
Note

Relationship Between Bactericidal Activity and the Hydrophobicity – Hydrophilicity Balance of Alcohol Solutions

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Alcohols showed bactericidal activity against Staphylococcus aureus, Micrococcus luteus, Escherichia coli, and Enterobacter cloacae suspended in 0.85% (v/v) saline in the order of n-butanol > n-propanol > ethanol > methanol. Against the bacteria suspended in n-octane, however, alcohols showed a reverse order of bactericidal effectiveness. When toluene as a hydrophobic solvent was mixed with a small amount of hydrophilic methanol (5 or 10%, v/v), or conversely, when hydrophilic methanol was mixed with a small amount of hydrophobic n-hexane (10 or 30%, v/v), the bactericidal activity of the mixture increased markedly. The pattern of the relative glucose-Sudan III solubility curve was similar to that of the bactericidal activity curve of ethanol solutions. The results obtained suggest that the bactericidal activity of an alcohol was not dependent on the logarithm of the partition coefficient of the alcohol but on the hydrophobicity-hydrophilicity balance of the solution.

Key words: Alcohol/Bactericidal activity/Hydrophobicity/Hydrophilicity.

Many studies on the toxic effects of ethanol at relatively low concentrations (below 20%, v/v) on microorganisms have been reported to date. Growing cells are more sensitive to the lethal effect of ethanol than nongrowing cells (Casey and Ingledew, 1986). The effects of ethanol on the growth, fermentation, and cell death of microorganisms become more pronounced at higher temperatures (Casey and Ingledew, 1986; Ingram and Buttke, 1984; Jones, 1989; Van Uden, 1989). Ethanol causes leakage of cellular constituents (Leao and Van Uden, 1982 and 1984) and inhibits the uptake of glucose and other nutrients (Van Uden, 1989). Price (1939 and 1950) and Shapero et al. (1978) have reported that the bactericidal activity of ethanol is the highest at a concentration range of 60–70% (v/v). Lockemann et al. (1941) have reported the bactericidal activity of methanol, ethanol, propanol and isopropanol. However, they have not mentioned the bactericidal mechanism of ethanol.

We have reported that Escherichia coli phages are more tolerant to alcohol than bacteria (Yamashita et al., 2000). We further found that the inactivation activity of ethanol on λ phages was the highest at a concentration range of 60–70% (v/v) and that the activity of the ethanol treatment in this range was not affected by temperature. The hydrophobicity-hydrophilicity balance of the alcohol solution also affected the phage-inactivation activity. In this study, we examined whether these phenomena could also be observed with bacterial cells.

The following bacteria were used: Staphylococcus aureus ATCC 12600, Micrococcus luteus ATCC 4698, E. coli W3110 and Enterobacter cloacae ATCC 13047. The bacteria were cultured to the stationary phase in LB broth containing 10g polypeptone, 5g yeast extract and 5g NaCl per l at pH7.0. For the bactericidal activity test, bacteria (10^7 cfu/ml) were suspended in 20ml of 0.1 M phosphate buffer plus 0.85% (w/v)
saline solution (pH7) with or without alcohol or toluene, n-hexane and n-octane. The bacterial suspension was incubated with shaking at 20 or 25°C. After 10 min, samples (1 ml) were taken out, diluted and plated on the LB agar (1.5%, w/v) medium. After cultivation at 30°C for 5 days, resultant colonies were counted and viability was determined. The log $P_{ow}$ of ethanol, n-propanol, n-butanol, toluene, n-hexane and n-octane were cited from Inoue and Horikoshi (1991) and the log $P_{ow}$ of methanol was calculated with the method of Leao, Hanisch and Eikins (1971).

Regarding the bactericidal activity of short alkyl chain alcohols, having a carbon number of one to four, against S. aureus suspended in saline, it was observed that the higher the number of carbons in an alcohol, the stronger its bactericidal activity: n-butanol > n-propanol > ethanol > methanol (Fig. 1). This order of alcohols according to their bactericidal activity was also obtained with E. coli, M. luteus and E. cloacae (data not shown). n-Butanol had the highest bactericidal activity among the short-chain alcohols (C1-C4), although its phage-inactivation activity was the weakest (Yamashita et al., 2000). E. coli was more sensitive to alcohol than S. aureus. All of the bacteria examined were killed within 10 min by 50-70% (v/v) ethanol solution at 20°C. The higher the temperature for ethanol treatment, the higher the bactericidal activity of ethanol against S. aureus and E. coli (data not shown). However, the bactericidal activity of ethanol was at the maximum at a concentration of 70% (v/v) irrespective of the treatment temperature (Fig. 2) as has been previously described (Lockmann et al., 1941; Price, 1939 and 1950; Shapero, et al., 1978). From these data, we suppose that the hydrophilicity-hydrophobicity balance of an alcohol solution is an important factor and influences the bactericidal action of the alcohol. The relative solubility curve of glucose plus Sudan III in ethanol was similar to the bactericidal activity curve of ethanol solutions against S. aureus (Fig. 2) and E. coli (data not shown), as observed with E. coli λ phages treated with ethanol (Yamashita et al., 2000).

When 5 or 10% (v/v) of methanol ($P_{ow}$, -0.4) was added to a hydrophobic solvent toluene ($P_{ow}$, 2.7), the bactericidal activity of the mixture against S. aureus increased markedly (Fig. 3), whereas 5 or
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**FIG. 4.** Effect of n-hexane on the survival of E. coli W3110 in methanol-saline mixture. The bacterial cells were incubated for 10 min at 25°C with methanol-saline mixture (●), methanol-saline mixture plus 10% (v/v) n-hexane (▲) or methanol-saline mixture plus 30% (v/v) n-hexane (■), n-hexane-saline mixture (○).

