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Orientation of clay particles sorbed on bacteria possessing different ionogenic surfaces

Previous studies in this laboratory have shown that cells of *Rhizobium* species with exclusively acidic (carboxyl) surface ionogenic groups sorb approximately twice as much sodium montmorillonite or sodium illite as cells of strains with more complex carboxyl-amino surface groups^{1,2}. It was suggested that this difference might reflect differences in the orientation of the clay particles on the cell surfaces, resulting from the fact that the broad face of clay particles is negatively charged while the edges of the particles may be positively charged³. LAHAV⁴ treated montmorillonite with $(\text{NaPO}_3)_6$ to block the positively charged groups on the particle edges in an attempt to assess the nature of the orientation of clay particles sorbed to *Bacillus subtilis*. In the present study, more precise information on the mechanism of sorption of clays on bacterial cells has been obtained by examining the effects of $(\text{NaPO}_3)_6$ -treated clays on a range of bacterial strains with different surface ionogenic properties.

Concentrated suspensions of sodium montmorillonite and sodium illite were treated with dilute $(\text{NaPO}_3)_6$ solution prior to dilution of the clay suspensions for use in electrophoretic studies. These clays are referred to subsequently as $(\text{NaPO}_3)_6$ -montmorillonite and $(\text{NaPO}_3)_6$ -illite. Using the microelectrophoretic techniques detailed previously^{1,5}, the electrophoretic mobilities of a number of strains of root-nodule bacteria were determined in the presence and absence of normal or $(\text{NaPO}_3)_6$ -treated clays in NaCl ($I = 0.05$). The cultures employed were: (a) Carboxyl type cells: *Rhizobium lupini* strains UT2 and UT12, *Rhizobium japonicum* QA372 and *Rhizobium trifolii* TA1; (b) Carboxyl-amino type cells: *Rhizobium trifolii* strains SU297A, SU297B and SU298D, and *Rhizobium leguminosarum* TA101. The results obtained using a range of clay concentrations are presented in Fig. 1.

All cultures responded uniformly to the untreated sodium montmorillonite, the electrophoretic mobility of the cells increasing with increasing clay concentration to a point where the mobility resembled that of sodium montmorillonite itself. This effect has been reported previously^{1,2}, when it was suggested that sorbed clay particles completely enveloped the bacterial surface. Although the $(\text{NaPO}_3)_6$ -montmorillonite exhibited a mobility similar to that of the untreated clay (Fig. 1), the response of cells to the presence of this clay depended on the surface charge characteristics of the different bacteria. Cells with carboxyl type surfaces showed no increase in mobility in the presence of $(\text{NaPO}_3)_6$ -illite, indicating a lack of sorption of the clay to the bacterial surface. The slight increase in mobility of cells with carboxyl-amino type surfaces in the presence of the $(\text{NaPO}_3)_6$ -montmorillonite suggests a limited degree of sorption of the clay to the bacterial surface.

Bacteria with characteristically low electrophoretic mobilities showed an increase in mobility with increasing sodium illite concentration (Fig. 1). On the other hand, bacteria with characteristically high mobilities exhibited a decrease in mobility with increasing concentrations of $(\text{NaPO}_3)_6$ -illite. These results with a clay of relatively low electrophoretic mobility emphasise the point that the mobility of cells observed at high clay concentrations is actually that of the clay envelope around the cells². As in the case of $(\text{NaPO}_3)_6$ -montmorillonite, no increase in the mobility of carboxyl

type cells was observed in the presence of $(\text{NaPO}_3)_6$ -illite. A slight, but definite, change in mobility of carboxyl-amino type cells was observed with increasing $(\text{NaPO}_3)_6$ -illite concentration. This change was expressed as an increase in mobility of *R. trifolii* SU297B and *R. leguminosarum* TA101, and as a decrease in mobility of *R. trifolii* SU298D.

