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(U) Molecular Specificity of Adsorption of Biofilm Macromolecules and Microbial Biofouling on Artificial Surfaces in the Sea

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

An ELIZA method using immune chicken IgG coated plates does not appear to be specific for microbial adhesion enhancing macromolecules (MAEM). Hyperimmune mouse IgG made against MAEM(but not normal mouse IgG) reacts significantly with coated plates in the presence or absence of MAEM antigens. Assays yield titers of approximately 100,000 for the hyperimmune mouse sera over that of normal control. Hyperimmune mouse sera consistantly showed this property despite modificationsin the assay method. The very high titer of hyperimmune mouse serum suggests that immunization has produced an antibody(ies) against MAEM antigens, however, the assay could not discriminate specific from nonspecific reactions. Efforts will continue to focus on the problem, by examining the effects of D-galactose and other simple sugars on this binding. Elevated salt concentrations will be examined as a means of minimizing non-antigen mediated reactions. To date

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ANNUAL REPORT ON CONTRACT N00014-88-K-0131

TITLE: The Molecular Specificity of Adsorption of Biofilm Macromolecules and Accumulation of Microbial Biofouling on Artificial Surfaces in the Sea.

PRINCIPAL INVESTIGATORS:

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RESEARCH OBJECTIVES:

The diversity and variability of macromolecular components that mediate initial microbial attachment to surfaces in ambient seawater is being determined employing immunological techniques. Antisera against microbial adhesion enhancing macromolecules (MAEM) have been raised in chickens and the immunoglobin-G (IgG) fraction utilized for isolation of crude MAEM from samples of cell free coastal seawater and marine microbial culture media by immunoaffinity chromatography. The objective of the present study is to produce monoclonal antibodies (MAbs) against MAEM and use these to chromatographically isolate and purify individual MAEM from mixtures of such components. Variability and diversity of MAEM produced by biofouling microorganisms, those found soluble in ambient seawater and on biofouled surfaces will be assessed employing a spectrum of MAEM MAbs. The specificity of the interactions of the various MAEM with glass and metallic surfaces will be assessed. Alterations in the physical characteristics of the test surfaces and changes in their susceptibility to microbial biofouling will be correlated with these interactions.

PROGRESS TO DATE:

A. Detection of monoclonal antibodies made against microbial adhesion enhancing macromolecular antigens.

Microbial adhesion enhancing macromolecules (MAEM) have been isolated by immunoaffinity chromatography from samples of cell free coastal seawater and from the laboratory culture growth media of marine microorganisms recovered from biofouled surfaces. MAEM were recovered from these samples by immunoaffinity chromatography employing polyclonal antibodies against these macromolecules. Recovered antigens were pooled for use in the production of monoclonal antibodies (MAbs).

The first objective of this project period was to standardize an ELIZA method which was suitable for a mass screening of hybridomas produced against MAEM antigens. A major problem encountered was the difficulty of effectively binding the MAEM polysaccharide(s) (PS) to an ELIZA plate, which is made of either polystyrene (ST) or polyvinyl chloride (PVC). An initial attempt was made to modify both ST and PVC plastic surfaces using 0.5-5% glutaraldehyde to increase their PS binding ability. A second approach was to coat ST plastic surfaces with with bovine serum albumin and poly-L-lysine, and conjugate PS antigens to the immobilized peptides using the Bio Rad EDAC or the cyanuric chloride procedures. A third approach was to utilize a primary antibody raised in the chicken to capture the PS antigens. Immune chicken IgG was isolated fom egg yolk by the polyethylene glycol method, subsequently purified by repreated precipitation with ammonium sulfate.

B. Isolation of hybridomas producing MAEM monoclonal antibodies.

The second project objective was to make and isolate hybridomas that produce MAbs against individual PS antigens. Hybridoma culture supernatants were to be screened by the ELIZA methods described above.

C. Results.

1. Detection of MAEM MAbs

(a) Treatment of PVC plates with glutaraldehyde.

Plates were pretreated with 0.5-5% glutaraldehyde for periods of 60 minutes and overnight at both room temperature and at 4°C. The plates were then coated with 1 ug/ml PS antigen either in a bicarbonate buffer (pH 9.6) or phosphate buffered saline (PBS) at a pH of 7.4, and blocked with 2% BSA in PBS. Pooled hyperimmune mouse sera and normal mouse sera were each serially diluted in buffer containing BSA and 0.5% Tween 20 and titrated using the prepared plates. While plates treated with higher concentrations of glutaraldehyde proportionally bound more immune IgG (but not normal mouse IgG), no significant increases in binding of immune mouse IgG were found on plates coated with PS antigen. Thus, treatment with glutaraldehyde increased the apparent "nonspecific" binding of immune mouse IgG and not that of normal mouse IgG, however, PS coated plates did not bind more immune IgG than that seen on the ccorresponding glutaraldehyde treated, uncoated plates. At present we have no clear explanation of why glutaraldehyde treatment increases the binding of immune mouse IgG, but these results were highly reproducible.

