MICELLE AND ACID-SOAP FORMATION OF LINOLEIC ACID AND 13-L-HYDROPEROXY-LINOLEIC ACID BEING SUBSTRATES OF LIPOXYGENASE-1

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Summary

Surface tension measurements of linoleic acid solutions in 0.1 M sodiumborate buffer pH 10 at 23°C showed that at increasing the linoleic acid concentration a sharp transition from monomers to micelles occurs at 167 μM. At pH 9 and 8 formation of acid-soap dimers from monomers starts at 60 μM and 21 μM respectively. The concentration range at which only monomers exist is therefore markedly reduced. For 13-L-hydroperoxylinoleic acid at pH 10 acid-soap formation still takes place, starting at approx. 220 μM. The total lipid concentration at which acid-soap or micelle formation starts in mixtures of linoleic acid and 13-L-hydroperoxylinoleic acid has been determined in relation to the molar ratio of both acids.

Introduction

Soybean lipoxygenase-1 (EC.1.13.11.12) converts linoleic acid in the presence of molecular oxygen to 13-L-hydroperoxylinoleic acid. In the absence of oxygen both linoleic acid and 13-L-hydroperoxylinoleic acid are converted in coupled reactions to various products [1, 2]. Recently the conversion of 9-D- and 13-L-hydroperoxylinoleic acid by lipoxygenase-1 under anaerobic and aerobic conditions has been reported [3]. Gatt et al. [4] have shown that micelle formation of substrates may lead to a false interpretation of enzyme-kinetic data. Evidently for a kinetic study of an enzyme acting on an amphiphilic substrate it is necessary to determine the cmc of the substrate under the same experimental conditions as the kinetic measurements. With regard to the substrates of lipoxygenase-1 cmc values reported so far for linoleic acid show a large diversity, whereas for 13-L-hydroperoxylinoleic acid or mixtures of it with linoleic acid no data are available. Several authors have shown that the hydrolysis of long-chain fatty-acid soaps is much more important than has to be expected on account of the pK_a values [6, 7]. This phenomenon is attributed to the formation of acid-soaps, being 1:1

dimers of dissociated and undissociated fatty acid [6, 7]. Therefore not only the formation of micelles but also the formation of acid-soaps has to be considered in kinetic studies on lipoxygenase-1.

Materials and Methods

Linoleic acid (purity > 99%) was obtained from Lipid Supplies (St. Andrews University, St. Andrews, Scotland). 13-L-Hydroperoxylinoleic acid was prepared as described previously [3]. Sodiumborate buffer (0.1 M) was used, which is the most commonly applied buffer in lipoxygenase-1 studies. Surface tension was measured according to the method of Wilhelmy as described by Harkins et al. [5] using a thin platinum plate (perimeter 3.94 cm) and a Beckman LM 600 electro-microbalance. Various amounts of surface active agent (in aqueous solution) were added to the solution in a 10 ml circular trough and the surface tension was continuously recorded with a Varian A25 recorder. After each addition a sufficiently long period of time was allowed to elapse for attaining a steady value of the surface tension. Cmc values were obtained by plotting the measured surface tensions versus the logarithm of the concentrations of the surface-active agent. The concentration at which a deviation from straight line was observed in the surface tension vs log [surface-active agent] plot was defined as the start of acid-soap formation.

Results

Fig. 2.1. shows plots of the surface tension (γ) versus log [linoleic acid] at pH 8, 9 and 10. At pH 10 the surface active agent is mainly the linoleate anion. Aggregation occurs over a very narrow concentration range, allowing a precise determination of the cmc. A value of 167 μM was found. However at pH 8 and pH 9 aggregation occurs over a much wider range. This gradual aggregation over a considerable range of concentration lacks the sharp discontinuity associated with the cmc. It was reported previously [6] that this pre-micellar aggregation can be attributed to the formation of dimeric acid-soap or higher aggregates. Acid-soap formation was found to start at linoleic acid concentrations of 21 μM at pH 8 and of 60 μM at pH 9.

