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Visual Detection of Formaldehyde by Highly Selective Fluorophore Labeling via Gold(III) Complex-Mediated Three-Component Coupling Reaction

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A novel method for visual detection of formaldehyde with excellent selectivity via a gold(III) complex-mediated three-component coupling reaction of resin-linked sterically bulky amines and fluorescent alkynes has been developed.

Formaldehyde is a reactive organic compound ubiquitously found in the environment and present in food mainly due to natural occurrence and/or release from food packaging materials. Common foods contain formaldehyde with an average level of 1-98 mg/kg. However, formaldehyde has been illegally added to food for preservation and bleaching with extreme cases of over 4,250 mg/kg.

Serious exposure to formaldehyde may induce acute poisoning, dermal allergy, and irritation problems. According to International Agency for Research on Cancer (IARC), formaldehyde is carcinogenic to humans in causing nasopharyngeal cancer. Chronic toxicities of formaldehyde include neurotoxicity, reproductive toxicity, hematotoxicity and genotoxicity. World Health Organization (WHO) establishes a tolerable daily intake of 0.15 mg/kg/day for formaldehyde.

A good linearity (R² = 0.9937) between the formaldehyde concentration and propargylamine formation was found by LC–MS/MS analysis. Using a hand-held UV lamp, the formaldehyde concentration (as low as 10 ppm) could be qualitatively determined by naked eyes. Excellent selectivity and tolerance have been demonstrated by application on formaldehyde detection of crude mushroom water extracts.

Herein we first report a visual detection method with excellent selectivity for formaldehyde based on the gold(III)-mediated A³-coupling reaction (Scheme 1). Using resin-linked sterically bulky amines, formaldehyde can be exclusively linked to fluorescent alkynes through propargylamine formation. A good linearity (R² = 0.9937) between the formaldehyde concentration and propargylamine formation was found by LC–MS/MS analysis. Using a hand-held UV lamp, the formaldehyde concentration (as low as 10 ppm) could be qualitatively determined by naked eyes. Excellent selectivity and tolerance have been demonstrated by application on formaldehyde detection of crude mushroom water extracts.

Scheme 1. Bis-cyclometallated gold(III) complex-mediated three-component coupling reaction for exclusive fluorescent detection of formaldehyde.
We hypothesized that sterically bulky amines would achieve excellent selectivity for formaldehyde in the step of iminium ion formation. Thus, sterically bulky 2,2,6,6-tetramethyl-piperidine (1a) bearing four alpha-methyl groups was chosen for the studies (Scheme 2). As a rapid detection method, we set out to conduct the A⁺-coupling reaction for 1 h. Heating of formaldehyde (0.1 mmol), amine 1a (0.11 mmol), phenylacetylene 2a (0.15 mmol) and gold(III) complex 3a (0.01 mmol) in water (0.6 mL) at 50 °C for 1 h gave propargylamine 4a in 6% isolated yield (ESI†). Under the same reaction conditions, no propargylamine formation for acetaldehyde, isobutyaldehyde, benzaldehyde, and acetone was detected by ESI-MS analysis of the reaction mixtures (ESI†). These findings indicated the excellent formaldehyde selectivity of sterically bulky 2,2,6,6-tetramethyl-piperidine 1a.

Scheme 2. Selectivity study of A⁺-coupling reaction with different carbonyl-containing compounds.

After identifying the sterically bulky amine component for exclusive formaldehyde selectivity, we studied the effects of different reaction parameters on the formation of propargylamine 4b by HPLC analysis using formaldehyde, amine 1b, and alkyne 2b (Scheme 3). A calibration curve of propargylamine 4b with excellent linearity (R² = 0.9996) was constructed for quantification (Figure S2, ESI†).

Scheme 3. A⁺-coupling reaction of formaldehyde with amines and alkynes catalyzed by different gold(III) complexes.

Three cyclometallated gold(III) complexes 3a-c commonly used by our research group were screened to study their catalytic activities on the formation of propargylamine 4b (Scheme 3) (Table S2, ESI†). Bis-cyclometallated gold(III) complex 3a gave the highest yield among the three catalysts that is in line with our previous work. In addition, we studied the effects of different solvents, solvent ratio, temperature and amounts of formaldehyde, amine 1b, alkyne 2b and bis-cyclometallated gold(III) complex 3a on the yield of propargylamine 4b (Tables S3-S6, ESI†). Up to 56% yield could be achieved at 50 °C in 1 h using formaldehyde (10 µmol), amine 1b (50 µmol), alkyne 2b (100 µmol), and gold(III) complex 3a (50 µmol) in 1,2-dichloroethane (450 µL).

