Note

Effects of Hydrodynamic Volume of Anionic Lipopolysaccharide, Emulsan, on Emulsifying Activity

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To understand the structure-function relationship of an anionic lipopolysaccharide emulsan, the effect of hydrodynamic volume on the emulsifying activity was investigated. As a result, it was found that the hydrodynamic volume of emulsan was an important factor in its emulsifying activity. The hydrodynamic volume was decreased by the addition of a positively charged polypeptide, and the emulsifying activity was decreased, but negatively charged or uncharged polypeptide had little effect. These results suggest that the conformation of the backbone of emulsan helps to govern its emulsifying activity.

Key words: emulsan; biosurfactant; lipopolysaccharide; conformation; emulsifying activity

Emulsan is a polymeric biosurfactant produced by *Acinetobacter calcoaceticus* RAG-1 with a mean molecular weight (M_r) of 1,000,000. Emulsan has a carbohydrate backbone as its hydrophilic moiety and fatty acids as hydrophobic moieties attached to the backbone *via* ester or amide bonds.¹⁾ Emulsan is an emulsion stabilizer rather than a reducer of interfacial tension.²⁾ Once an emulsion is formed, it is stable and has high affinity for oil-water interfaces, so only a low concentration is needed for emulsification.³⁾ Because of these properties, emulsan can be used commercially for emulsion stabilization and transportation of heavy oils.⁴⁾ The compound has the disadvantage that its composition and properties vary with the culture conditions of the bacterium.⁵⁾ Because of this, factors that affect its emulsifying activity are of interest. As one such factor, the hydrodynamic volume of emulsan was examined here. This volume was changed by the addition of charged polypeptides and the relation between hydrodynamic volume and emulsifying activity was investigated.

Emulsan was purchased from Emulsan Biotechnologies Inc. (Green Farms, USA), and was purified further by a hot-phenol extraction method for removal of contaminating protein.^{6,7)} The protein was measured with a protein assay kit (BCA-1, Sigma). The emulsifying activity of emulsan was measured by the method of Zuckerberg *et al.*⁸⁾ In a 125-ml Erlenmeyer flask, 7.5 ml of TM buffer (20 mM Tris-HCl, pH 7.2, and 10 mM MgSO₄), 0.1 ml of test oil (hexadecane:2-methylnaphthalene, 1:1), and 1 mg of emulsifier were mixed. After reciprocal shaking (150 strokes/min, 30°C) for 1

h, the optical density at 600 nm was measured. One unit of optical density was defined as one unit of emulsifying activity. The hydrodynamic volume of the emulsan was measured with a capillary viscometer (size 75, Cannon Instruments, State College, PA) in a 25°C water bath. The emulsan concentrations used were in the range of 0.1 to 0.5 g/l.

The protein concentration, emulsifying activity, and hydrodynamic volume of the purchased emulsan before purification were 11.6%, 1.81 units/mg, and 2.42 dl/g, respectively. Four steps of extraction decreased the protein concentration to 0.8%. The purified emulsan with 0.8% protein was used in this study. To change the hydrodynamic volume of the emulsan, polypeptides of different charges were added to give a uniform protein concentration of 11.6% together with the commercial emulsan. Mixtures of emulsan and a polypeptide were incubated for 10 min in TM buffer (pH 7.2), after which the emulsifying activity and hydrodynamic volume were measured. When positively charged poly-DL-lysine (Sigma; M_r , 47,900) was present, the emulsifying activity decreased to 0.41 units/mg compared with 1.58 units/mg of the control after purification (Fig. 1). The activity changed little with negatively charged poly-L-glutamic acid (Sigma; M_r , 36,200) or uncharged poly-L-phenylalanine (Sigma; M_r , 16,800). The hydrodynamic volume decreased from 2.14 dl/g to 1.04 dl/g only when the positively charged polypeptide was added. The backbone of the negatively charged emulsan probably was removed from ionic interaction by the positively charged polylysine. To check this idea, the emulsifying activities of emulsan with and without polylysine were measured at various pHs (Fig. 2). The samples were left for 10 min in a buffer at pH 3 to pH 9.5 before the measurement. With added polylysine, the protein concentration was again 11.6%. The emulsifying activity was little changed by the pH when polylysine was not added, but with added polylysine, it changed considerably. In the neutral range of pH, both the polylysine and emulsan would be charged; if so, ionic binding would occur and both hydrodynamic volume and emulsifying activity would decrease. At acidic or alkaline pH, however, either emulsan or polylysine probably became uncharged, so the emulsifying activity would be found. We concluded that the hydrodynamic volume decreased when polylysine was added because of ionic interac-

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Fig. 1. Effects of Polypeptides on the Emulsifying Activity and Hydrodynamic Volume of Emulsan.

Control, emulsan after purification without polypeptide. The final protein concentration of polylysine, polyglutamate, and polyphenylalanine was 11.6%. Emulsifying activity was measured at pH 7.2. The columns show means and the bars show standard deviation.

Fig. 2. Effects of pH on the Emulsifying Activity of Emulsan with and without Polylysine.

Emulsan without polylysine, ○; emulsan with polylysine concentration of 11.6%, ●. Polylysine was added to give a protein concentration of 11.6%. Samples were incubated at a certain pH for 10 min before the emulsifying activity was measured. Means $\pm$ standard deviation.

tions.

To investigate in more detail the effects of the positively charged polypeptide on the emulsan, polylysine was added at various concentrations with the mixture at pH 7.2. The emulsifying activity was lower with more polylysine, and was close to zero at 23.1% or higher (Fig. 3). The hydrodynamic volume of emulsan decreased only slightly with polylysine of up to 9.1%, but decreased greatly with 11.6% or more. When little polylysine is

Fig. 3. Effects of the Polylysine Concentration on the Emulsifying Activity and Hydrodynamic Volume of Emulsan.

Control, emulsan after purification without polylysine. The emulsifying activity was measured at pH 7.2.

bound to the emulsan, a conformational change other than shrinkage may occur interfering with emulsification.

In conclusion, the change in the hydrodynamic volume of emulsan when polylysine is added, affected the emulsifying activity although the relation was not linear. This finding suggests that the conformation of the lipopolysaccharide backbone is an important factor in the emulsifying activity of emulsan, which is anionic. The measurement of hydrodynamic volume cannot directly show changes in conformation, so direct evidence is needed for a firm conclusion.

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