Fig. 2.2. shows the surface tension versus the log [linoleic acid], log [13-L-hydroperoxylinoleic acid] or the logarithm of the total lipid concentration of mixtures of both acids, at pH 10.0. In general, micelle formation is negatively influenced by polar groups in the apolar chain of the soap molecule leading to higher cmc values. It appeared that at pH 10.0 dimeric acid-soap formation from 13-L-hydroperoxylinoleic acid starts at 220 μM. Also in this case this phenomenon starts before the cmc is reached. In mixtures of linoleic acid and 13-L-hydroperoxylinoleic acid acid-soap formation occurs at almost all molar ratios. In Fig. 2.3 the concentrations of the linoleic acid/13-L-hydroperoxylinoleic acid mixtures at which acid-soap or micelle formation starts at pH 10, are plotted against the mole fraction of linoleic acid. From this figure for

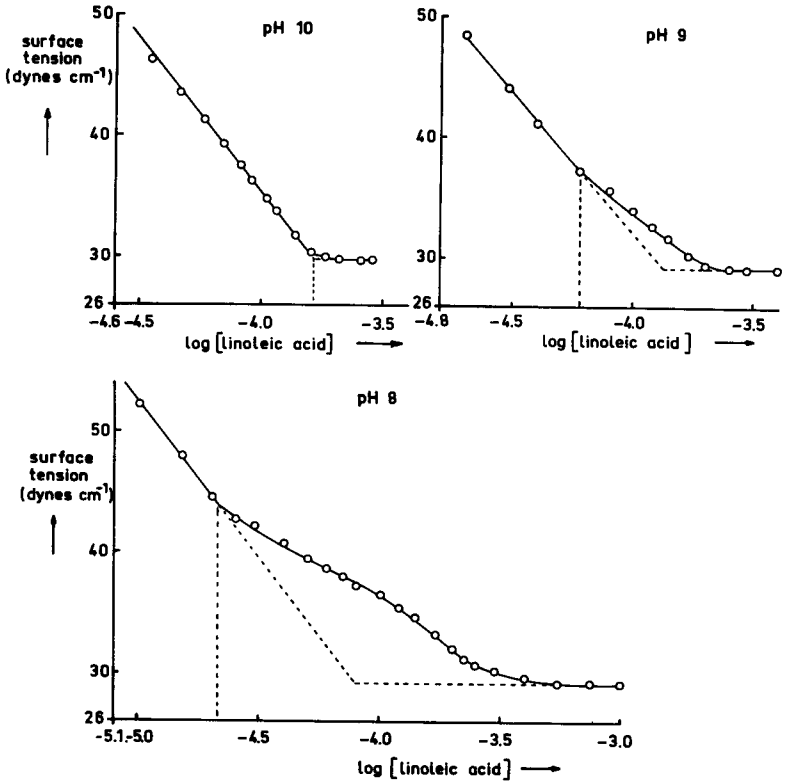

Fig. 2.1. Surface tension of linoleic acid solutions in 0.1 M sodiumborate buffer (pH values : 10.0, 9.0 and 8.0) against log [linoleic acid]. t = 23°.

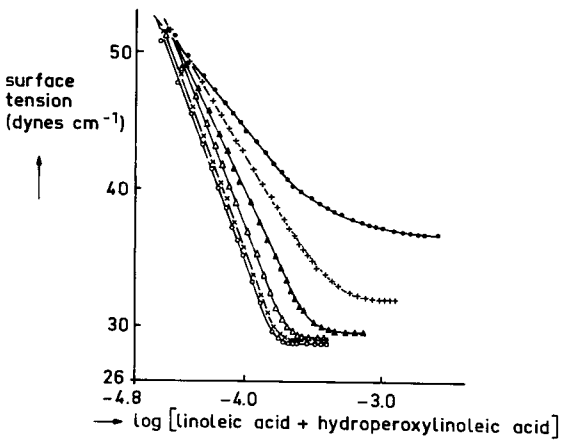

Fig. 2.2. Surface tension of linoleic acid, 13-L-hydroperoxy-linoleic acid and mixtures of both acids in 0.1 M sodiumborate buffer (pH 10.0) against the logarithm of the total lipid concentration. t = 23°. Mole fraction linoleic acid: O : 1.00, X : 0.88, Δ : 0.71, ▲ : 0.44, + : 0.21, ● : 0.00.

a given mixture the maximum total lipid concentration can be obtained at which both acids occur exclusively in the monomeric form, which is of the utmost importance for kinetic studies on lipoxygenase-1.

