Rapid, Sensitive, and Selective Assay for Tryptophan in Serum

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We have developed a rapid, selective, and sensitive fluorometric procedure for measuring tryptophan in serum. This assay is based on the facile reaction of tryptophan with aqueous formaldehyde; the concentration of the amino acid is measured from the diminution in its fluorescence as the reaction proceeds. Compared to other assay procedures, this procedure has the advantage of being sensitive to the amino acid, but unresponsive to other indole derivatives such as tryptamine and 5-hydroxytryptamine. Concentrations of tryptophan as low as 0.5 mg/liter may be assayed in 15 min.

Additional Keyphrases: monitoring dialysis of uremics • tryptophan fluorescence assay

Efficient use of the artificial kidney on patients afflicted with chronic uremia requires knowledge of the concentration of various compounds in serum during hemodialysis. The dialysis procedure requires about 8 h; therefore, assay for such compounds should be rapid and specific.

Patients suffering from chronic uremia have lower than normal concentrations of tryptophan in their serum, while the concentration of tryptophan metabolites is increased (1); therefore tryptophan is a useful thing to measure during hemodialysis to follow the effectiveness of the procedure.

Several methods for assay of tryptophan in biological systems have been reported, but they lack selectivity [e.g., direct measurement of tryptophan fluorescence (1, 2)], are slow [e.g., conversion to norharman (3)], or require equipment not commonly found in clinical laboratories [e.g., digital integration methods (4) to observe fluorescence decay].

We have developed a rapid fluorometric assay for tryptophan and have applied this technique to blood serum. Our test is based on the facile reaction of tryptophan with aqueous formaldehyde to form the imine (1); diminution in fluorescence observed in a given time is proportional to tryptophan concentration.

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\text{H} - \text{N} = \text{C} - \text{COOH}
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\text{H} \quad \text{CH}_{2}
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Materials and Methods

Reagents

Tryptophan, 5-hydroxytryptamine, and tryptamine were obtained from Sigma Chemical Co., St. Louis, Mo. 63178, and were used as received.

Indole-3-acetic acid was obtained from Aldrich Chemical Co., Milwaukee, Wis. 53233, and was recrystallized from ethanol before use.

Formaldehyde (12 mol/liter, “analyzed reagent”) was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J. 08865. Our samples of formaldehyde had a pH of about 3.5 (even immediately after opening); consequently, the pH was adjusted with dilute NaOH to 7.30, the optimum pH for the assay (see below).

Trichloroacetic acid, obtained from Eastman Organic Chemicals, Rochester, N. Y. 14650, was used as received.

All fluorometric measurements were made with an Amino-Bowman Spectrophotofluorometer (Model 4-8202, American Instrument Co., Silver Spring, Md. 20910).

Procedures

Tryptophan assay. One milliliter of serum, mixed with 1 ml of water and 5 ml of a trichloroacetic acid solution (100 g/liter), was shaken occasionally for 5 min to ensure total deproteinization. The precipitate was then removed by centrifugation. The pH of 2 ml of the supernatant liquid was adjusted to 7.30 and diluted (with water adjusted to pH 7.30) to 10 ml. Five milliliters of this solution was mixed rapidly with 5 ml of a formaldehyde solution prepared by diluting the reagent formaldehyde solution (12 mol/liter) with one part of distilled water to two parts of the formaldehyde and subsequently adjusting to pH 7.30 with 0.1 molar NaOH. A sample of the deproteinized serum-formaldehyde mixture was then placed in the fluorometer, to observe the decay in fluorescence at 360 nm (on irradiation at 280 nm). The change in fluorescence (Δf) was measured for 1.0 min, beginning 15 s after the cell was placed in the fluorometer.

Preparation of the calibration curve. A series of 15 tryptophan solutions, ranging in concentration from 1 to 120 mg/liter, was prepared and treated as described in the assay procedure. The corresponding relationship between the change in fluorescence and concentration is shown in Figure 1. A blank (water only) was run with no change in the signal from the fluorometer over a period of 3 min.
Results

Twenty sera were treated as described above. Their tryptophan content ranged from 8 to 13 mg/liter; the frequency with which 8, 9, 10, 11, 12, or 13 mg/liter concentrations were found was 1, 4, 7, 5, 2 and 1 times, respectively.

The average tryptophan concentration was 10.3 mg/liter (SD, 1 mg/liter), well within the generally accepted range of 11 ± 2 mg/liter (5).

Three of the samples were repeated five times each; data for one of these are typical: 10, 11, 11, 11, and 10 mg of tryptophan per liter.

Tryptophan (a 10 mg/liter solution) was added to serum to alter the concentration of this amino acid and the corresponding changes in fluorescence ($\Delta f$) were measured. The tryptophan solution replaced a corresponding volume of water, so as to maintain a constant volume. For increments of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the solution, the respective $\Delta f$ values were 39, 47, 55, 61, 68, and 76 fluorometer units/min, respectively.

Addition of 1 ml of a 10 mg/liter solution of serotonin, tryptamine, or indoleacetic acid (in place of an equal volume of water) had no effect on the fluorescence change, indicating that under the conditions of the assay, these metabolites do not react with the formaldehyde at a rate rapid enough to affect the result. Aqueous (nonserum) solutions of these compounds (10 mg/liter) did not undergo a significant diminution in fluorescence during the time span of the assay. Very high concentrations of 5-hydroxytryptamine (70 mg/liter) afforded a $\Delta f$ that was within the experimental error of the assay.

Discussion

Fluorometric procedures are commonly used to assay tryptophan in biological specimens, because it is one of only three amino acids that fluoresce.

Udenfriend proposed a method for assay of tryptophan based on its native fluorescence (1, 2). We think that this method suffers a lack of sensitivity; any compound containing an indole ring will absorb and fluoresce in the same region of the spectrum as tryptophan. This might lead to possible errors in the assay if tryptamine or serotonin, for example, were present in moderate concentrations, although admittedly this is unlikely, because these substances are normally present in 100-fold smaller concentrations.

The condensation of tryptophan with formaldehyde, followed by heating and oxidation with $H_2O_2$ to produce norharman has been suggested as an assay for the amino acid in biological systems (3). This test is more selective than measurement of the native fluorescence of tryptophan, but the procedure requires about 1 h (after protein is removed from the serum). Our assay procedure is as selective as the norharman procedure, as evidenced by the lack of response of other indole-ring compounds as discussed above, and much faster.

Fitzgerald (4) has recently proposed that integration of the decay of fluorescence from tryptophan be used as an analytical technique. This procedure has not been applied to biological systems, but there is no reason why it should not be applicable to such systems. This technique, however, requires equipment not normally available in the clinical laboratory.

The change in fluorescence in the presence of formaldehyde was first reported by Konev et al. (6), who observed an increase in fluorescence instead of the diminution we observe, probably because the concentration of tryptophan they used was larger than the concentration region in which fluorescence intensity is proportional to concentration; thus, the condensation product, which actually has a lower fluorescence quantum yield, appeared to have a higher quantum yield.

Our assay procedure is useful for tryptophan samples of 1 to 120 mg/liter. The useful range can be extended to 0.5 mg/liter if necessary by omitting the dilution of the serum with water. The procedure can be used to analyze tryptophan in more concentrated samples by additional dilution and preparation of an extended calibration curve and provides the clinical analyst with a rapid and selective test for tryptophan.

We acknowledge with thanks the support of the NIAMD, NIH, USPHS (Contract No. 72-2216). We also thank the clinical laboratory of the Methodist Hospital, New Orleans, for serum samples.

References