Simple and rapid determination of histamine in food using a new histamine dehydrogenase from *Rhizobium* sp.

Tsuneo Sato *, Tatsuo Horiuchi, Ikuko Nishimura

Research and Development Division, Kikkoman Corporation, Noda City, Chiba 278-0037, Japan

Abstract

A colorimetric enzyme assay for the quantitative analysis of histamine in food has been developed using a new histamine dehydrogenase (HDH) from *Rhizobium* sp. The HDH specifically catalyzes the oxidation of histamine but not other biogenic amines such as putrescine and cadaverine. The principle of our photometric assay is as follows. The HDH catalyzes the oxidative deamination of histamine in the presence of 1-methoxy PMS (electron carrier), which converts WST-8 (tetrazolium salt) to a formazan. This product is measured in the visible range at 460 nm. The correlation between the histamine level and absorbance was acceptable, ranging from 0 to 96 μM with histamine standard solutions, corresponding to 0 to 30 μM of the reaction solution ($r = 1.000$, $CV = 1.0\%$ or less). Assays of canned tuna (in oil and soup) and raw tuna with 45–675 μmol/kg histamine added showed good recoveries of 96–113, 98–108, and 100–106%. The histamine contents of a commercial canned tuna and fish meal containing histamine at high concentrations were determined using the new method and other reference methods (HPLC method, Association of Official Analytical Chemists official method, and two commercial enzyme immunoassay test kits). This simple and rapid enzymatic method is as reliable as the conventional methods.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Histamine; Histamine dehydrogenase; Fish; Enzymatic method

The consumption of foods containing a large amount of histamine (a biogenic amine) has been implicated in causing allergy-like food poisoning known as scombroid poisoning [1–4]. Symptoms of flushing and nettle rash appear on the face from 30 min to 1 h after the meal, followed by headache, diarrhea, and throbbing. Scombroid fish, including sardine, tuna, and mackerel, contain high concentrations of free histidine [4–7], which under certain conditions is decarboxylated by bacteria to produce high levels of histamine [8,9]. In addition, a large amount of histamine has been detected in fermented foods such as cheese, wine, and fish sauce [7,10–15]. It is thought that histidine becomes histamine during fermentation in response to the decarboxylation action of lactic acid bacteria [16,17].

Allergy-like food poisoning generally occurs when food containing 1000 ppm (9000 μmol/kg) or more of histamine is consumed [1]. However, poisoning may be caused in some individuals even when histamine has not reached this level. It has been reported that this is due to the synergistic action of amines other than histamine contained in food, such as putrescine and cadaverine [18–20], but the mechanism of food-based histamine allergies has not yet been determined. In the United States, the toxic level that poses a risk to health is set at 500 ppm (4500 μmol/kg), and the caution level is 50 ppm (450 μmol/kg). The European Union has also set a level of 100–200 ppm (900–1800 μmol/kg) for seafood, and Codex has also proposed regulations at this level [21–23].

---

* Corresponding author. Fax: +81 4 7123 5550.
E-mail address: 8901@mail.kikkoman.co.jp (T. Sato).

An Association of Official Analytical Chemists (AOAC)[1] official method of analysis based on fluorescent measurement has been established [6,24] and is recognized as the most suitable method for the determination of histamine contained in fish and fermented food. The method uses *o*-phthalaldehyde as a fluorescent reagent, which yields a fluorophore, and the intensity of the fluorophore is measured by a photofluorometer. However, to obtain derivatives from this fluorophore and histamine, impurities in the sample must be removed. Thus, the labor and time required to carry out "cleanup" procedures are unavoidable.

High-performance liquid chromatography (HPLC) and liquid chromatography are suitable methods of histamine analyses [10–15,25–29]. In addition to histamine, biogenic amines, such as putrescine and cadaverine, can be simultaneously and quantitatively determined by HPLC, but HPLC analyses are time-consuming and an advanced operating technique is required.

Recently, commercial enzyme immunoassays (EIAs) have become available [30–32]. However, these kits are expensive. Moreover, a calibration curve must be determined for each measurement, for which roughly five measurements are required.

Histamine assays based on enzyme method using histamine oxidase [5,33–38] or histamine dehydrogenase (HDH) [39] are simple to perform and have recently been suggested to permit rapid measurement. However, these histamine oxidase and HDH also react with putrescine and tyramine [33–45]. Because these amines may be present in food at the same level as histamine [1,10,14,23,26,29], it is difficult to selectively detect histamine by using above histamine oxidase and HDH.

