ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1975, p. 733-736
Vol. 8, No. 6

Antimicrobial Effect of Simple Lipids and the Effect of pH and Positive Ions

KÅRE LARSSON,* BÖRJE NORÉN, AND GÖRAN ODHAM

Department of Food Technology, Chemistry Center, S-220 07 Lund 7, and Ecology Center, University of Lund, S-223 62 Lund, Sweden*

Received for publication 21 August 1975

Various branched fatty acids, particularly those of *iso*-configuration, have been shown to possess fungistatic and bacteriostatic properties. On the basis of their swelling effect on hyphae of *Fusarium roseum* it was suggested that this is due to an increase in the permeability of the plasma membrane. The solubilization of fatty acids in membranes should be expected to be influenced by the degree of dissociation and the presence of counter ions. Therefore, the effects of pH and K^+, Na^+, and Ca^{2+} ions were studied. It is demonstrated that the presence of the univalent ions, Na^+ and K^+, markedly enhances the fungistatic effect of *iso*-tetradecanoic acid, whereas the opposite effect is noted for the divalent ion, Ca^{2+}. The effects are particularly pronounced at high pH. Furthermore, the antimicrobial effect obtained from the combination of fatty acid and tetramethylthiuramdisulfide is significantly enhanced in the presence of 0.1 and 0.2% KCl.

Certain simple lipids, mainly fatty acids, have been studied with regard to antimicrobial activity (1, 2), suggested as due to an increase in the permeability of the plasma membrane. It was found that the strongest effect was obtained when there is a hydrophobic group of moderate size at the end of the hydrocarbon chain tail (2). It is also known that certain antibiotic agents act by increasing the permeability of different ions through the membrane (for example, gramicidin and valinomycin), and therefore it was natural to study the effect of these antimicrobial fatty acids in the presence of different ions. Fatty acids incorporated in the plasma membranes of most microorganisms should be expected to be almost completely dissociated, as the physiological pH's usually are above the dissociation constant (pK_a) of fatty acids (about 4.8). If the fatty acid molecules are dissociated and arranged close enough, an expansion effect on the membrane due to electrostatic repulsion can be expected. Such a repulsion depends also on the binding of counterions to the charged groups. Furthermore the pH has a fundamental effect on the solubility of fatty acids in aqueous media and, thus, on the partition between a lipid membrane and its aqueous environment. As in the previous work the biological activity was correlated with the surface film behavior, and an *iso*-branched fatty acid, 12-methyltridecanoic acid, was the lipid component selected for these studies.

MATERIALS AND METHODS

The preparation of the *iso*-fatty acid, the biological activity measurements, and the surface film technique have been described in a previous paper (1). The measurements were performed at pH 5, 6.5, and 8 and at concentrations of Na^+, K^+, and Ca^{2+} corresponding to 0.1, 1, and 2% by weight in the nutrient media. As the nutrient solution used earlier contains quite high amounts of K^+ (cf. reference 1), a modified nutrient solution was used consisting of K_2HPO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4$ (trace), and water (1,000 ml). Then, the critical salts were removed, except for a small amount of K^+ (0.45 mg/ml) as K_2HPO_4. The adjustment of pH was achieved by addition of 0.1 N HCl or NaOH. The ionic concentration introduced in this way is negligible compared to the ionic concentrations that were studied. The only test organism used in this work was *Fusarium roseum*.

RESULTS AND DISCUSSION

The effect of pH on the inhibition of the conidia germination by 12-methyltridecanoic acid, alone and in combination with various concentrations of $CaCl_2$, NaCl, or KCl, is demonstrated in Fig. 1. At an incubation period of 3 h, the addition of 2% NaCl or KCl caused germination inhibition at pH 5, and to some extent also at pH 6.5. However, when the incubation was prolonged to 4 h this inhibition was overcome, and in all series, where the salts were present alone, the relative number of germinating conidia was 90 or above. The results