10% (v/v) methanol by itself demonstrated no activity. When a hydrophilic methanol (log $P_{ow}$, -0.4) solution was mixed with 10 or 30% (v/v) n-hexane (log $P_{ow}$, 3.9), the bactericidal activity of the mixture against E. coli increased markedly (Fig. 4). 10 or 30% (v/v) n-hexane by itself demonstrated no activity. n-Octane (log $P_{ow}$, 4.9) demonstrated no bactericidal activity against S. aureus and E. cloacae. n-Hexane (log $P_{ow}$, 3.9) also had no bactericidal activity against S. aureus but demonstrated slight bactericidal activity against E. coloacae. However, n-

**FIG. 5.** Effects of n-hexane, n-octane, n-hexanol and n-octanol on the survival of S. aureus ATCC 12600. The bacterial cells were treated for 10 min at 25°C with n-octane-saline mixture (▲), n-hexane-saline mixture (○), n-octanol-saline mixture (■), or n-hexanol-saline mixture (●).

n-hexanol (log $P_{ow}$, 2.5) and n-octanol (log $P_{ow}$, 3.3) exhibited higher bactericidal activity against S. aureus (Fig. 5) and E. coloacae (data not shown). n-Hexane and n-octane possess no hydroxyl groups, while n-hexanol and n-octanol do. When bacterial cells were treated with n-octane alcohol-mixture, the bactericidal activity increased markedly (Fig. 6). Mixed with n-octane, alcohols at concentrations lower than 40% showed bactericidal activity in the order of methanol > ethanol > n-propanol > n-butanol (Fig. 6). This order of alcohols based on their bactericidal activity when mixed with n-octane corresponds to the hydrophilicity of alcohol and varies inversely with the order of that when bacteria were suspended in 0.85% (v/v) saline (Fig. 1). However, at concentrations higher than 40%, alcohols mixed with n-octane showed an inverse order of bactericidal activity to that of alcohols at concentrations lower than 40% (v/v) (Fig. 6). The bactericidal activity of alcohol at concentrations lower than the maximum was proportional to the log $P_{ow}$ of the alcohol (Fig. 1) with log $P_{ow}$ values as follows: methanol - 0.4, ethanol 0.1, n-propanol 0.8 and n-butanol 2.5. The order of alcohols to their bactericidal effectiveness at concentrations lower or higher than the maximum was inversely proportional to the order of that when bacteria were suspended in 0.85% (v/v) saline (Fig. 1).

**FIG. 6.** Effect of alcohols on the survival of S. aureus ATCC 12600 in n-octane-saline mixture. The bacterial cells were treated for 10 min at 20°C with methanol (▲), ethanol (●), n-propanol (■), n-butanol (○), or n-octane-saline mixture (○).
The degree of growth inhibition which organic solvents exhibit on bacteria has been indicated to correlate with the log $P_{ow}$ of solvents (Aono et al., 1994; Inoue and Horikoshi, 1991). Toxic solvents including toluene are believed to break down the microbial membrane (De Smet et al., 1978; Jackson and De Moss, 1965). Phospholipid liposomes are swollen by the intercalation of various organic solvent molecules (Sikkema et al., 1994). Although, it was seen that the higher the carbon number of alcohol (larger log $P_{ow}$), the higher the bactericidal activity (Figs. 1 and 5), when cells were treated with a solvent, the lower the log $P_{ow}$, the greater the toxicity was to microorganisms (Aono et al., 1994; Inoue and Horikoshi, 1991).

If the bactericidal activity of an alcohol was proportional to the log $P_{ow}$, the bactericidal curve of the alcohol solution must be linear. However, the bactericidal activity curve of alcohol solutions exhibited a U shape with an optimum concentration (Figs. 2 and 6; Lockmann, 1941; Price, 1939 and 1950). The mixtures of alcohol and solvent exhibited higher bactericidal activity than the alcohol or solvent alone (Figs. 3, 4 and 6). It is considered that solvents are too hydrophobic and short-chain alcohols are too hydrophilic to demonstrate bactericidal activity. Glycerol and diols showed weak bactericidal activity by their hydrophilicity (Yamashita, 2001). Bull and Breese (1978) reported that the alcohol denaturation of egg albumin appears to have a large temperature coefficient, that the rate of denaturation depended critically on the concentration of alcohol, that the protein was dehydrated by the alcohol, and that the protein denaturants increased greatly as the length of the carbon chain of alcohol increases. Mitaku et al. (1988) reported that organic solvents without any hydrogen-bonding groups do not destroy the structure of bacteriorhodopsin but that solvents which contain a hydrogen-bonding group as well as hydrocarbon chains exhibit a significant degree of denaturation, that solvent molecules attach the hydrogen bonds between helices and cause denaturation cooperatively, that glycerol and diol did not cause the denaturation of bacteriorhodopsin, and these solvents are so hydrophilic that they do not penetrate into bacteriorhodopsin. In this study, we have found that the relative solubility of glucose and sudan III correlate with the bactericidal activity of the alcohol solution, that n-hexane, n-octane did not have a bactericidal activity but n-hexanol and n-octanol had a larger bactericidal activity, and that the larger the carbon number of alcohol, the smaller the maximum bactericidal alcohol concentration, and that the bactericidal activity of alcohol appears to have a large temperature coefficient. These features of the bactericidal activity by alcohols and solvents are very similar to those associated with the denaturation of bacteriorhodopsin and egg albumin by an alcohol and solvent. From these data, it is suggested therefore that the hydrophilicity-hydrophobicity balance of an alcohol and solvent solution is the primary factor for the bactericidal activity, and that the action of alcohol on a bacterial cell targets which exhibit both hydrophilic and hydrophobic characteristics such as protein.

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REFERENCES


