

C
540
PRI

C-4)

GELATINOLYTIC ACTIVITY IN HUMAN WHOLE SALIVA

A PROJECT REPORT PRESENTED BY

NALIN PRIYANTHA

**to the Board of Study in Chemical Sciences
POSTGRADUATE INSTITUTE OF SCIENCE**

**in partial fulfillment of the requirement
for the award of the degree of**

MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

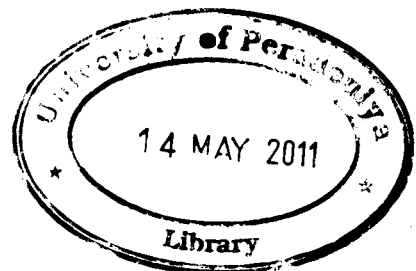
of the

UNIVERSITY OF PERADENIYA

SRI LANKA

2010

645666



Abstract

GELATINOLYTIC ACTIVITY IN HUMAN WHOLE SALIVA

R.G.Nalin Priyantha

Department of chemistry

University of Peradeniya

Sri Lanka

Matrix Metalloproteinases (MMPs) are a family of structurally related, Zinc containing enzymes that have the ability to breakdown connective tissue. So far 26 MMPs have been identified. Their expression is known to increase in various inflammatory, malignant and degenerative diseases. However the complex roles of these MMPs in diseases are not fully understood yet. The new challenge in MMP research is to better understand the complex roles these enzymes play in human diseases. In this research, the objective was to optimize conditions for zymography procedure to visualize gelatinolytic -activity in human saliva and to identify major MMPs present in human saliva, responsible for the gelatinolytic activity.

Saliva was collected from systemically healthy individuals aged between 20-40 years of age (n=30). Saliva samples were centrifuged and supernatants were stored at -20°C until analyze. Experiments were conducted to find optimum conditions of zymography procedure for visualization of gelatinolytic activity with varying sample size, voltage, washing period and incubation period. Gelatin zymography of human saliva were done at optimum condition for identification of major MMPs present in human saliva.

Optimum conditions for zymography were determined as sample size ($10\ \mu\text{l}$), voltage (125 mv), incubation time (14 hours) and 4 x 45 minutes intervals washing period to visualize clear gelatinolytic activity of human saliva. Gelatinolytic activity was observed at bands of molecular weights at 72 and

92 kDa. MMP-2 and MMP-9 are the MMPs that are reported to show gelatinolytic activity at 72 and 92 kDa in gelatin zymography respectively. Therefore, the gelatinolytic activity observed in human saliva may be due to presence of MMP-2 and 9.