the reduction of physiological activities of bacteria caused by hydrostatic pressure is a pressure not-*in-situ* effect. When treated but before being shifted into media, the samples Nos. 1—5 (table 1) have been in normal environment for 95 h. They are fairly inactive and far from normal physiological activities. In other words, the pressure effect on physiological activities of bacteria in milk still exist after depressurization for 95 h.

The pressure restraining effects on growth and breeding of bacteria are promising in application to preservation of fresh milk. The raw milk treated by pressure has unique advantages. First, pressure treatment does not produce any chemical change and does not change its original nutrition and flavor, which is impossible for high temperature "sterilization". Second, the milk treated by pressure can be preserved in normal environment for longer time without spoiling.

The experimental results indicate that the pressure characteristics of coliform group inoculated in milk are completely different from that of most other original bacteria in milk; the hydrostatic pressure of only 20 MPa can restrain the physiological activities of most other bacteria in milk. However, the hydrostatic pressure of 40 MPa has no obvious influence on the breeding of coliform group. In other words, the pressure of 40 MPa cannot reduce the physiological activities of coliform group effectively. But hydrostatic pressure of 100 MPa lasting for 5 min can kill more than 90% of the coliform group inoculated to milk, leading to a survival rate of less than 10% of the total population.

The results above show that although the breeding of coliform group is not easy to be influenced by pressure below 40 MPa compared with most other bacteria in milk, the lethal pressure of coliform group is, in fact, lower.

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Reference

1 Tamura, K., Muramoto, Y., Kourai, H., Hydrophobicity of the cell surface and drug susceptibility of *Escherichia coli* cultivated at high pressures up to 30 MPa, *Biotech. Lett.*, 1993, 15:1189.

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Room temperature phosphorescence study on supramolecular system of cyclodextrin/surfactants

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THE microenvironmental properties of cyclodextrin/surfactants supramolecular system have been investigated with a variety of methods[1—4]. Usually, a 1:1 stoichiometry of cyclodextrin/surfactant and noneffectiveness of cyclodextrin on the micellization behavior are reported

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in most cases. But, β-cyclodextrin (β-CD)-induced aggregation of the surfactants below CMC has been recently assumed to take place in the cyclodextrin/surfactant mixed system[3]. Although the room temperature phosphorescence (RTP) emission can be only observed under the condition of deaerated solution and (or) rigid supports in most cases, recently, the RTP emission in aerated solution has been observed based on the relatively rigid inner cavity of cyclodextrins. The RTP enhancement is related to the formation of rigid inclusion complex and its effectiveness in shielding photoexcited lumophores from oxygen[7, 8]. In the present note, the intense RTP of α-bromonaphthalene(α-BrN) in β-CD solution induced by surfactants has been observed. The properties of supramolecular system of cyclodextrin/surfactant and aggregation of surfactant in the presence of β-CD are investigated by using RTP spectra of α-BrN as a probe.

1 Experimental

A Shimadzu RF-5000 fluorescence spectrophotometer equipped with a thermostat cell housing was employed.

α-BrN was the chemical reagent from the First Shanghai Reagent Factory and was vacuum distilled prior to use. Sodium dodecyl sulfate (SDS) was received from Sino-American Biotechnology Co. Cetyltrimethylammonium bromide (CTAB) was the analytical reagent from the Third Shanghai Reagent Factory. Cetyltrimethylammonium chloride (CTAC) was TCI-EP from the Tokyo Kasei. Triton X-100 (TX-100) was the analytical reagent from Rohn-Mass. Tween-20 was received from Guangzhou Chemical Reagent Co. β-CD was purchased from Suzhou Gourmet Power Factory and thrice recrystallized from boiling water. Water was doubly deionized and then distilled.

The solutions were allowed to equilibrate for 6 h before analysis. All measurements were performed at (30 ± 0.5)℃.