We recently found a new HDH from *Rhizobium* sp. 4–9 that acts more specifically on histamine than does the histamine-degrading bacterial enzyme mentioned previously. Using this enzyme, we developed a colorimetric enzyme method that can quantitatively determine histamine that is simpler, more rapid, and more economical than the conventional histamine assay methods. The sensitivity of the current procedure is equivalent to that of conventional methods for the detection of histamine in seafood. The current method provides a histamine assay that is easier to perform than previous methods, can be performed in a short time period, and permits the determination of multiple samples without expensive equipment or extensive technical skills.

Materials and methods

Chemicals

All reagents were of analytical grade or of the highest grade available. Histamine dihydrochloride, putrescine

dihydrochloride, cadaverine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, and agmatine sulfate salt were obtained form Sigma–Aldrich (Japan). Glucose, K_2HPO_4, KH_2PO_4, agar, $(NH_4)_2SO_4$, and glycine for producing HDH were obtained from Wako Pure Chemical (Japan).

Isolation and identification of HDH-producing microorganisms

Microorganisms with potent HDH were obtained from soil samples collected from several locations in Japan. Soil (1 g) was added to 10 ml of histamine broth (glucose 0.1% [w/v], yeast extract [0.2%, w/v, Difco, USA], histamine dihydrochloride [0.1%, w/v], and K_2HPO_4 [0.05%, w/v] in tap water at, pH 6.75) and cultivated at 30 °C for 1 week. Then, 0.1 ml of the culture was spread onto histamine agar plates (2% agar in histamine broth) and incubated at 30 °C for 1 week. Approximately, 500 colonies on the culture plates were transferred to 10 ml of histamine broth and cultured at 30 °C for 4 days. The cells were then collected by centrifugation at 18,000g for 10 min, suspended in 1 ml of 20 mM potassium phosphate buffer (pH 8.0), and sonicated. The homogenate was centrifuged at 18,000g for 10 min to remove intact cells and sonicated cell debris. The activities of histamine oxidase and HDH in the supernatant were then determined according to the method of Shimizu and coworkers [42].

Identification of isolated strain 4–9 was based on its 16S rDNA sequences, phylogenetic analysis, and morphological and physiological tests [46,47].

Purification of HDH

Strain 4–9 was grown aerobically at 30 °C for 48 h in 20 L of histamine medium. Washed microorganisms were then suspended in 20 mM potassium phosphate buffer (pH 8.0) and sonicated. The homogenate was centrifuged at 18,000g for 30 min to remove intact cells and cell debris. Then $(NH_4)_2SO_4$ was added to the above supernatant to 40% saturation and the precipitate formed was pelleted by centrifugation at 18,000g for 10 min. The supernatant to which $(NH_4)_2SO_4$ was added to 60% saturation was centrifuged at 18,000g for 10 min, and the resultant precipitate was dissolved in 20 mM potassium phosphate buffer (pH 8.0) containing 13% (w/v) ammonium sulfate and dialyzed. The dialyzed enzyme solution was applied to a Toyopearl-Butyl-650 (Tosoh, Japan) column equilibrated with the same buffer, and the absorbed protein was eluted with a linear $(NH_4)_2SO_4$ gradient (13–0%). The active fractions were then pooled and subjected to ultrafiltration such that the $(NH_4)_2SO_4$ was exchanged for 20 mM potassium phosphate buffer (pH 8.0). The semipurified preparation was then applied to a DEAE–Sephacel (Amersham Pharmacia) column equilibrated with 20 mM potassium phosphate buffer (pH 8.0). The absorbed protein was finally eluted with a linear NaCl gradient (0–1.0 M) in 20 mM potassium phosphate buffer (pH 8.0).

---

[1] *Abbreviations used:* AOAC, Association of Official Analytical Chemists; HPLC, high-performance liquid chromatography; EIA, enzyme immunoassay; HDH, histamine dehydrogenase; DEAE, diethyl aminoethyl; 1-methoxy PMS, 1-methoxy-5-methylphenazinium methylsulfate; WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitorophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt; EDTA, ethylenediamine tetraacetic acid; DDBJ, DNA Data Bank of Japan; CV, coefficient of variation.