Development of an Amperometric L-Alanine Sensor Using L-Amino Acid Oxidase from
Neurospora crassa

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An L-alanine (Ala) sensor was developed using partially purified L-amino acid oxidase
(EC 1.4.3.2, L-AAOx) from Neurospora crassa (N. crassa). The amount of L-Ala was
amperometrically determined by flow injection analysis (FIA) involving an L-AAOx
reactor, the pyruvate oxidase (EC 1.2.3.3, PyOx) electrode and the contrast electrode.
Pyruvic acid, formed from L-Ala through the action of L-AAOx, was further oxidized by
PyOx via the L-AAOx reaction. The amount of oxygen consumed in the PyOx reaction,
proportional to the amount of L-Ala present, was monitored by the oxygen electrode. The
L-Ala concentration was calculated from the difference (C. D.) between the PyOx and
contrast electrode output. Optimum assay conditions consisted of 50 mM Tris-HCl (pH
7.4) and a transported buffer flow rate of 0.18 ml min⁻¹. Moreover, TPP and FAD at final
concentrations of 1 mM and 10 µM, respectively, were added to the buffer as activators of
PyOx. The sample injection volume was fixed at 50 µl. A single assay could be completed
in approximately 10 min and the assays were stable for up to 50 repetitions. A linear
relationship was obtained between C. D. and the L-Ala concentration range of 0.05 to 0.7 mM (correlation coefficient of 0.994). The relative
standard deviation (R.S.D.) was 4.42% (n = 10) at 0.4 mM L-Ala. The L-Ala content of
four beverages was also determined using the proposed sensor system. The results obtained
indicated a linear relationship between the amount of L-Ala determined by the proposed
sensor and that determined by the conventional method. Thus it was possible to develop a
biosensor for the determination of L-Ala from its oxidative product, pyruvic acid. The
system utilized L-AAOx, an enzyme with a low substrate specificity and isolated from N.
crassa.

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