The Effect of Surfactant Structure on Ribonuclease A—Surfactant Interactions

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The enzymic activity of ribonuclease A was measured in the presence of several surfactants at pH 7.2. Cationic surfactants with trimethylammonium and pyridinium head groups do not deactivate or denature the enzyme, whereas *n*-dodecylamine hydrochloride, like the anionic surfactant sodium *n*-dodecyl sulphate, deactivates and denatures ribonuclease A.

The binding of surfactants to proteins and the role of surfactants as protein denaturants have led to investigations into the nature of the interactions involved in these processes. Jones *et al.* (1973) have shown that sodium *n*-dodecyl sulphate binds to pancreatic RNAase A* [ribonucleate pyrimidine nucleotido-2'-transferase (cyclizing), EC 2.7.7.16] in aqueous solution at pH 7.3 and initiates chain unfolding. In contrast no interaction between RNAase A and the cationic surfactant *n*-dodecyltrimethylammonium bromide could be detected under comparable conditions. It was suggested that the chemistry of the surfactant head group is a determining factor in surfactant-protein interactions and that the formation of strong ionic links between the surfactant head group and charged sites on the surface of the protein molecule precedes chain unfolding. Steric hindrance of the methyl groups prevents ionic links from forming between *n*-dodecyltrimethylammonium bromide and RNAase A.

We now report kinetic and spectroscopic studies on the interactions between RNAase A and a range of surfactants with different head groups and hydrocarbon chain lengths. These results have a direct bearing on the previous suggestions.

Materials and methods

The preparation and purification of sodium *n*-dodecyl sulphate ($C_{12}H_{25}OSO_3^-Na^+$) have been previously described (Jones *et al.*, 1973), as have the preparation and purification of *n*-tetradecyltrimethylammonium bromide [$C_{14}H_{29}N^+(CH_3)_3Br^-$] (Jones & Reed, 1968). *n*-Dodecylamine ($C_{12}H_{25}NH_2$, puriss grade; Fluka A.-G. Chemische Fabrik, Buchs, Switzerland) and *n*-dodecanoic acid ($C_{11}H_{23}CO_2H$, specially pure; BDH Chemicals Ltd., Poole, Dorset, U.K.) were used as supplied. *n*-Hexadecyltrimethylammonium bromide [$C_{16}H_{33}N^+(CH_3)_3Br^-$; BDH Chemicals Ltd.] was recrystallized

twice from acetone-ethanol (3:1, v/v). *n*-Hexadecylpyridinium chloride ($C_{16}H_{33}N^+C_5H_5Cl^-$) was prepared from *n*-hexadecyl chloride (purum grade; Fluka A.-G. Chemische Fabrik) by refluxing with excess of pyridine. The crude product which separated out on cooling the reaction mixture was purified by recrystallization from acetone.

The RNAase A and cytidine 2':3'-cyclic monophosphate (sodium salt) were obtained as previously described (Jones *et al.*, 1973).

The enzymic activities of RNAase A at 25°C in the presence of the surfactants were measured with cytidine 2':3'-cyclic monophosphate as substrate by the method of Crook *et al.* (1960). A standard solution of substrate at a concentration of 3.0 g/l was made up in dimethylformamide containing just sufficient water for dissolution. The substrate solution (0.1 ml) was added to the two spectrophotometer cuvettes containing (2.9-*y*) ml and (2.9-*y*-*x*) ml of the appropriate buffer solution respectively. To each cuvette was added *y* ml of the required surfactant stock solution. The reaction was started by the addition of *x* ml of an enzyme solution (0.3 g/l) in the same buffer. The extinction was continuously recorded at 284 nm with a Beckman DB-GT spectrophotometer. Experiments with *n*-dodecanoic acid were done in 0.1 M-Tris-HCl buffer, pH 7.2. For the experiments with all the other surfactants 10 mM-Tris-HCl buffer, pH 7.2, was used. The stock surfactant solutions were either 0.1 M, 10 mM or 0.1 mM.

U.v. difference spectra were recorded in the range 200–400 nm with a Beckman DB-GT spectrophotometer at 25°C. To each cuvette was added 0.3 ml of the enzyme solution (3 g/l) in the appropriate buffer. To the reference cuvette was added 2.7 ml of buffer solution and to the sample cuvette 2.7 ml of surfactant solution of concentration 10 mM.

Results and discussion

Fig. 1 shows the initial rate of change of extinction for the reaction between RNAase A and cytidine 2':3'-cyclic monophosphate as a function of the

* Abbreviation: RNAase A, ribonuclease A.