2 Results and discussion

2.1 Room temperature phosphorescence of α-BrN/β-CD/SDS ternary inclusion complex

It is seen in fig. 1 that the intensity of RTP of α-BrN in the presence of β-CD initially increases with increasing SDS concentration and then decreases sharply to reach zero at higher SDS concentration. It indicates that the microenvironmental properties for α-BrN in the presence of β-CD are changed with the increase of SDS concentration. Because the RTP emission of α-BrN depends on the formation of rigid inclusion complex and its effectiveness in shielding photoexcited α-BrN from oxygen[5,6], the intense RTP can be observed when a ternary inclusion complex of α-BrN/β-CD/SDS is formed with the addition of SDS surfactant, leading to much higher rigidity and effectiveness in shielding excited α-BrN from oxygen. Upon the addition of increasing amounts of SDS, SDS micelles or/and premicelles are formed; α-BrN is dislodged from the β-CD cavity and subsequently incorporated into the interior of micelles[1]; the intensity of fluorescence of α-BrN slightly decreases and the intensity of RTP of α-BrN decreases sharply. When the concentration of SDS is above the CMC value (8.1×10^{-3} mol/L), the intensity of RTP decreases to reach zero. This result indicates that the inner core of micelles composed of alkyl chain of surfactants is a more flexible, less rigid microenvironment for α-BrN with higher concentration of oxygen than in the cavity of β-CD.

Because the higher fluorescence polarization value can, in fact, represent the more rigid microenvironment for fluorophore, the measurements of fluorescence polarization of the

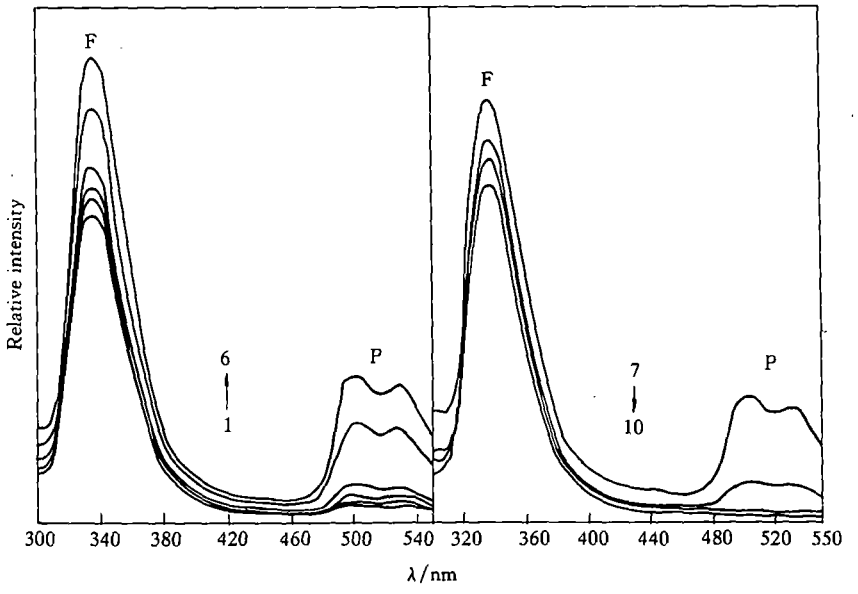

Fig. 1. Fluorescence and RTP spectra of α-BrN in aqueous β-CD solution with different concentrations of SDS. [SDS] = curve 1, 0 mol/L; 2, 5×10^{-5} mol/L; 3, 1×10^{-4} mol/L; 4, 2×10^{-4} mol/L; 5, 5×10^{-4} mol/L; 6, 8×10^{-4} mol/L; 7, 1×10^{-3} mol/L; 8, 3×10^{-3} mol/L; 9, 7.5×10^{-3} mol/L; 10, 1×10^{-2} mol/L. [β-CD] = 5×10^{-3} mol/L, [α-BrN] = 10^{-5} mol/L.

ternary inclusion complex provide direct evidence for the above-mentioned mechanism. Table 1 shows that the change in fluorescence polarization of α-BrN is related to the change in the intensity of RTP of α-BrN in the presence of β-CD and SDS surfactant.

