Sensors and Materials, Vol. 15, No. 4 (2003) 225–238
MYU Tokyo

S & M 0524

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

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(Received April 17, 2003; accepted July 25, 2003)

Key words: L-alanine, L-amino acid oxidase, pyruvate oxidase, *Neurospora crassa*, FIA system

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 with an 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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