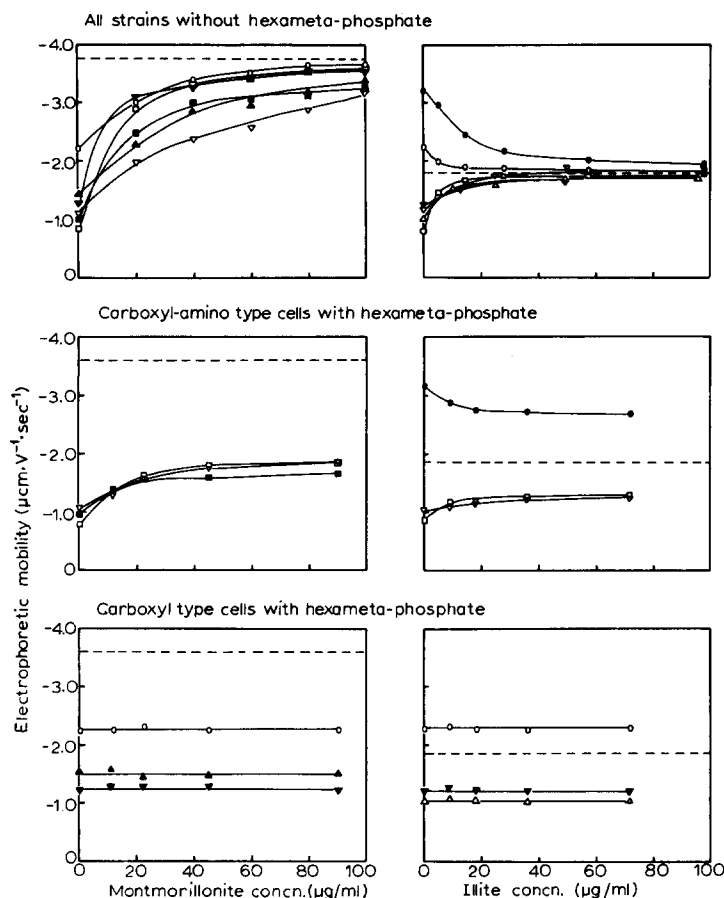


Fig. 1. Effects of increasing concentrations of untreated and $(\text{NaPO}_3)_6$ -treated montmorillonite or illite on the electrophoretic mobility of different strains of root-nodule bacteria. ■, *R. trifolii* SU297A; □, *R. trifolii* SU297B; ●, *R. trifolii* SU298D; ▽, *R. leguminosarum* TA101; ▼, *R. japonicum* QA372; ▲, *R. lupini* UT2; △, *R. lupini* UT12; ○, *R. trifolii* TA1. -----, electrophoretic mobility of appropriate untreated or $(\text{NaPO}_3)_6$ -treated clay.

The results obtained with both $(\text{NaPO}_3)_6$ -montmorillonite and $(\text{NaPO}_3)_6$ -illite indicated that these clays are not sorbed on to cells with carboxyl type ionogenic surfaces. This supports the suggestion made previously^{1,2} that the orientation of clay particles at such cell surfaces is entirely edge-to-face. That is, the positively charged edges of the clay particles are attracted to the negatively charged groups on the bacterial surface. This edge-to-face orientation is prevented by $(\text{NaPO}_3)_6$ blocking the positive groups at the edge of the clay particles.

With carboxyl-amino type cells, the mobility studies indicated a limited

amount of sorption of the $(\text{NaPO}_3)_6$ -treated clays. The actual amounts of either untreated or $(\text{NaPO}_3)_6$ -treated montmorillonite sorbed per cell for *R. trifolii* SU297B and *R. leguminosarum* TA101 were calculated by the method of NEVO *et al.*⁶. The sorption isotherms presented in Fig. 2 show that only about 0.6 pg of $(\text{NaPO}_3)_6$ -montmorillonite per cell saturates the available surface on cells of both bacterial strains. This level is attained at equilibrium concentrations of the $(\text{NaPO}_3)_6$ -clay of about 10 $\mu\text{g}/\text{ml}$, while saturation of the cells with untreated montmorillonite¹ was

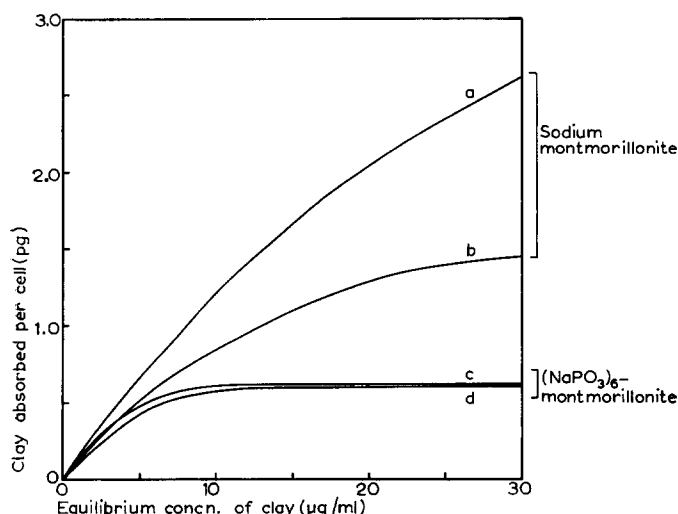


Fig. 2. Sorption isotherms of (a) montmorillonite on *R. leguminosarum* TA101; (b) montmorillonite on *R. trifolii* SU297B; (c) $(\text{NaPO}_3)_6$ -montmorillonite on *R. trifolii* SU297B; (d) $(\text{NaPO}_3)_6$ -montmorillonite on *R. leguminosarum* TA101.

achieved only with equilibrium concentrations in excess of 30 $\mu\text{g}/\text{ml}$. Since the positive charges on the edges of the clay particles are blocked by the $(\text{NaPO}_3)_6$, these results suggest a degree of face-to-face association between the negatively charged face of the clay particles and the positively charged amino groups on the surfaces of these cells.

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