REACTION OF ALKYL GALLATES WITH DIPHENYL PICRYLHYDRACYL RADICALS IN SDS MICELLES

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ABSTRACT

Diphenylpicrylhydracyl is a stable free radical that readily reacts with phenol groups. In the present work we have measured the reaction of DPPH with three alkyl gallates of different hydrophobicity: methyl gallate, propyl gallate, and octyl gallate. Experiments were carried out in acetate buffer (pH 5.0) in presence of several SDS concentrations. At surfactant concentrations higher than 20 mM, the gallates are almost totally associated to the micellar pseudophase. Under these conditions the rate of the reaction becomes almost independent of the length of the alkyl group. This indicates that the average localization of the gallate reactive group is almost independent of the solute hydrophobicity.

INTRODUCTION

Rates of intramolecular bimolecular processes are extremely dependent on the characteristics of the micellar pseudophase and the hydrophobicity of the reactants. In particular, if one of them is highly hydrophobic and, hence, totally incorporated to the micelles, the rate of the process will depend upon the partitioning of the other reactant and its average intramolecular distribution¹. It can be envisaged that fastest reaction rates should be obtained when both reactants are totally incorporated, they are located in the same micellar region and the characteristics of the intramolecular media are favourable to the reaction. When these conditions are met, it is observed a large micellar catalysis with a characteristic decrease in rate when the surfactant concentration increases, due to a direct "dilution" effect.

DPPH, a hydrophobic free radical², readily reacts with phenolic compounds^{3,4}. In micellar solutions, it can be considered that DPPH is mostly associated to the micellar pseudophase, while the distribution and location of the phenolic compound will depend upon their characteristics, mostly its hydrophobicity. In particular it can be assumed that an increase in the solute hydrophobicity will increase its incorporation to the micelles, particularly in the micelle hydrophobic core^{5,6}.

Hydrophobicity of alkyl gallates can be tuned by changing the length of the alkyl chain^{7,8}. We have measured intramolecular rate constants in the reaction of three gallates of widely different hydrophobicity with DPPH incorporated into Triton X-100 micelles¹. It was found that intramolecular rate constants are independent of the length of the gallate alkyl chain. This result suggests that distribution of the reactive moieties, the phenol groups, is not influenced by the length of the alkyl chain. In order to see if this conclusion also applies to ionic micelles, we have measured the rate of reaction between DPPH and gallates in presence of SDS micelles. The results indicate that also in this system intramolecular rate constants are not influenced by the length of the gallate alkyl chain (from methyl to octyl).

EXPERIMENTAL

Chemicals. Sodium dodecyl sulphate (SDS) and ethanol were purchased from Merck. Methyl, propyl and octyl gallate, DPPH, and 1,1-diphenyl-2-picrylhydrazilo (DPPH-H) were obtained from Sigma-Aldrich. All chemicals were employed as received.

Solutions. Stock solutions of the gallates and DPPH were prepared daily in ethanol. All SDS solutions were prepared in acetate buffer (5 x 10⁻³ M, pH 5.0). Experiments at pH 3.0 were performed in citrate buffer (5 x 10⁻³ M). All experiments were carried out in buffer solutions containing 2.3 % (V/V) ethanol in order to assure complete solubilization of the reactants.

Kinetic Measurements. The reaction was followed by measuring the decrease in DPPH absorbance in the visible band (529 nm) employing a Hewlett-Packard spectrophotometer. A small aliquot of the gallate solution was added to the cell containing the SDS and the DPPH to initiate the reaction. All the measurements were carried out at room temperature (21.0 ± 0.5 °C) employing surfactant concentrations in the 20-200 mM range.

RESULTS AND DISCUSSION

DPPH is a highly hydrophobic free radical of very low water solubility² that, in micellar solutions, can be considered to be almost quantitatively incorporated to the dispersed microphase¹. In these systems, DPPH radicals react with a wide range of substrates, ranging from very hydrophilic (urate⁹) to very hydrophobic (octyl gallate) compounds.^{1,8}

DPPH has been widely employed to evaluate the reactivity of antioxidants towards free radicals.¹⁰ These studies have been carried out mostly in homogeneous solvents (ethanol) and hence the measured reactivity is unrelated to the antioxidant hydrophobicity. In presence of SDS micelles, DPPH readily reacts with the gallates at rates that are strongly dependent of the pH. Typical results are given in Fig. 1. The faster reaction at pH 7.4 suggests that, at this pH, deprotonated gallate is the reactive species. On the other hand, similar rates are obtained at pHs 3.0 and 5.0 (data not shown). This independence suggests that, in this pH range, the reactive moiety is the prevailing fully protonated polyphenolic group. All kinetic measurements were carried out at pH 5.0 and involve the protonated gallates. The initial step (and rate determining process) can be then represented by

Figure 1. Time profile of DPPH (50 μM) absorbance diminution following addition of methyl gallate (30 μM) to a SDS solution (100 mM). (○) Data obtained at pH 5 (acetate buffer); (●) Data obtained at pH 7.4 (phosphate buffer).