(b) Conjugation of PS antigen to peptide coated plastic surfaces.

There was no significant increase in the binding of immune IgG as compared to normal mouse IgG on plates coated with BSA to which PS antigens had been conjugated with using the Bio Rad EDAC method. Conjugation of PS antigen to poly-L-lysine was equally unsuccessful. It is of interest to note that again, immune mouse IgG bound to poly-L-lysine, even in the absence of PS antigens, much more effectively than normal mouse IgG.

(c) Primary chicken antibodies made to PS antigens.

Hens were hyperimmunized by repeated subcutaneous inoculation with PS antigens and their eggs collected. Chicken IgG was isolated using polyethylene glycol and subsequently purified by repeated precipitation wih ammonium sulfate. ST plates were individually coated with either normal or immune chicken IgG. Normal and immune mouse sera were serially diluted and the mouse IgG binding to the coated plates tested by an immune peroxidase ELIZA method. Results are graphically summarized in Figures A, B and C. Hyperimmune mouse serum reacted very strongly with the immune chicken IgG coated plate wells, even in the absence of PS antigens (Figure C). Those plate wells coated coated with normal chicken IgG showed some reactivity with hyperimmune mouse serum, however, to a significantly reduced extent (Figure B). The cause of the apparent nonspecific binding of immune chicken IgG and the hyperimmune mouse sera is not clear at this time. Further modifications of the ElIZA assay are being examined.

(d) Effect of galactose on ELIZA results.

MAEM PS antigens can be eluted from immunoaffinity columns composed of either immobilized chicken or toad antibody by D-galactose (100 ug/ml) (Tosteson, T.R. et al., Journal of Colloid and Interface Science 104:60-71, 1985). The effect of D-galactose on the apparent nonspecific binding of hyperimmune mouse serum with either normal, and more importantly immune chicken IgG. ELIZA plates coated with both normal and immune chicken IgG were exposed to normal and hyperimmune mouse serum diluted with diluent buffer, with or without added D-galactose (100 ug/ml). Initial experiments indicated that the presence of D-galactose had no effect on the observed interaction of immune chicken IgG and hyperimmune mouse serum. Further experiments are now in progress.

2. Isolation of MAEM MAbs producing hybridomas.

BALB/c mice were injected (i.p.) with 100 ug of MAEM PS antigens dissolved in PBS. These animals were sacrificed 4-5 days after inoculation, their spleens removed aseptically and lymphocytes collected by the Ficol-Hypaque method. Somatic hybridization of lymphocytes with mouse plasmacytoma SP2/0 cells was performed by the polyethylene glycol method using PEG 6000-7000 (Matheson, Coleman & Bell). To date, 28 clones have been shown to produce reactive IgG, detectable by the method described above (section C. Results, Part 1, c). using immune chicken IgG coated plates. These clones are stored frozen in liquid nitrogen, awaiting further refinement of the ELIZA method.

WORKPLAN (Year 2):

The current ELIZA method using immune chicken IgG coated plates does not appear to be specific. Hyperimmune mouse IgG (but not normal mouse IgG) reacted significantly with coated plates in the presence or absence of MAEM PS antigen. The assay yielded a titer of approximately 100,000 for the hyperimmune serum over that of the normal control. It is important to note that the hyperimmune mouse serum, which was a pool from four mice, consistently showed this property despite differences in assay methods. The success of our approach to this

project heavily relies on the specificity of the assay. The very high titer of hyperimmune mouse serum suggests that immunization has produced an antibody(ies) against MAEM PS antigens, however our assay at present could not discriminate specific from nonspecific reactions. Efforts will continue to focus on this problem, by examining the effects of higher concentrations of D-galactose and other simple sugars. The effects of elevated salt concentrations will be examined as a means of minimizing non-antigen mediated reactions. Concurrent with these efforts we are going to rescreen the hybridoma supernatants using our current immune chicken IgG method, including those modifications outlined here.

INVENTIONS:

None

PUBLICATIONS AND PRESENTATIONS:

Diversity and Seasonality of Microbial Aggregation-Adhesion Enhancing (AAE) Biofouling Macromolecules in Coastal Seawater
T.R. Tosteson
Presented at the 7th International Congress on Marine Corrosion and Biofouling, Polytechnical University of Valencia, Valencia, Spain, November 7-11, 1988.

Adhesion of Microorganisms in the Marine Environment
T.R. Tosteson
Presented at a Symposium on Adhesion in Biological Systems held in conjuntion with the ASCB/ASBMB Meeting, San Francisco, January 29-February 2, 1989.

TRAINING ACTIVITIES:

Mr. Luis Cruz, a graduate student (Ph.D.) has been workin on this project in the laboratory of Dr. T.R. Tosteson.

