Communications to the Editor

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FLUOROMETRIC DETERMINATION OF L-TRYPTOPHAN AND TRYPTAMINE WITH CHLOROACETALDEHYDE

Hideaki Iizuka* and Takehiko Yajima
School of Pharmaceutical Science, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274, Japan

L-Tryptophan (Trp) and tryptamine (Trp-NH_2) react with chloroacetaldehyde (CAA) to form derivatives with strong fluorescence (Ex=305 nm and Em=455 nm for Trp; Ex=304 nm and Em=452 nm for Trp-NH_2) in a dilute hydrochloric acid solution under optimal conditions. Other biogenic indolylethylamines and phenylethylamines do not form fluorescent derivatives with CAA.

KEYWORDS——chloroacetaldehyde; fluorogenic reagent; L-tryptophan; tryptamine; fluorometric determination

Axelsson[1] examined various carbonyl compounds to obtain fluorogenic reagents which produce fluorescence with biogenic monoamines to use in histochemical analyses of those amines. The most sensitive reagent was reported to be glyoxylic acid (GA) which formed fluorescent products with phenylethylamines as well as indolylethylamines.

We observed in our laboratory that CAA induced fluorescence in the presence of amino acids, of which Trp produced the most intense fluorescence. On the basis of these observations, a new fluorometric method for the determination of Trp was developed as follows. As shown in Fig. 1, the fluorescence maximum of the Trp-adduct with CAA was at 455 nm (excitation at 305 nm).[2] The fluorescence intensities were higher under acidic conditions especially in the presence of hydrochloric acid than under neutral or basic conditions. Figure 2 shows the relationship between concentrations of hydrochloric acid and fluorescence intensities.[3] It has been reported that Trp-NH_2 reacts with GA to form β-carboline derivatives with strong fluorescence in the presence of hydrochloric acid as catalyst.[4] In the CAA method, it was therefore assumed that hydrochloric acid acted also as catalyst. In a hydrochloric acid solution, amino acids other than Trp and cystein did not react with CAA. However, the fluorescence intensity of cystein was much lower than that of Trp.[5] Among phenylethylamines and indolylethylamines tested, CAA reacted

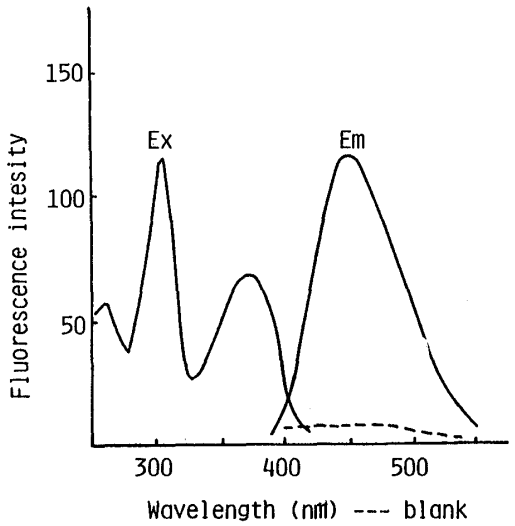

Fig.1. Excitation and Emission Spectra of L-Tryptophan

only with Trp and Trp-NH_2.[6] Trp-NH_2-adduct showed a fluorescence maximum at 452 nm (excitation at 304 nm) and its excitation and emission spectra were almost the same as those of Trp. In Fig. 3, fluorescence intensities at 80°C and 100°C are shown as a function of reaction time. Fluorescence intensities reached the same maximum level after 50 min at 100°C and 2.5 h at 80°C and the maximum level was maintained thereafter. On the basis of these data, the reaction time of 1 h and the reaction temperature of 100°C were adopted for the proposed method.

