AN ELECTROCHEMICAL APPROACH TO ORGANIC AGGREGATION

V. Zutic, N. Ivosevic*, S. Kovac and V. Svetlicic

Center for Marine and Environmental Research, Ruder Boskovic Institute, PO Box 1016, 10 001 Zagreb, Croatia

Abstract

At an electrode/seawater interface, surface-active organic molecules can be detected and characterized through their adsorption, while surface-active organic particles ($\geq 1\ \mu m$) can be simultaneously detected and characterized through their attachment. Frequency of electrical attachment signals reflects the abundance of particles. Amplitude and shape of each attachment signal depends upon the reactivity and interfacial area of attachment (size) of a single particle. For a population of particles, potential of appearance of attachment signals serves to estimate the critical interfacial tension of attachment and energy of adhesion. We investigate attachment signals of single cells, of exopolymeric particles and aggregates formed in bacterial cultures and compare them with signals of surface active particles that are abundant (up to 10^5/ml) in Northern Adriatic. The electrochemical approach, although *a priori* lacking molecular specificity, has an advantage over other recently developed techniques in directly measuring interfacial properties of single particles that are critical for the onset of aggregation phenomena.

Key-words: electrochemistry, bacteria, adsorption, Adriatic Sea

Introduction

The complex interfacial processes responsible for aggregation can be decoupled using a model fluid interface, mercury electrode/seawater, where the interfacial energy and charge density are controlled by applied potential. Fast dropping mercury electrode (DME), among other surface attributes such as hydrophobicity and variable surface charge, mimics interfacial dynamics of natural fluid interfaces.

Adsorption of organic molecules at DME is manifested as a regular suppression of current of polarographic maxima in the potential range of adsorption, while adhesion of fluid particles ($\geq 1\ \mu m$) yields pronounced current spikes - attachment signals. Average frequency of electrical attachment signals reflects the abundance of particles. Amplitude and shape of each attachment signal depends upon the reactivity and interfacial area of attachment (size) of a single particle. The critical potentials of appearance of attachment signals serve to estimate the energy of adhesion (1). We present typical attachment signals for single phytoplankton cells and for aggregated bacterial cells and compare them with signals of surface-active particles (SAP) that were identified in stratified Mediterranean estuaries (2-4) (up to 10^5/ml) and in the Northern Adriatic (5) that are closely related to transparent exopolymeric particles, identified more recently (6).

Experimental

Electrochemical measurements. The electrochemical technique used is chronoamperometry at the DME at potentials of streaming maximum of oxygen reduction (7-9). Laboratory experiments were performed in diluted seawater (1:5). The maximum contact time between a sample and mercury surface is 2 s. The potentials are referred to Ag/AgCl reference electrode.

Phytoplankton cells: naked microflagellate Isochrysis galbana was grown in seawater sterilized and enriched with F-2 nutrients in batch cultures. Cells were separated after 6 days of growth. Viability of cells was controlled in all stages of the experiment by microscopic observation of cell motility. Bacterial suspensions: marine bacteria isolated as attached (strains S3 and LHAT1) or free-living (strain BF2) in natural habitat (Scripps Pier 10,11) and filamentous bacteria *Saprospira grandis A* (12) were grown as batch monocultures. The cells were harvested after 3 days and separated from the growth medium. Marine snow: samples from Northern Adriatic were taken by scuba diver, at a depth of 16 m, in August 1994.

Results and discussion

The dropping mercury electrode is used here as a model interface to identify physico-chemical interactions in the interfacial process involving phytoplankton cells, marine bacteria and extracellular polymers. Amperometric curves were recorded at two characteristic potentials where the mercury surface is positively charged (E = -400 mV, $\sigma = +3.8\ \mu C/cm^2$), negatively charged (E = -800 mV, $\sigma = -6.5\ \mu C/cm^2$) and at E = -550 mV where the electrode is uncharged.

In Fig. 1 we compare attachment signals of North Adriatic surface-active aggregates contained in a marine snow sample with the signals of single phytplankton cells and aggregated bacteria. The model unicellular organism we used *(Isochrysis galbana)* is a marine nanoflagellate without cell wall. The cell size of 4-7 μm and flexibility of cell membrane are features of choice to obtain characteristic electrical signals for attachment of single cells. We selected yellow pigmented bacterium S3 related to *Cytophaga/Flavobacteria* (11) that are associated with particles and have surface dependent gliding motility.13 The cell dimensions were 1.4-4.0 μm in length and 0.4-0.6 μm in width. The cells appear mucoid in colonies, and in liquid medium they exist as a mixture of single cells and stable aggregates, up to 200 cells.

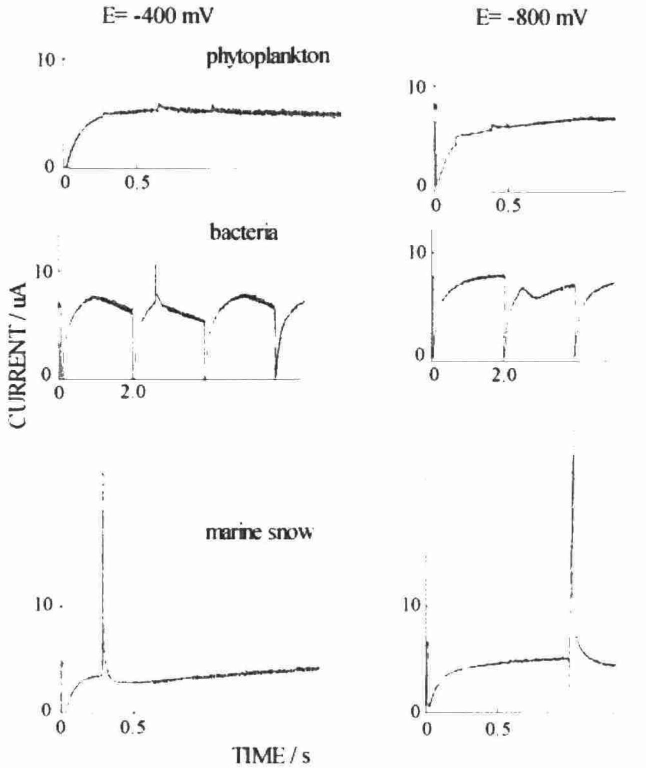

Fig. 1. Current-time curves of oxygen reduction in dispersion of phytoplankton cells, (*I. galbana*, 1.6×10^6/ml), aggregated bacterial cells (S3 strain, 5.4×10^8/ml) and samples of marine snow (N. Adriatic, 08/18/94, 16 m depth) recorded at potentials - 400m mV (positively charged electrode, $+3.8\ \mu C/cm^2$) and at - 800 mV (negatively charged electrode, $-6.5\ \mu C/cm^2$).

Surface-active particles (SAP) yield typical attachment signals that can be clearly distinguished from signals of single phytoplankton cells and aggregated bacteria. Signals of individual aggregates in the sample of marine snow recorded at two potentials (Fig. 1) show distinct features corresponding to a fast attachment and spreading (t~100ms). Note that the signal at -800 mV commences with a spike of the opposite sign corresponding to the displacement of the negative surface charge at the electrode/seawater interface by attachment and spreading of the aggregate. The surface charge displacement is direct evidence for the molecular contact between the aggregate and the mercury surface.

The effect of aggregation on the form of electrochemical attachment signals was studied in suspensions of bacteria that appear in different association state: single cells, single cells + clumps, and filaments.