2008 TAIWAN INTERNATIONAL SCIENCE FAIR

CATEGORY : Biochemistry

PROJECT : Effects of Macromolecular Crowding on Model in vitro Antigen-Antibody Reactions

AWARDS : Biochemistry First Award

SCHOOL : Hwa Chong Institution

FINALISTS : Leong Yuan Chang

COUNTRY : Singapore

Effects of Macromolecular Crowding on Model *in vitro* Antigen-Antibody Reactions

Abstract

Many biological processes in the human body take place in intracellular environments under crowded conditions. This means that such cellular activities occur in the presence of inert macromolecules. These macromolecules are thought to have large effects on reaction rates and equilibria. However, under conventional conditions, these same processes are studied *in vitro* under uncrowded conditions in dilute buffers and therefore not reflective of physiological conditions. Hence, this project aims to study the effect of macromolecular crowding on antigen-antibody reactions in an attempt to mimic intracellular environments. It also aims to highlight the importance of introducing crowders as a factor of study in conventional *in vitro* experiments involving cellular reactions. The model reaction investigated involves human collagen type 1 on skin samples and specific antibodies against these antigens. Inert macromolecules of defined hydrodynamic radius and charge were added into the reaction media while quantitative tests are conducted to determine the effect of crowding on the rate and extent of reaction. The macromolecular crowders used were Dextran Sulphate, Ficol170, Ficol1400 and Polyvinylpyrrolidone360. Results were than analysed with the Metamorph 7 imaging system to determine signal strength under crowded and non-crowded conditions. At high primary antibody dilutions, skin samples incubated with a primary antibody solution crowded with a combination of inert macromolecules showed improve signal strength. This could be because crowding tends to shift reaction equilibria such as to minimise the amount of excluded volume, leading to association of the antigen and its specific antibody. This is known as the excluded volume effect. In addition, crowding the primary antibody solution helped increase the rate of reaction. The results also suggest that the addition of crowders may reduce the amount of antibodies needed per sample and decrease the necessary incubation time. Therefore, antigen-antibody reactions can be done more cost-effectively, However, the presence of charged macromolecules such as Dextran Sulphate has an inhibitory effect. Further work is also needed to establish whether macromolecular crowding enhances specificity and thereby reduce autoflourescence of the skin sample.

評語

1) Good motivation and clear presentation.
2) Rational experimental design.
3) Significant results.