

CHARACTERISATION OF MITOCHONDRIAL PROTEINS IMPLICATED IN APOPTOSIS

J.A. Burgess, A. Smail, M.M. Sheil and M.R. Wilson

Chemistry Department, University of Wollongong, Wollongong, N.S.W. 2522

Apoptosis, a highly regulated process leading to cell death, is important in the maintenance of tissue homeostasis, growth and selection of lymphocytes and embryogenesis. It has also been implicated in disease states such as rheumatoid arthritis, acquired immunodeficiency syndrome (AIDS), Alzheimer's disease and cancers.

The involvement of mitochondria in the steps leading to apoptosis has been the focus of many recent studies. It is known that in some apoptotic pathways mitochondria undergo permeability transition (PT). PT involves the formation of large protein pores at points of contact between the inner and outer mitochondrial membranes. This results in the collapse of the electrochemical gradient established by the respiratory electron transport chain.

The release of proteins from mitochondria that have undergone PT has been recognised. It is known that isolated mitochondria release cytochrome c and other proteins when undergoing PT *in vitro*. The isolation and characterisation of proteins released from the mitochondria following PT is crucial for better understanding the mechanisms involved in the apoptotic pathway.

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) has become a very useful technique for the separation and visualisation of the protein cocktail in a biological sample. Spots on the gel that increase or decrease in intensity compared to a control may identify proteins involved in apoptosis. Such spots can then be removed and analysed by MALDI or electrospray mass spectrometry.

PT of mitochondria isolated from mouse liver was induced by a variety of means *in vitro* and the proteins released analysed by 2-D PAGE. Differences between the composition of proteins released for the different treatments and preliminary work to identify specific proteins of interest will be presented.