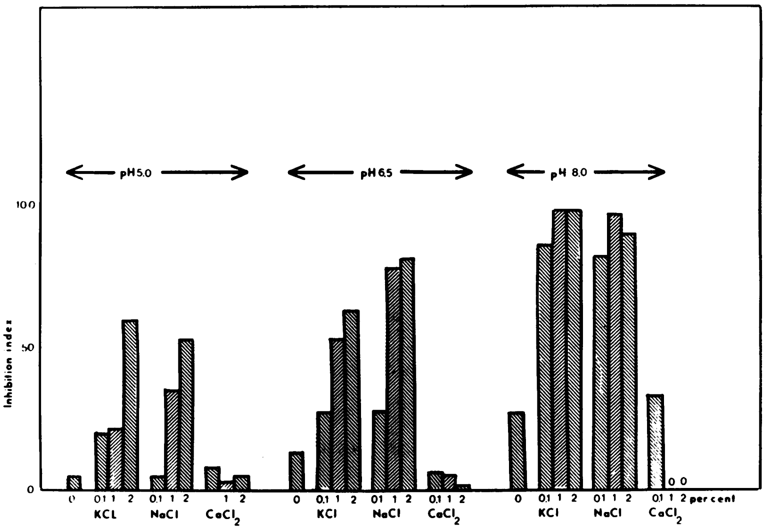

FIG. 1. *Influence of pH and various concentrations of Na^+, K^+, and Ca^{2+} on the inhibition efficiency of 12-methyltridecanoic acid (100 μg/ml) on Fusarium roseum. Relative number of germinating conidia in nutrient solution (control) was equal to 100. The corresponding inhibition indices when no iso-fatty acid was supplemented were all less than 10.*

obtained after 4 h of incubation are given in Fig. 1 as the synergistic effects of salts; the *iso*-acid was most evident after this time.

12-Methyltridecanoic acid alone produced its strongest effect at pH 8.0 and weakest at pH 5.0. After 3 h of incubation, the inhibition indices at pH 5.0, 6.5, and 8.0 were 21, 48, and 72, respectively. As seen from Fig. 1, the inhibition 1 h later was strongly reduced. This is taken as an indication of a fungistatic effect under these conditions, the difference illustrating the growth from 3 to 4 h of incubation.

The influence of pH on the effect of the fatty acid was accentuated by the presence of KCl and NaCl (Fig. 1). Thus, the presence of either of these salts at a concentration of only 0.1% increased the inhibition index at pH 8.0 by a factor of about 3, whereas the effect at lower pH was less striking (Fig. 1). At higher concentrations, however, an enhancement of the efficiency of the fatty acid inhibition was much stronger, and it was evident also at pH 6.5 and 5.

The effect of Ca^{2+} is opposite to that of the univalent ions. A decrease in the effect of the 12-methyltridecanoic acid was seen at pH 6.5, even at a concentration of only 0.1% $CaCl_2$. The negative effect of $CaCl_2$ used in higher concentration was most pronounced at pH 8.0. The influence of K^+ and Ca^{2+} on the germination inihibition of 12-methyltridecanoic acid (100 μg/ml of medium) was also studied in the nutrient solution used in our previous work. The experiments also included series with media supplied with thiram (tetramethylthiuramdisulfide) (0.5 μg/ml), alone or in combination with the *iso*-fatty acid. It was found that, after 3 h of incubation at 25 C, the germination inhibition caused by the fatty acid, and the fatty acid plus tetramethylthiuramdisulfide, was significantly enhanced in the presence of 0.1 and 0.2% KCl. At a prolonged incubation (4 h) the enhancement effect remained only in the medium with the fatty acid plus tetramethylthiuramdisulfide. In all cases, the presence of $CaCl_2$ did not influence or decrease the effect of the inhibitory substances.

