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Selective and sensitive determination of peptides using 3,4-dihydroxyphenylacetic acid as a fluorogenic reagent

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ABSTRACT

A novel fluorescence (FL) reaction for *N*-terminal Gly-containing peptides has been developed using 3,4-dihydroxyphenylacetic acid (3,4-DHPAA). The reaction of the peptides with 3,4-DHPAA was carried out in borate buffer (pH 8.0) in the presence of sodium periodate at 37 °C for 10 min, and the FL was measured with a spectrofluorimeter at the excitation and emission wavelengths of 370 nm and 465 nm, respectively. The 3,4-DHPAA reagent generated particularly strong FL for peptides containing Gly at their *N*-termini. When various other bio-substances, such as amino acids, sugars, nucleic bases, nucleotides, and proteins, were reacted with 3,4-DHPAA, no FL was observed. Under optimized reaction conditions, the lower detection limit of 0.25 μmol L^{-1} was obtained for the *N*-terminal Gly-containing peptides of Gly-Pro (GP) and Gly-Pro-Pro (GPP), which gave 3 times greater FL intensity than that observed for the reagent blank. The proposed reaction with 3,4-DHPAA as a fluorogenic reagent is selective and sensitive for the detection of *N*-terminal Gly-containing peptides, and therefore, this method could be a useful tool for the determination of these particular oligopeptides.

1. Introduction

Various peptides play crucial roles in the physiological and biochemical functions essential for life. Peptide studies in the last decade have explored many fields of scientific research, such as peptidomics [1], peptide-based drug development [2,3], peptidomimetics [4], peptide–membrane interaction [5], and peptide engineering [6]. Many biologically active peptides have a specific amino acid at their *N*-termini. For instance, opioid peptides such as enkephalins, dynorphins, and endorphins contain Tyr at their *N*-termini [7], and growth factors such as insulin-like growth factor-I (IGF-I) and liver cell growth factor, GHK, have Gly at their *N*-termini [8,9]. In addition, *N*-terminal Gly-containing small oligopeptides such as GPE and GPR have physiological roles, including neuro-protective activity [10–12]. Furthermore, various *N*-terminal Gly-containing small oligopeptides are derived from the enzymatic digestion of collagen with collagenase; these oligopeptides might be associated with pathophysiological disorders [13].

Approaches for selective identification and sensitive quantification of particular peptides require superior analytical methods, because facile and sensitive determination of peptides will provide significant information in physiological and pathological studies. In conventional analytical methods, several amine-reactive fluorogenic reagents have been used for the sensitive detection of peptides, i.e., *o*-phthalaldehyde (OPA) [14,15], dansylchloride [16], fluorescamine [17], and 4-fluoro-7-nitro-2,1,3-benzoxadiazole [18]. However, these reagents are not selective for peptides, because they react with primary amino groups, and thus, with a large number of other bio-substances such as amino acids, catecholamines, nucleic acids, amino sugars, and proteins. Although this wide reactivity is advantageous for the simultaneous determination of various bio-substances when using a superior separation system, a peptide-specific chemical reaction would be more helpful for the facile identification of particular peptides.

Recently, we developed a unique FL reaction for detecting peptides with 1,2-dihydroxybenzene as a fluorogenic reagent [19]. This reaction required heating at 100 °C for 10 min in a neutral borate solution (pH 7.0) in the presence of sodium periodate, and generated strong FL for peptides containing Leu, Ala, or Phe at their *N*-termini. When studying this reaction, we found that 3,4-DHPAA selectively reacts with *N*-terminal Gly-containing peptides at a low temperature and generates strong FL (Fig. 1). In this article, we report for the first time the FL reaction of peptides with 3,4-DHPAA as a fluorogenic reagent. The peptides in a final reaction

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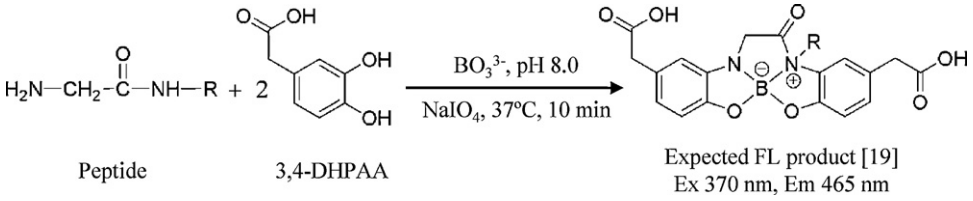

Fig. 1. FL reaction of an *N*-terminal Gly-containing peptide with 3,4-DHPAA.

mixture were reacted with 0.03–1.87 mmol L^{-1} 3,4-DHPAA at 37 °C for 2 min–24 h in 15.6–93.6 mmol L^{-1} borate solution (pH 6.0–9.0) in the presence of 0.06–0.44 mmol L^{-1} sodium periodate.