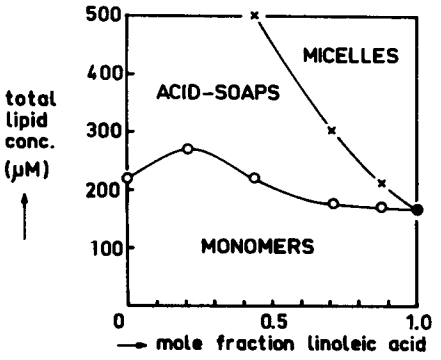

Fig. 2.3. The concentration ranges of monomers, acid-soaps and micelles in mixtures of linoleic acid and 13-L-hydroperoxylinoleic acid in 0.1 M sodiumborate buffer pH 10.0 as a function of the mole fraction of linoleic acid. t = 23°.

Finally from the slopes ($\delta\gamma / \delta \log c = - RT\Gamma / \log e$) of the straight lines in Fig. 2.2 values for the surface excess (Γ) of $4.76.10^{-10}$ mol. cm^{-2} and $2.19.10^{-10}$ mol. cm^{-2} are calculated for linoleic acid and 13-L-hydroperoxylinoleic acid respectively. Therefore the values for the area per molecule surface active agent in the monolayer are: 0.35 nm^2 per molecule linoleic acid and 0.74 nm^2 per molecule 13-L-hydroperoxylinoleic acid.

Discussion

Comparison of the present results with cmc values reported previously shows that a cmc for linoleic acid of 150 μM was found by Lagocki et al. [8] at 20°C in 0.05 M sodiumborate buffer (pH 9). However, these authors did not take into consideration the premicellar aggregation, which is probably due to the absence of measurements in the range of 120 μM to 200 μM linoleic acid. The results of Tookey et al. [9] suggest a value of 200 μM at 20°C in 0.043 M sodiumborate buffer pH 9, which contained 4.5% ethanol. On the other hand Allen [10] found cmc values of 24 μM, 22 μM and 22 μM in 0.2 M Tris buffers pH 7.5, 8 and 9 respectively. Differences in ionic strength of the used buffers alone cannot explain the diversity of the found cmc values. As the pH has a profound influence on the cmc of a fatty acid [11], the uniformity of the values found by Allen for linoleic acid (apparent pK_a : 7.9 [12]) at different pH values is rather surprising. In fact the values reported by Tookey et al. and by Allen seem to be doubtful, because the authors obtained their values by plotting the surface tension (γ) of linoleic acid as a function of its concentration. The cmc was defined by these authors as the intersection of the two extrapolated (pseudo) linear limbs of the plot at low and high linoleate concentrations. This unusual method leads to erroneous cmc values. The generally accepted procedure for obtaining cmc values from surface tension

measurements is based on the Gibbs adsorption isotherm:

$$\left(\frac{\delta\gamma}{\delta \ln c}\right)_T = -RT\,\Gamma_x \quad [\,\Gamma_x : \text{surface excess of compound x}\,]$$

This equation may be used, provided that the ionic strength of the buffer is relatively high in respect to the concentration of the surface active agent. The cmc is obtained by plotting the measured surface tension versus the logarithm of the linoleic acid concentration. Semilogarithmic replots of the data given by Tookey et al. and by Allen produce either significantly different cmc values or no value at all.

With respect to the study of enzyme kinetics on soybean lipoxygenase-1, it can be concluded from Fig. 2.1 that measurements at pH 10 offer the widest range of concentrations of substrate in the monomeric form. For kinetic experiments with mixtures of linoleic acid and 13-L-hydroperoxylinoleic acid Fig. 2.3 depicts the concentration ranges at which only monomers occur. The study of the kinetics of the anaerobic reaction of soybean lipoxygenase-1 with linoleic acid and 13-L-hydroperoxylinoleic acid will be published shortly.

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