The surface film experiments were run under the same conditions as the biological activity measurements to compare the collapse behavior of the film with the antimicrobial activity. It was suggested in the previous papers that these types of lipids act by increasing the permeability of the plasma membrane, and, on the basis of the surface film studies, it was proposed that this effect is related to the instability of the condensed monolayer phases on water. The effects of pH and different ions can be due to a change in the structure of this active lipid component due to changes of the degree of ionization, or in the counterion association. An alternative explanation of the strong effects observed by the presence of K^+ and Na^+ is that the increased permeability of the membrane results in a facilitated diffusion of these ions, disturbing the active K^+/Na^+ transport mecha-

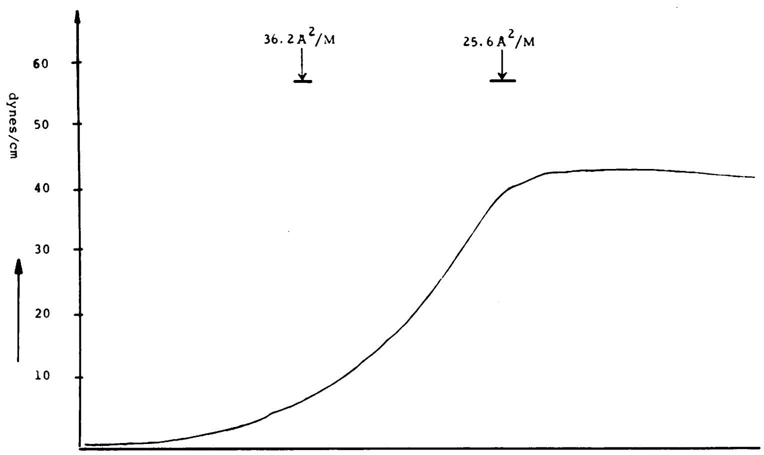

FIG. 2. *π-A isotherm of 12-methyltridecanoic acid at 21 C at a $CaCl_2$ concentration of 0.1% in the substrate (pH = 6.5).*

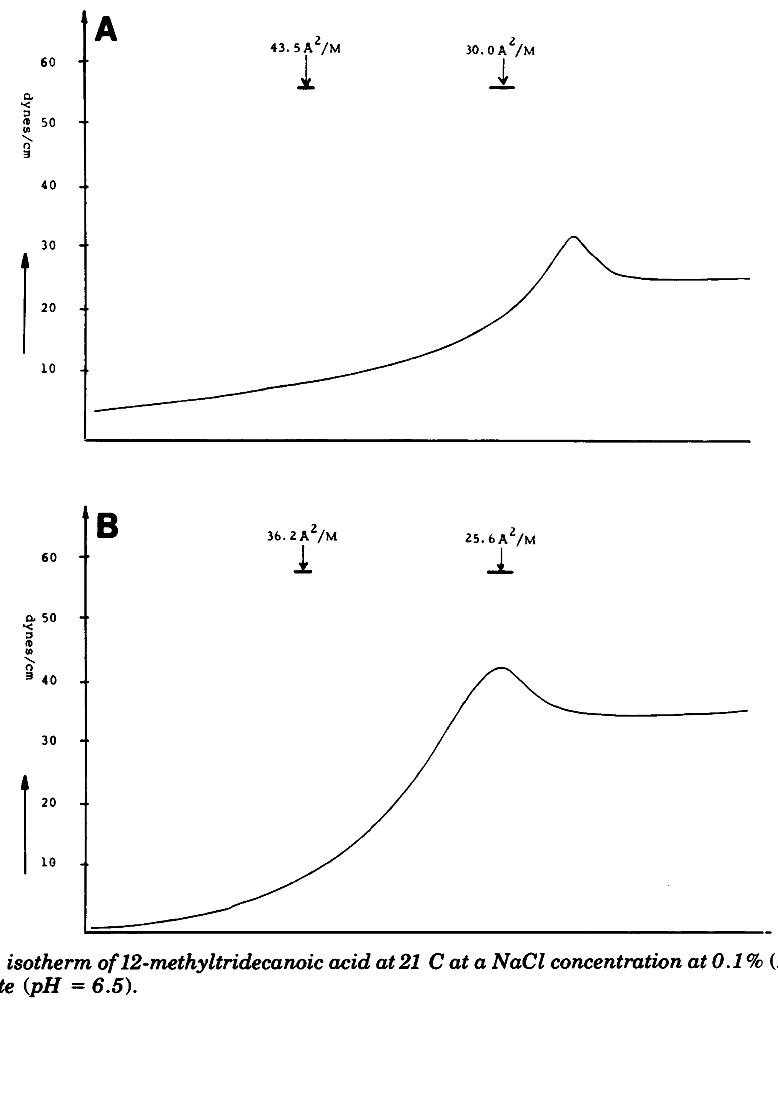

FIG. 3. *π-A isotherm of 12-methyltridecanoic acid at 21 C at a NaCl concentration at 0.1% (A) and 2% (B) in the substrate (pH = 6.5).*