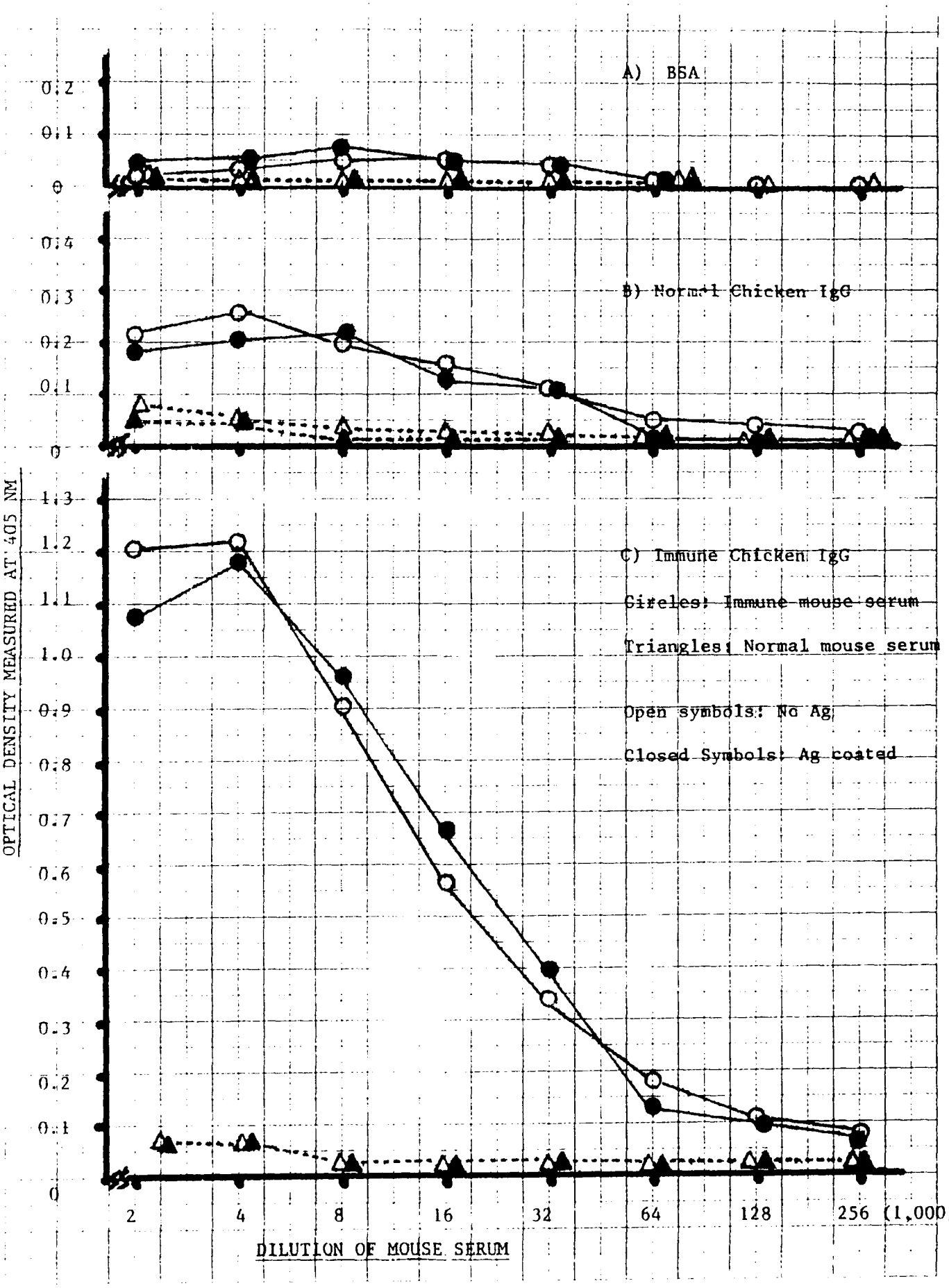
AWARDS AND FELLOWSHIPS:

T.R. Tosteson received a Scholarly Productivity Award from the Univerisity of Puerto Rico NSF-EPSCOR Program in October, 1988.

Figure Legend

TITRATION OF HYPERIMMUNE MOUSE ANTI-MAEM SERUM BY ELIZA

Polystyrene (ST) ELIZA plates (Beckman) were coated with (A) 2% BSA, (B) normal chicken IgG, and (C) immune chicken IgG, by incubating at 4°C overnight. The plates were subsequently blocked with 2% BSA for 60 minutes at room temperature. Half of each respective plate was subsequently reacted with 1 ug/ml MAEM PS antigen in PBS for 60 minutes at 37°C. The other half of each plate was reacted with PBS alone. Normal and hyperimmune mouse sera were serially diluted and their IgG binding tested using goat-anti-mouse IgG peroxidase conjugate (Jackson Immunochemicals).


A) BSA
0.2
0.1
0
B) Normal Chicken IgG
0.4
0.3
0.2
0.1
0
C) Immune Chicken IgG
Circles: Immune mouse serum
Triangles: Normal mouse serum
Open symbols: No Ag
Closed Symbols: Ag coated
OPTICAL DENSITY MEASURED AT 405 NM
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
8
16
32
64
128
256 (1,000)
DILUTION OF MOUSE SERUM