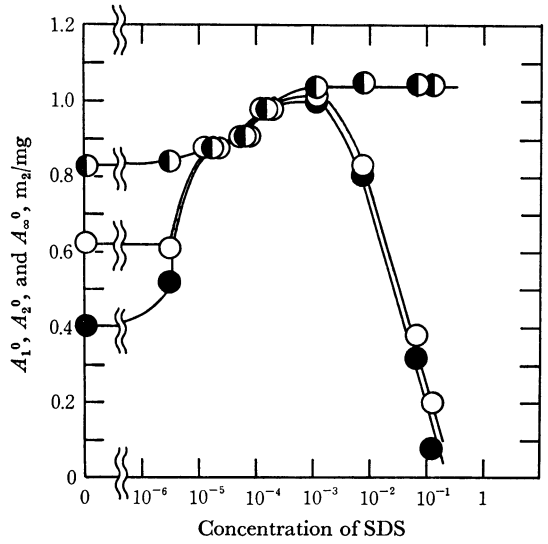

Fig. 3. Effects of SDS concentration in spreading solution on limiting areas, A_1^0 (●), A_2^0 (○), and A_∞^0 (◐) of ovalbumin monolayers.

Similar treatments were applied to the system involving SDS added to the spreading solution. Results are shown in Fig. 3, in which A_i^0's ($i=1$, 2, and ∞) are plotted against SDS concentration in the spreading solution containing protein for monolayer. It can be seen that the addition of SDS to the spreading solution affects in two different stages. For concentrations lower than *ca.* 3×10^{-4}M, it causes increments in A_i^0's with the coincident value of 0.83—1.04 m²/mg, which is higher than the A_∞^0 value (0.83 m²/mg) obtained for the protein monolayers without SDS.[9] For concentrations beyond this the addition of SDS results in the constant A_∞^0 value and the marked lowerings in A_1^0 and A_2^0.

These two stages seem to reflect the different fashions in interacting SDS molecules with protein. It should be noted for the first stage that the rate constants, k_1 and k_2, are almost the same as those for the protein without SDS. These phenomena can be interpreted by the attachment of SDS molecules to protein, which causes the increase of limiting area in such a manner as not to influence the easiness of its uncoiling processes.

At the concentration of around 3×10^{-4}M, uncoiling is completed instantaneously. This means that the interaction reaches a critical point, so that the association is made between SDS and protein molecules. A similar association has been proposed for explaining the shifting of optical absorption spectra of a mixed solution of *p*-aminoazobenzene and SDS when bovine serum albumin is added to it.[12] Electrophoretical studies[13] on the mixed solution of SDS and ovalbumin have also shown the appearance of a shift due to the formation of SDS-protein complex at around 10^{-5}M, which is in accord with the threshold value of the coincident region in Fig. 3.

The second stage is characterized by k_2 values 1.4—3.3 times larger than those for the first, with the constant A_∞^0 and the lowering A_1^0 and A_2^0 values. The increment beyond the saturation concentration seems to contribute to the interaction in such a manner as to decrease the A_1^0 and A_2^0 values but not to affect the saturated A_∞^0, which does not much differ from that obtained in the first stage. Probably, the attached SDS is increased by a small amount throughout the second stage. The role of these SDS molecules in the second stage should be different from that in the first. Blei[12] has proposed a solubilizing complex comprised in SDS molecules located to hydropohbic residue of the molecules of bovine serum albumin. A similar structure may be taken into account for elucidating the uncoiling processes of ovalbumin in the second stage of SDS concentration.

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