3636777 SCBC/D : MAJOR :BIOCHEMISTRY ; Ph.D. (BIOCHEMISTRY)
KEY WORD : CHEMILUMINESCENCE / LINAMARIN / LINAMARASE / GLUCOSE OXIDASE / PEROXIDASE / LUMINOL / CASSAVA

SUTARNTHIP RUENGPRAPAVUT : A NEW CHEMILUMINESCENT ASSAY FOR LINAMARIN. THESIS ADVISORS: MONTRI CHULAVATNATOL, Ph.D., JISNUSON SVASTI, Ph.D., PICHIT TOSUKHOWONG, Ph.D., BHINYO PANIJPAN, Ph.D., UDOM CHANTHARAKSRI, Ph.D. 156 P. ISBN 974-662-339-7

A new chemiluminescent assay was developed for the quantitative determination of linamarin (2-β-D-glucopyranosyl-2-methylpropane nitrile), the predominant cyanogenic glucoside present in cassava. The assay involved hydrolysis of linamarin by a specific enzyme, linamarase (EC 3.2.1.21), releasing glucose which was then measured by using a chemiluminescent system, consisting of glucose oxidase (EC 1.1.3.4)-peroxidase (EC 1.11.1.7)-luminol. The light measured using a luminometer was proportional to linamarin in the range of 1-8 μM. This method was more sensitive than the conventional spectrophotometric method (alkaline picrate) because it can determine linamarin at levels as low as 50 pmole. There was good agreement between the results of spectrophotometric method and chemiluminescent method for determining linamarin content of cassava tissue extracts, with the exception of extracts of mature leaves, for which the two methods did not produce agreeing results. However, several agents were found to interfere with the new assay. Vanadate, Mg^{2+} and Cu^{2+} were inhibitory to the luminescence of the H_2O_2-peroxidase-luminol system used in the coupling reaction while EDTA or EGTA activated the system. In addition, Hg^{2+}, NEM, PCMBS which inhibited glucose oxidase and Tris ion which inhibited linamarase all interfered with the new assay. Selected sugars, namely glucosamine, galactose, sucrose, sorbitol, fructose and N-acetylglucosamine showed no effect on the chemiluminescence.

A preparation in the extract of mature leaves (called "factor X") was found to inhibit the chemiluminescence using either $K_3Fe(CN)_6$ or peroxidase. The inhibiting factor was negatively charged at pH 8.5 and had an absorbance at 280 nm but contained no detectable protein. Its molecular weight was low (less than 6,500) but it was non-dialyzable. It was heat labile and probably consisted of no less than 3 components.

Chemiluminescence can be detected by using photographic film detection. As little as 15 μM of H_2O_2 was detected in PVC microtiter plate using Kodak Tri-Xpan (ASA 400) or Kodak Ektachrome (ASA 100).