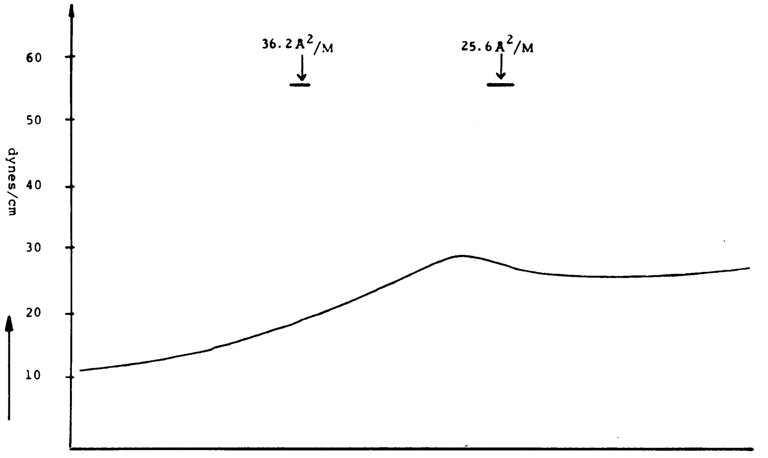

FIG. 4. *π-A isotherm of 12-methyltridecanoic acid at 20 C at pH = 8 in the substrate.*

nism. It was felt that the surface film behavior would provide information on this problem.

The presence of small amounts of Ca^{2+} has a drastic effect on the surface film properties of the *iso*-fatty acid (Fig. 2). The liquid-condensed phase persists to a much higher pressure when Ca^{2+} is present, and at a molecular cross-section area of 25.4 Å²/molecule there is a transition to a solid-condensed phase. Furthermore, there is no reduction in pressure after the collapse point at 22.1 Å/molecule, showing that the condensed monolayer phase is stable. This behavior is in agreement with the reduction in inhibition shown by this lipid (Fig. 1). It should be pointed out, however, that the general stabilizing effect of Ca^{2+} on membranes should give an effect in the same direction.

The effect of Na^+ is demonstrated in Fig. 3. No difference between the effect of K^+ and Na^+ was seen. With increasing salt concentrations the collapse pressure of the liquid-condensed phase increases, as well as its compressibility. At the lowest concentration the collapse starts at about 26 Å²/molecule, whereas it increases to about 27 A²/molecule at a concentration of 2% in the substrate. The reduction in pressure after collapse is about the same. The strong enhancement of the antimicrobial activity when these ions are present therefore can hardly be explained by an increase in the expanding effect of this lipid. It seems more probable that an osmotic diffusion of these ions by increased membrane permeability due to the lipid component is the result of their strong enhancement of the biological activity.

The effect of pH is a more complex problem. Increased dissociation should be expected to give an expansion of a lipid bilayer in which the fatty acid is incorporated, due to electrostatic repulsion. This is quite pronounced when the molecules are closely packed, as in a condensed monolayer phase of the acid, which is illustrated by the isotherm given in Fig. 4. On the other hand, the solubilization of the fatty acid in the lipid layer of the membrane might be expected to be reduced with increased dissociation as the water solubility increases.

It is believed that the enhancement of the antimicrobial effect of *iso*-branched fatty acid and related simple lipids (cf. references 1, 2) by the addition of small amounts of univalent ions can be utilized in antimicrobial preparations for applications in food preservation and in topical ointments.

LITERATURE CITED

1. Larsson, K., B. Norén, and G. Odham. 1972. Antimicrobial effect of simple lipids and its relation to surface film behavior. Biochem. Pharmacol. 21:947–962.
2. Larsson, K., B. Norén, and G. Odham. 1975. Antimicrobial effect of simple lipids with different branches at the methyl end group. Antimicrob. Agents Chemother. 8:742–750.