Table 1 Variation of fluorescence polarization of α-BrN with SDS concentration at fixed 5×10^{-3} mol/L β-CD

$C_{SDS}\times10^{-4}$ /mol·L^{-1}	0	0.5	1	2	5	8	10	30	75	100
P	0.107	0.083	0.085	0.104	0.105	0.143	0.154	0.090	0.081	0.045

It is interesting to note (fig. 1) that the RTP intensity of α-BrN decreases when the concentration of SDS (8×10^{-4} mol/L) is far smaller than its CMC value in pure water. It indicates that the microenvironmental properties for α-BrN are changed at about the SDS concentration of 8×10^{-4} mol/L, suggesting a transformation of α-BrN from the cavity of β-CD ternary inclusion complex to a microenvironment with poorer rigidity and higher oxygen concentration than in the cavity of β-CD. As the β-CD concentration is fixed at 2×10^{-3} mol/L or 7×10^{-3} mol/L, the same results are observed. These results imply that the aggregation of SDS below its CMC is induced by β-CD[3, 7, 8]. An association constant on the order of 10^4 L/mol has been reported for the ionic surfactant/β-CD inclusion complex, which is one order of magnitude higher than that (10^3 L/mol) for the α-BrN/β-CD inclusion complex[2, 6]. Thus, in the present system, β-CD will preferentially include the surfactant molecule. Under the present circumstances, the α-BrN molecule, of course, is not in the cyclodextrin cavity any more, and is dislodged from the cavity of β-CD, then, is instead solubilized in an aggregate consisting of surfactant and β-CD, leading to decrease in RTP intensity. Usually, it is observed that the CMC is near equal to or lower than the sum of the CMC in pure water and [β-

CD][3]. But, in the present system, the concentrations of β-CD are 2×10^{-3}, 5×10^{-3} and 7×10^{-3} mol/L, respectively, which indicates not only the aggregation of SDS surfactants is induced by β-CD below its CMC, but also the "critical aggregate concentration" (8×10^{-4} mol/L) of SDS in the presence of β-CD is smaller than the concentration of β-CD in solution. It shows also that the aggregation of SDS surfactants below CMC can be induced by β-CD even if the concentration of SDS is not higher than the concentration of β-CD. Although the "critical aggregate concentration" of SDS is not dependent on the concentration of β-CD in the present system, that the RTP intensity of α-BrN at higher β-CD concentration decreases more slowly than that at lower β-CD concentration implies that SDS surfactant molecules in the presence of β-CD become not easy to aggregate to micelles with increasing β-CD concentration.

2.2 In the presence of β-CD, the aggregation of different kinds of surfactants

It can be seen (fig. 2) that the intense RTP emission of α-BrN is induced by nonionic-, anionic-surfactant molecules, and the weaker RTP emission of α-BrN is induced by cationic-surfactant molecules. These results indicate that the head group of different kinds of surfactants significantly affects the formation of α-BrN/β-CD/surfactant ternary inclusion complex and RTP intensity induced. In a surfactant/β-CD inclusion complex, it is impossible that the same or near enhancement of RTP intensity is not observed if only the hydrophobic alkyl chain of surfactant molecules is included within the cavity of β-CD, and the polar head group of surfactant molecules simply protrudes into the bulk aqueous phase. On the other hand, the diameters of the alkyl chain and β-CD cavity make it impossible for β-CD cavity to completely include both surfactant and α-BrN. The ternary inclusion complex should be assumed to form by partial inclusion of both surfactant and α-BrN within β-CD cavity.

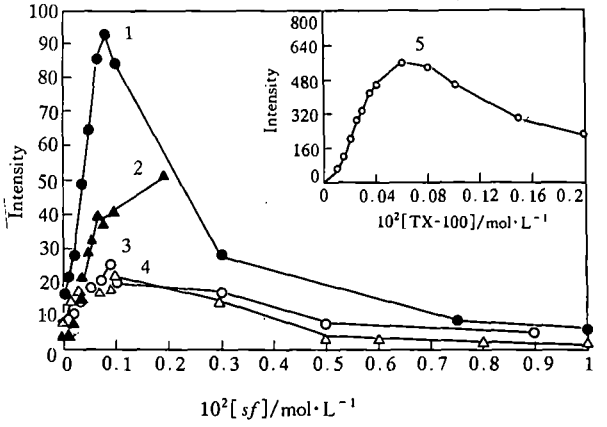

Fig. 2. RTP intensity of α-BrN as a function of concentrations of different kinds of surfactants in aerated β-CD aqueous solution. 1, SDS, 2, Tween-20, 3, CTAB, 4, CTAC, 5, TX-100.

