A167 Microplate reader based determination of alpha-amylase: screening of plants with an antidiabetic impact

I. Funke and M.F. Melzig
Institut für Pharmazie, Humboldt-Universität zu Berlin, Goethestr. 54, D-13086 Berlin, Germany.

Several methods for the determination of alpha-amylase (EC 3.2.1.1) have been described, whereas the determination of alpha-amylase using maltooligosaccharides of defined chain length with a 4-nitrophenyl or 2-chloro-4-nitrophenyl group as a chromophore are in current use. The used substrates are cleaved by alpha-amylase to yield free chromophores which can be continuously monitored at 405 nm (kinetic determination of alpha-amylase activity) (1, 2).
One aim of our work is the adaptation of this reaction to analysis with a microplate reader in 96-well-plates. We tested different substrates (*p*-nitrophenyl-α-D-maltoheptaoside, *p*-nitrophenyl-α-D-maltopentaoside) and different assay conditions (temperature, time).
While most of the described examinations of alpha-amylase activity are significant in the diagnosis of pancreatic diseases we established a test for screening plants with an antidiabetic relevance.
The inhibition of alpha-amylase activity was standardized with Acarbose (Glucobay®), which is used in the treatment of diabetes mellitus type II. We are using the test for the examination of numerous plants concerning a possible influence of alpha-amylase activity. First tests with aqueous extracts of *Phaseolus vulgaris* L. showed a distinct inhibition in a concentration-dependent way.

References: 1. Gella, F.-J. et al. (1997) Clin. Chim. Acta 259: 147-160. **2.** Soor, S.K., Hincke, M.T. (1990) Anal. Biochem. 188: 187-191.

A168 Possible antidiabetic effects of *Eucalyptus globulus* leaves aqueous extract

F. Gonzalez-Mujica, N. Motta and J. Capote
Sección de Bioquímica Médica. Facultad de Medicina. Universidad Central de Venezuela. Apartado de correos 50.587 Sabana Grande, Caracas, Venezuela.

In the folk medicine *Eucalyptus globulus* is used for the empirical treatment of diabetes. In the present work we studied the effects of *E. globulus* leaves aqueous extract on mechanisms that participate in the glycaemia regulation, such as, hepatic neoglucogenesis and glucose-6-phosphatase (G-6-Pase) activity. *E. globulus* leaves aqueous extract concentration was quantified by its absorption at 264 nm (UA_{264nm}). Liver slices neoglucogenic capacity was measured as early described (1) in the absence (control) and in the presence of 4 $UA_{264\ nm}$/assay of the plant extract. The G-6-Pase activity with 2 substrates, glucose-6-phosphate (G-6-P) and pyrophosphate (PPi), was assayed (2) in the absence and in the presence of 1.5 $UA_{264\ nm}$/assay of the plant extract. The hepatic neoglucogenic capacity was strongly inhibited by *E. globulus* leaves aqueous extract, being in control 57.11 ± 2.03 and in treated 28.46 ± 2.04 µmol of glucose/ h x g liver dry weight, difference statistically significant at $p< 0.0005$. In intact microsomes, using G-6-P as substrate of G-6-Pase, *E. globulus* leaves aqueous extract decreased the V_{max} (from 9.57 ± 0.69 to 6.34 ± 0.49 phosphate µmol/h) and increased the K_M (from 5.95 ± 1.29 to 14.06 ± 1.11 mM), both modifications were statistically significant at $p< 0.05$. In disrupted microsomes there was a small increase in K_M (from 0.84 ± 0.08 to 1.12 ± 0.06 mM), without change in $V_{max.}$ The G-6-Pase activity, using PPi as substrate, showed a small increase in the K_M of intact microsomes (from 1.41 ± 0.19 to 2.39 ± 0.24 mM), the other kinetic parameter were not changed neither in intact nor in disrupted microsomes. The plant extract behaves like a mixed non-competitive inhibitor of the G-6-P transporter (T1) with less effect on the catalytic subunit and the phosphate/pyrophosphate transporter (T2) of the G-6-Pase system. The G-6-Pase modifications by *E. globulus* leaves aqueous extract could explain the liver neoglucogenic inhibition. The decrease in the hepatic glucose production by *E. globulus* leaves aqueous extract could be useful in diabetes treatment.

Acknowledgements: This work was supported by grants CDCH-UCV 09-33-4788-00 and 09-33-4706-00.

References: 1. Gonzalez-Mujica, F. et al. (1998). Phytother. Res. 12: 291-293. **2.** Burchell, A. et al. (1988). Clin. Chem. Acta 173: 183-192.