Fig. 1. Initial rate of change of extinction for the reaction between RNAase A and cytidine 2':3'-cyclic monophosphate in the presence of surfactants at 25°C

----, *n*-Tetradecyltrimethylammonium bromide, *n*-hexadecyltrimethylammonium bromide and *n*-hexadecylpyridinium chloride (the experimental points have been omitted for clarity); ●, *n*-dodecylamine hydrochloride; ○, sodium *n*-dodecyl sulphate; ▲, *n*-dodecanoic acid. In all the experiments the RNAase concentration was 20 μg/ml and the cytidine 2':3'-cyclic monophosphate concentration was 0.1 mg/ml. All the experiments were done in 10 mM-Tris-HCl buffer, pH 7.2, except for those with *n*-dodecanoic acid, when 0.1 M-Tris-HCl buffer, pH 7.2, was used.

logarithm of the surfactant concentration. In these experiments the enzyme and substrate concentrations were kept constant. The cationic surfactants *n*-tetradecyltrimethylammonium bromide, *n*-hexadecyltrimethylammonium bromide and *n*-hexadecylpyridinium chloride do not deactivate the enzyme over the concentration range 10 μM–0.05 M. The initial rate of change of extinction in the absence of surfactants under the same conditions as pertain to the data in Fig. 1 was $0.054 \pm 0.001 \text{ min}^{-1}$. Both sodium *n*-dodecyl sulphate and *n*-dodecylamine hydrochloride deactivate RNAase A. The contrast between *n*-dodecylamine hydrochloride and the other cationic surfactants is very marked and clearly illustrates the importance of the chemistry of the surfactant head group in the deactivation process. These and our previous results (Jones *et al.*, 1973) show that increasing the hydrocarbon chain length of the *n*-alkyl trimethylammonium surfactants from C₁₂ to C₁₆ has no effect on their interaction with RNAase A, but replacing the $-\text{N}(\text{CH}_3)_3$ head group by $-\text{NH}_3^+$ results in an interaction with the enzyme which is apparently similar to that which occurs with the anionic surfactant sodium *n*-dodecyl sulphate. The effect of *n*-dodecanoic acid on the activity of

RNAase A is distinctly different from the effects of *n*-dodecylamine hydrochloride and sodium *n*-dodecyl sulphate. The activity is significantly decreased at low concentrations and the change in activity with concentration is more gradual, indicating that the mechanism of deactivation is probably different in this case. We repeated the kinetic measurements at enzyme concentrations of one-half and one-quarter of those used for the experiments shown in Fig. 1 for the systems involving *n*-dodecylamine hydrochloride, sodium *n*-dodecyl sulphate and *n*-dodecanoic acid. The shapes of the curves were identical with those shown but were displaced vertically downwards to lower (dE/dr) values. However, the critical concentrations at which *n*-dodecylamine hydrochloride and sodium *n*-dodecyl sulphate first decrease the enzymic activity (as shown by the arrows in Fig. 1) were independent of the RNAase A concentration. This would be expected because the ratio of the concentrations of surfactant to enzyme in the solutions at the critical concentrations is very high, so that even allowing for binding of the surfactant to the enzyme the total and free surfactant concentrations are almost equal. With sodium *n*-dodecyl sulphate the critical concentration at which the enzymic activity falls steeply corresponds to a degree of binding at which chain unfolding occurs (Jones *et al.*, 1973). The initial rate of change of extinction, however, is lowered at surfactant concentrations below the critical concentration. This may arise because of binding of some surfactant molecules in the vicinity of the active site of RNAase A. Since the active site is positively charged binding of *n*-dodecylamine cations to it is less likely to occur and unless the chain unfolds the rate of change of extinction will be unaffected by this surfactant.

The u.v. difference spectra of RNAase A in the presence of *n*-dodecylamine hydrochloride and sodium *n*-dodecyl sulphate gave peaks at 287 nm which can be attributed to a change in the environment of the three abnormal tyrosine residues on chain unfolding (Bigelow & Sonenberg, 1962). In contrast no such peak was observed in u.v. difference spectra of RNAase A in the presence of *n*-dodecanoic acid or *n*-hexadecyltrimethylammonium bromide.

We deduce by analogy with our previous studies on the RNAase A–sodium *n*-dodecyl sulphate interactions that *n*-dodecylamine hydrochloride deactivates RNAase A by first binding to ionic sites and then initiating chain unfolding. *n*-Dodecanoic acid does not unfold RNAase but probably deactivates it by binding in the vicinity of the active site. The failure of carboxylates to unfold bovine serum albumin has been observed by Reynolds *et al.* (1968). The cationic surfactants with bulky head groups such as trimethylammonium and pyridinium groups do not interact significantly with RNAase A because the formation of strong ionic links with negatively

charged groups on the surface of the enzyme which must precede chain unfolding does not take place.

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