In order to assess if the back reaction of process (1) is kinetically relevant, we carried the reaction between octyl gallate (30 μM) and DPPH (50 μM) in SDS (100 mM, pH 5.0) in presence of DPPH-H (5 μM). Addition of this excess of DPPH-H only reduces in 33 % the rate of the process.

DPPH consumption, at fixed pH and DPPH and gallate concentrations, markedly depends upon the surfactant concentration. Typical profiles are given in Figure 2. From these representations was obtained the initial rate of the

process. The values obtained, employing the three gallates and different SDS concentrations are collected in Figure 3. These results show that:

Figure 2. Effect of SDS concentration on the time profile of DPPH (50 μ M) absorbance decay following addition of methyl gallate (30 μ M) at pH 5.0. (●) 20 mM SDS; (○) 100 mM SDS.

Figure 3. Initial slopes of A/Ao vs time plots as a function of SDS analytical concentration. Data obtained employing DPPH (50 mM) and gallates (30 mM) in 50 mM acetate buffer (pH 5). (●) methyl gallate; (D) propyl gallate; (□) octyl gallate.

i) The rate of the process decreases when the surfactant concentration increases. This is due to dilution of the gallates in the micellar ensemble;

ii) Similar rates are observed, at a given SDS concentration, for the three employed gallates. In fact, the only difference is observed for methyl gallate at the lowest SDS concentration employed (20 mM).

Point ii) is compatible with an almost total incorporation of propyl gallate and octyl gallate at all the SDS concentrations. The lower value for methyl gallate at low (20 mM) SDS concentration can be explained in terms of incomplete association to the micelles. This consideration is compatible with reported data regarding the partition of the gallates between the external solvent (water plus 1 % ethanol) and SDS micelles. In this system, the reported partition constants were 8.7 and 157 M^{-1} for methyl gallate and octyl gallate, respectively.

The similarity among the rates of the different gallates with DPPH incorporated into SDS micelles indicates that intramicellar second rate constants are almost independent of the gallate alkyl chain. In order to stress these points, data were plotted according to Eqn 2¹:

$$\frac{[DPPH^{\bullet}][\Phi-OH]}{Rate} = \frac{V^o}{k_{Mic}K_{\Phi-OH}} + \frac{1}{k_{Mic}}V^o[SDS] \quad (2)$$

Plots obtained are shown in Figure 4 as a function of the micellized surfactant concentration. To obtain these values a free surfactant concentration of 3 mM was considered under our experimental conditions. In this figure are included results obtained only up to 100 mM SDS to avoid changes in the micellar characteristics produced at concentrations higher than the second CMC.¹¹ The intercepts and slopes of these plots are collected in Table 1.

Figure 4. Plot of the experimental data according to Eqn. 2. (A) methyl gallate; (B) propyl gallate; (C) Octyl gallate.

Table 1. Intercepts and slopes of the plots given in Figure 4.

Gallate	R	Slope (s)	Intercept (mM/s)
Methyl	0.9949	0.048 ± 0.004	-0.023 ± 0.194
Propyl	0.9946	0.046 ± 0.003	0.12 ± 0.19
Octyl	0.9953	0.051 ± 0.004	-0.15 ± 0.20

The values of the intercepts are very close to zero. This precludes a meaningful evaluation of partition constants but is consistent with an almost total incorporation of the gallates to the micelles in the SDS concentration range considered. The slopes of the plots correspond to

$$\text{Slope} = V^{\circ} / k_{\text{Mic}} \quad (3)$$

Where V° is the surfactant molar volume (250 mL/mole)¹² and k_{Mic} is the intramolecular second order rate constant. The data show that the three rate constants are similar, with differences inside their experimental errors. This suggests that the average intramolecular localization is independent of the length of their alkyl chain. Similar conclusion has been obtained when Triton X-100 is employed instead of SDS.

From Eqn. 2 it can be obtained the intramolecular rate constant. If V° is taken as 250 mL/mole the data of Table 1 render

$$k_{\text{Mic}} = 5.0 \text{ M}^{-1} \text{ s}^{-1}$$

a value very close to that reported for the reaction inside Triton X-100 micelles¹.

CONCLUSIONS

At SDS concentrations large enough to incorporate them quantitatively, methyl, propyl and octyl gallates react with DPPH at the same rate. This indicates that the average position of the reactive moieties, the hydroxyl groups, is not affected by the length of their alkyl chains.

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