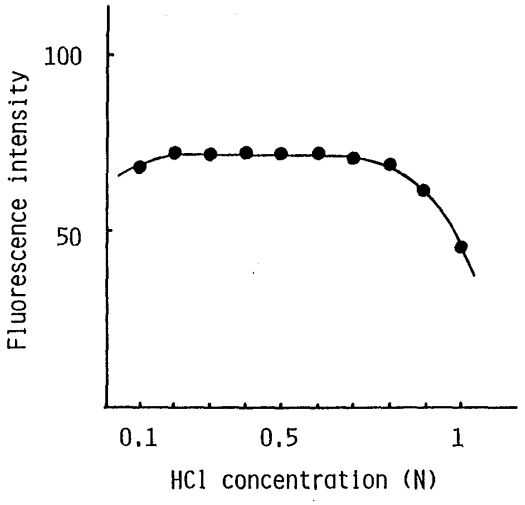

Fig.2. Relationship between HCl Concentration and Fluorescence Intensity

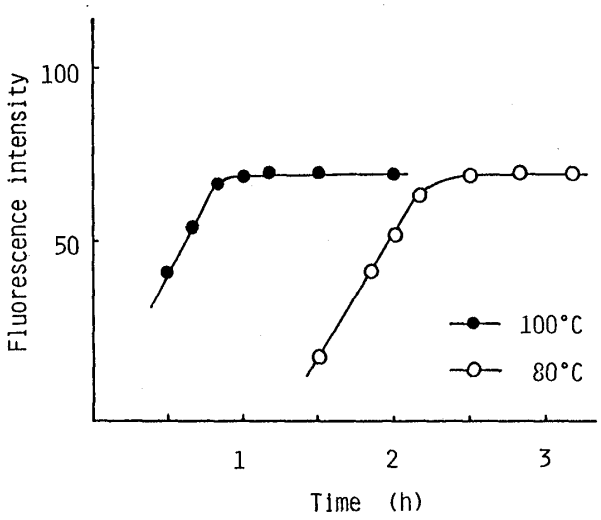

Fig.3. Effects of Reaction Time and Temperature on the Fluorescence Intensity

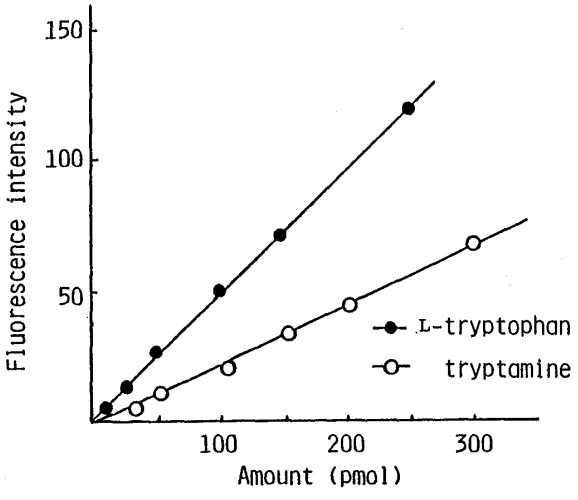

Fig.4. Working Curves for L-Tryptophan and Tryptamine

Working curves for Trp and Trp-NH_2 are shown in Fig. 4. The detection limits were 5 pmol and 20 pmol for Trp and Trp-NH_2, respectively. The reproducibility of the CAA method was determined by assaying 150 pmol aliquots of the compounds. The coefficient of variation of the measurements (n=5) were 1.1 % and 1.8 % for Trp and Trp-NH_2, respectively.

It was thus shown that the proposed method was highly sensitive and specific in determining Trp and Trp-NH_2. The application of the method for the determination of Trp in peptides is now in progress.

REFERENCES AND NOTES

1) S. Axelsson, A. Björklund and O. Lindvall, J. Histochem. Cytochem., 20, 435 (1972).
2) The reaction was carried out as follows; To a 0.5 ml aqueous solution containing 250 pmol of Trp in a stoppered test tube, 2 ml of 0.5N HCl and 0.1 ml of 0.5M aqueous solution of CAA were added in that order. Then the reaction mixture was heated for an hour in the boiling water bath. Fluorescence intensity was measured with a Hitachi 650-10S fluorescence spectrophotometer.
3) In Figs. 2, 3 and 4, fluorescence intensities were measured at Ex=305 nm and Em=455 nm. An aqueous solution containing 150 pmol of Trp was used in Figs. 2 and 3; reaction conditions are given in note 2).
4) A. Bjjörklund, O. Lindvall and L. Svensson, Histochemie, 32, 113 (1972).
5) Relative fluorescence intensity of L-cystein (150 nmol) was 90 % of that of Trp (150 pmol).
6) Phenylethylamines tested were L-dopa, dopamine, L-epinephrine and L-norepinephrine; indolylethylamines tested were Trp, Trp-NH , 5-hydroxy-tryptophan and 5-hydroxy-tryptamine.

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