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CELL SURFACE ENERGY, CONTACT ANGLES AND PHASE PARTITION

II. BACTERIAL CELLS IN BIPHASIC AQUEOUS MIXTURES

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Summary

Partition coefficients in biphasic mixtures of poly(ethylene glycol) and Dextran are compared to cell surface energies obtained from contact angles of each liquid phase on cell layers. Linear relationships are observed between these two independent measurements for a variety of bacterial cells. The results demonstrate the importance of interfacial phenomena and contact angles in the phase-partition process.

Introduction

Physical phenomena involving the cell surface may be characterized as being dominated either by surface energy or ionic effects. Non-ionic van der Waals' forces are collectively known as the surface energy, and changes in the surface free energy regulate intimate physical contacts at the cell surface such as are involved in phagocytosis, the attachment of cells to non-ionic surfaces such as air/water or air/hydrocarbon interfaces [1—4], or to the surfaces of certain cells and tissues [5]. Ionic and electrostratic forces at the cell surface regulate long-distance interactions between cells and other charged surfaces, and have been found to be of greatest importance to the maintenance of cell dispersal in the blood and other fluids, and to the adhesion of cells to highly charged surfaces. An excellent discussion of ionic interactions is provided by Sherbet [6].

Cell surface hydrophobicity is a qualitative term which tends to describe the characteristics of the cell which can be measured as the cell surface energy. Two independent methods of measuring cell surface energy have been related

in the previous paper of this series [1] and are: (1) the measurement of contact angles of drops on the surface of a layer of cells and (2) the measurement of partition coefficients between immiscible liquid phases. The fundamental relationship investigated in that paper is that between the difference in surface energies and the free energy of partition (Eqn. 1.):

$$-\log K_{eq} = \alpha\Delta\gamma + \beta \quad (1)$$

where $\Delta\gamma$ = the difference in interfacial energy between the cells and the indicated phases ($\gamma_{2c} - \gamma_{1c}$), K_{eq} = equilibrium partition coefficient of the cells between phases 1 and 2 ($[\text{cells}]_2/[\text{cells}]_1$), and α and β = empirical constants.

In the previous paper, the difference between the interfacial energies at the cell surface in the two phases, $\Delta\gamma$, was measured directly by placing drops of one liquid phase on layers of cells immersed in the second liquid phase. This is the most direct measurement of this difference which is possible. However, such a situation is not always experimentally feasible, and it is useful to compare contact angles taken with each liquid phase on the cells with reference to another bulk phase. Use of air as the bulk phase has been advocated by van Oss et al. [3]. This method has the advantage of simplicity and ease, but, certainly, cell surfaces are altered by drying, and the measurements are hampered by evaporation from the drop being measured. In addition, use of air or any other medium against which γ_{12} is high reduces the sensitivity of the measurement. The results obtained by van Oss et al. [3] were, nevertheless, predictive of the phagocytosis of bacteria by neutrophils, and we have used their methods of measurement and analysis to study the partition of bacterial cells between immiscible aqueous phases.

Methods

Surface measurements. Contact angles were measured by the projection technique [1], and the values given are the averages of the angles made by the edges of 10 or more drops on a lawn of bacterial cells. Cells were collected on a 0.45 μm pore diameter Millipore filter to a density of 10^6—10^7 cells/mm^2 and allowed to dry in air until the surface glossiness was reduced to a dull matt appearance. The filters were still damp at this time. The filter was then cut in half to provide a straight edge, placed on a microscope slide and placed in the light beam of the contact-angle apparatus. Drops of the appropriate sensing liquids were placed on the cell layer and contact angles were measured immediately to minimize the effects of evaporation. Surface tension was measured with a Fisher Autotensiomat, a du Nuoy surface tensiometer equipped with a strain transducer. Surface tension was measured either with a 6 cm platinum ring or with strips of hard-surfaced, ashless filter paper, following the recommendations of Gaines [7].

These measurements were then used to compute values for the cell/air and cell/liquid interfacial energies using the methods and computer program of Neumann et al. [8]. Cell/liquid interfacial energies from each of the two liquid phases used for partitioning were used to calculate $\Delta\gamma$.

Cells and other methods. Corynebacterium lepus, a hydrocarbonoclastic microbe, was grown on a kerosene consisting primarily of C-14 isomers accord-

ing to the methods outlined earlier [9,10]. *Saccharomycopsis lipolytica* (B121 × 74 c, diploid), a gift of Dr. R.C. von Borstel, was grown in the following medium: 0.06% (w/v) yeast extract (Difco), 0.01% peptone and 0.2% glucose. *Chlorella vulgaris* was grown in air-lift columns according to the method of Pirt and Wallach [11]. *Micrococcus luteus*, and *Staphylococcus epidermidis* (biotype 3) were grown in the following mineral salt medium plus 1% peptone and 1% nutrient broth: $NaNO_3$ (0.2%), K_2HPO_4 (0.1%), KH_2PO_4 (0.05%), KCl (0.01%), $MgSO_4$ (0.05%), $CaCl_2$ (0.001%), $FeSO_4$ (0.001%) and EDTA (0.00015%). Glucose broth of the following composition was used for the cultivation of *Serratia marscescens*: K_2HPO_4 (0.7%), glucose (0.5%) and $MgSO_4$ (0.01%). All bacteria were grown in 500-ml shake-flasks at room temperature.