It should be reasonable to infer from the above experiments that the more rigid microenvironment for α-BrN is interpreted in terms of favorable inclusion effects produced by the inclusion of ion pairs of monomers for ionic surfactants and polar head of monomers for nonionic surfactants at the open ends of β-CD torus, probably smaller end of β-CD[2], where the hydrophobic long alkyl chain is assumed to be partially included in the cavity of β-CD by a coiling conformation[7]. Nonionic- or anionic-surfactants monomer caps the ring of the primary OH side through hydrogen bond between the primary OH of β-CD and oxygen atom of head group of surfactant, leading to much higher rigidity and its effectiveness in shielding photoexcited α-BrN from quenching effects. The intense RTP induced is observed. But, ammonium-containing cationic surfactants monomer does not cap the ring of the primary OH side through hydrogen bond. The weak RTP induced is observed. RTP emission with the addition of TX-100 is near 10 times stronger than that observed in the presence of SDS. It is because the phenyl moiety of TX-100 is located inside the β-CD cavity, resulting in more rigidity and effectiveness

in shielding the excited α-BrN from oxygen quenching and nonradiative decay process[6].

It is interesting to note (fig. 2) that, except for nonionic surfactant Tween-20, the appearance in the variation-curve of the α-BrN RTP intensity vs. other surfactants' concentration in the presence of β-CD is similar to that of SDS surfactant. Results show that a similar variation trend and turning point between 5×10^{-4} and 10^{-3} mol/L is observed. This implies as if the surfactant with β-CD as a head group is formed because the polar head group of surfactants caps the open end of β-CD cavity and electrostatic repulsion of polar head of surfactants is effectively shielded. The surfactant with β-CD as a head group is the hydrophobic source for inducing the aggregation of different kinds of surfactants between 5×10^{-4} and 10^{-3} mol/L, making these show similar "critical aggregate concentration". Although the decrease in RTP intensity is not observed with increasing Tween-20 concentration, RTP intensity increases rapidly with Tween-20 concentration up to about 10^{-3} mol/L, and then increases more slightly. It also indicates that the conformation of the Tween-20 molecule in the presence of β-CD has been changed at about the concentration of 10^{-3} mol/L.

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References

1 Hashimoto, S., Thomas, J. K., Fluorescence study of pyrene and naphthalene in cyclodextrin-amphiphile complex systems, *J. Am. Chem. Soc.*, 1985, 107:4655.

2 Wan-Yunus, W. M. Z., Taylor, J., Bloor, D. M. *et al.*, Electrochemical measurements on the binding of sodium dodecyl sulfate and dodecyltrimethylammonium bromide with α- and β-cyclodextrins, *J. Phys. Chem.*, 1992, 96: 8979.

3 Jiang, Y. -B., Wang, X. -J., Direct evidence for β-cyclodextrin-induced aggregation of ionic surfactant below critical micelle concentration, *Appl. Spectrosc.*, 1994, 48:1428.

4 Jiang, X. -K., Gu, J. -H., Cheng, X. -E. *et al.*, The effect of hydrophobic-lipophilic interactions on chemical reactivity, *Acta Chimica Sinica* (in Chinese), 1987, 45:159.

5 Hamai, S., Inclusion complexes and the room-temperature phosphorescence of 6-bromo-2-naphthol in aerated aqueous solution of α-cyclodextrin, *J. Phys. Chem.*, 1995, 99:12109.

6 Xie, J. -W., Xu, J. -G., Chen, G. -Z. *et al.*, Room temperature phosphorescence of α-bromonaphthalene induced by β-cyclodextrin in the presence of cyclohexane, *Science in China*, Ser. B, 1996, 39(4):416.

7 Jiang, X. -K., Hydrophobic-lipophilic interactions, aggregation and self-coiling of organic molecules, *Acc. Chem. Res.*, 1988, 21:362.

8 Xu, C. -B., Tung, C. -H., Hydrophobic effects on photophysical and photochemical processes (Ⅵ), *Acta Chimica Sinica*, 1989, (1): 72.

Effects of high temperature treatment at initial stage of anther culture on culture responses in wheat

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Keywords: wheat, anther culture, culture temperature.

CULTURE temperature is an important factor in anther culture. In our laboratory we have made intensive studies on this problem, and found that the responses of anther culture to culture temperature were not only sensitive but also complicated, and varied with genotypes and even

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