2. Experimental

2.1. Chemicals and reagent solutions

3,4-DHPAA was purchased from TCI (Tokyo, Japan). Boric acid and sodium periodate were obtained from Wako Pure Chemicals (Osaka, Japan). Peptides, proteins, nucleic acid-related compounds, amino acids and sugars were purchased from Sigma (St. Louis, MO, USA), Wako Pure Chemicals, and Bachem (Bubendolf, Switzerland). All the chemicals were of the highest purity available and used as received. Deionized water was purified by a Milli-Q system (Millipore, MA, USA).

Stock solutions (0.5–4.0 mmol L^{-1}) of peptides or other substances were prepared in water or in 50% aqueous solution of 2-methoxyethanol and stored at −20 °C. The solutions were further diluted with water to obtain the desired concentrations before use. Borate buffer was prepared by dissolving boric acid in water and adjusting the pH with 1.0 mol L^{-1} sodium hydroxide. 3,4-DHPAA and sodium periodate were dissolved in water and kept at 4 °C.

2.2. FL reaction with 3,4-DHPAA

To a 1.5-mL tube, 250 μL of 40 μmol L^{-1} peptide, 250 μL of 0.75 mmol L^{-1} 3,4-DHPAA, 250 μL of 125 mmol L^{-1} borate buffer

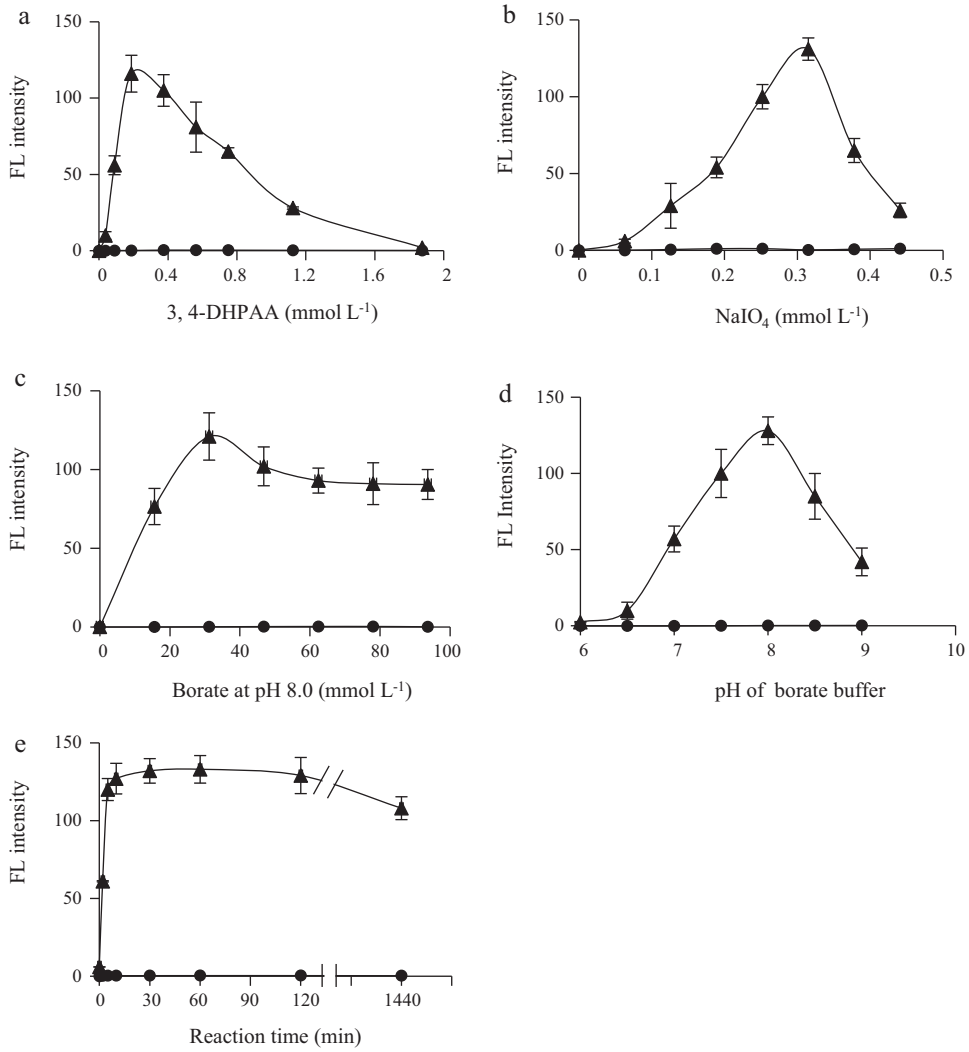

Fig. 2. The effects of the concentrations of: (a) 3,4-DHPAA, (b) $NaIO_4$ and (c) borate buffer (pH 8.0), and of: (d) pH of 31.3 mmol L^{-1} borate buffer and (e) reaction time at 37 °C on the production of FL from GP (–▲–) and the reagent blank (–●–). Each measurement was performed in triplicate.