

In Vitro* Biosynthesis of Caffeine: the Stability of *N*-Methyltransferase Activity in Cell-Free Preparations from Liquid Endosperm of *Coffea arabica

F. M. GILLIES¹, G. I. JENKINS¹, H. ASHIHARA², A. CROZIER¹

¹Bower Building, Division of Biochemistry and Molecular Biology,
Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, U.K.

²Department of Biology, Faculty of Science, Ochanomizu University,
Ohtsuka, Bunkyo-ku, Tokyo 112, Japan

SUMMARY

A cell-free system has been developed from *Coffea arabica* that is a rich source of the *N*-methyltransferase activity which catalyses the transfer of the methyl group from S-adenosyl-L-methionine to methylxanthines producing caffeine. Purification of the enzyme by anion-exchange chromatography results in low activity yields. Part of the reason was found to be inhibition of the *N*-methyltransferase activity by KCl and NaCl, which are used to elute the protein during anion-exchange chromatography. The enzyme was found to have a half life at 4°C of approximately 90 min. An extensive study of a wide range of protease inhibitors failed to show any effective stabilisation of activity suggesting that the losses are not due to the action of endogenous proteases in the extract. The stability of the enzyme has been shown to be improved substantially with the incorporation of 20% (v/v) glycerol or 20% (v/v) ethylene glycol in the buffers used. Incorporation of 20% (v/v) glycerol in buffers during anion-exchange chromatography resulted in 54-78% yield of *N*-methyltransferase activity and a *ca.* 10-20 fold purification.