Partitioning experiments were performed by thoroughly mixing a sample of cells with the relevant two-phase mixture of Dextran and poly(ethylene glycol), made in distilled water, and allowing the mixture to separate spontaneously overnight in the cold. The number of cells in each phase was determined with a Coulter Counter Model TA-II. In some of the studies, partition was performed under sterile conditions, and samples from each phase were diluted, plated on agar, and colonies were counted with a Fisher Count-All.

A comparison was made of $\Delta\gamma$ and K_{eq} for several different bacterial species in two different biphasic systems. Using the phase system consiting of 6% Dextran (M_r 500 000) and 6% poly(ethylene glycol) (M_r 20 000), $\Delta\gamma$ and K_{eq} were determined for four different bacterial species and one algal species (Fig. 1). Three species were compared in the system composed of 10% Dextran (M_r 100 000) and 10% poly(ethylene glycol) (M_r 20 000). In both cases, the data were normalized for cell surface area, since this varies so greatly between the different species. This was done using the mean values obtained by the Coulter Counter model TA-II. There is a linear relationship between $\Delta\gamma$ and K_{eq} for each biphasic system. The slopes of the lines are quite similar, but the intercepts are not, possibly indicating a difference in the electrostatic contribution to partition in these biphasic systems [1]. Thus, we can see that for a given

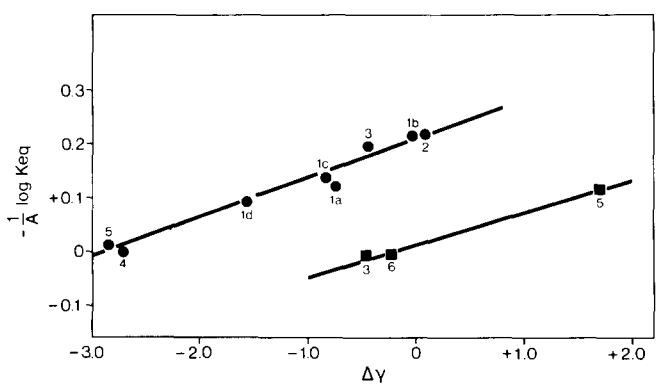

Fig. 1. The relationship between $-(\log K_{eq})/A$ and $\Delta\gamma$ for various cell types: (1) *C. Lepus*, a = day 1, b = day 2, c = day 3 and d = day 4 of culture; (2) *M. luteus*; (3) *S. epidermidis*; (4) *S. lipolytica*; (5) *S. marscescens*; and (6) *C. vulgaris*. Two biphasic mixtures were used: 6% poly(ethylene glycol) (M_r 20 000) and 6% Dextran (M_r 500 000) (●), and 10% poly(ethylene glycol) (M_r 20 000) and 10% Dextran (M_r 100 000) (■).

biphasic system, there is a relatively constant relationship between $\Delta\gamma$ and K_{eq}, and that in well characterized situations it should be possible to obtain one of these parameters from the other.

In the biphasic systems containing 6% Dextran and 6% poly(ethylene glycol), the more negative $\Delta\gamma$, the greater the hydrophobicity of the cell surface. Thus, the order of cell surface hydrophobicities in the species studied is: *S. marscescens* $\simeq$ *S. lypolytica* > *S. epidermidis* > *M. luteus*. *C. lepus* poses a special problem, since its surface hydrophobicity changes continuously throughout its growth cycle due to the combined effects of its liquid hydrocarbon growth substrate and the production of copious quantities of surface-active material [2,9,10]. Under the growth conditions used here, *C. lepus* had an intermediate hydrophobicity after 1 day of growth (Fig. 1, 1a), became more hydrophilic as the culture entered the exponential phase (1b), and then the cells became progressively more hydrophobic (1c, 1d). This pattern is consistent with the partition of the bacterium between the hydrocarbon and aqueous phase of its growth medium which was observed earlier [9]. In those studies, the inoculum adhered to the oil/water interface for a short period following inoculation, then separated completely into the aqueous phase. Later, the bacteria partitioned into the hydrocarbon phase.

In conclusion, the results presented here offer further support to the relationship which was developed earlier [1] between (a) the equilibrium partition coefficient of a particle between two immiscible liquid phases and (b) the difference between the interfacial energies of the particle with each phase.

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