To achieve fluorescent detection of formaldehyde, we decided to attach a fluorophore to the alkyne component. The amount of formaldehyde can then be determined by measuring the fluorescent intensity of the resulting propargylamines. On the other hand, to minimize the background fluorescent signal coming from the unreacted fluorescent alkynes, excess reagents have to be removed after the A⁺-coupling reaction. To facilitate the washing step, the sterically bulky amines were attached to polymer-bound 2-chlorotrityl chloride resins to give resin-linked sterically bulky amine 1e according to literature. The A⁺-coupling reaction of formaldehyde, resin-linked sterically bulky amine 1e, and coumarin-linked alkyne 2c gave fluorescent propargylamines (Scheme 4).

Scheme 4. A⁺-coupling reaction of formaldehyde, resin-linked sterically bulky amine 1e and coumarin-linked alkyne 2c via propargylamine formation and cleavage of propargylamine 5a from the resin 4c.

The A⁺-coupling reactions of formaldehyde (0.02 mmol), resin-linked sterically bulky amine 1e (15 mg), coumarin-linked alkyne 2c (0.02 mmol) and bis-cyclometallated gold(III) catalyst 3a (0.01 mmol) were conducted in 1,2-dichloroethane at 50 °C for 1 h to give resin-bound propargylamine 4c (Scheme 4). To confirm the product formation, propargylamine 5a was cleaved from the respective resins by treatment with TFA/CH₂Cl₂ and confirmed by ESI-MS analysis (ESI†). Upon excitation at 355 nm, propargylamine 5a exhibited a maximum emission wavelength at 461 nm that is consistent with a coumarin chromophore (Figure 1A).

Figure 1. (A) Fluorescent spectrum of propargylamine 5a; (B) A good linearity (R² = 0.9937) between formaldehyde concentration (4-103 ppm) and the peak area of the propargylamine 5a determined by LC–MS/MS.
LC–MS/MS was used to determine the amount of the propargylamine 5a. The peak area, which represented the amount of propargylamine 5a, increased with the concentration of formaldehyde (from 4 to 103 ppm) (Figure 1B). A good linear relationship ($R^2 = 0.9937$) between the peak area and formaldehyde concentrations was observed, suggesting the readout from the LC–MS/MS can be used for quantification.

Under irradiation by a hand-held UV lamp at 365 nm, the brightness of the resin-bound propargylamine 4e was related to the formaldehyde concentrations. The observable change of the brightness could be straightforwardly differentiated by naked eyes to qualitatively detect the formaldehyde concentration as low as 10 ppm (Figure 2).

**Figure 2.** Resin-bound propargylamine 4e in dichloromethane under irradiation of UV lamp at 365 nm.

The selectivity experiments were performed by using resin-linked sterically bulky amine 1e and coumarin-linked alkyne 2e with acetaldehyde (250 µmol, ~18,400 ppm), isobutyraldehyde (250 µmol, ~30,000 ppm) and benzaldehyde (125 µmol, ~26,500 ppm), respectively (ESI†). No propargylamine was detected after cleavage of the resins by ESI-MS analysis. As a competitive experiment, only the corresponding propargylamine 5a derived from formaldehyde was detected by ESI-MS analysis in the A¹-coupling reaction of a mixture of 5,000 ppm of each of formaldehyde, acetaldehyde, isobutyraldehyde, and benzaldehyde. These experiments indicated the excellent formaldehyde selectivity of this method.

To study the applicability of this method for formaldehyde detection in food, crude water extract of dried Shiitake mushroom was used. It was found that formaldehyde in the mushroom water extract could be exclusively captured by the resin-linked sterically bulky amine 1e after reaction at 50 °C for 1 h as confirmed by the detection of propargylamine 5a in ESI-MS analysis (ESI†). In this connection, the A¹-coupling reaction is of excellent tolerance towards complex matrix in food samples.

**Conclusion**

In summary, a visual detection method for formaldehyde based on a gold(III) complex-mediated three-component coupling reaction of formaldehyde, amines and alkynes has been developed. No expensive instruments and sophisticated operation skills are required with fast reaction time of 1 h at 50 °C. Exclusive formaldehyde selectivity was achieved by using resin-linked sterically bulky amines. Signal readout can be achieved by using LC–MS/MS analysis or naked eyes. Excellent tolerance in complex reaction mixtures has been demonstrated by formaldehyde detection of crude mushroom water extracts. This highly selective method opens up a new direction for the development of rapid on-site detection of formaldehyde with diverse applications.

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**Notes and references**

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c00000x/


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