Biobased Synthesis of Gold Nanoparticles by Methanobactin

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Abstract. Preparation of gold nanoparticles with a narrow size distribution has enormous importance in nanotechnology. Methanobactin (Mb) is a copper-binding small peptide that appears to function as an agent for Cu sequestration and uptake in methanotrophs. Mb can also bind and catalytically reduce Au (III) to Au (0). In the presence of hydroquinone as a electron donator, Mb can catalyze Au(Ⅲ) reduce to Au(0) and yield gold nanoparticles continuously. In this paper, a quantitative assay method for the content of Mb has been used. Continuous reduction of Au (III) by Mb can be achieved by using hydroquinone as the reducing agent. Effect of pH, reaction temperature, reaction time on Mb-mediated synthesis of gold nanoparticles was studied. The optimal conditions of gold nanoparticles synthesis were as follows: the optimum pH value was 5.1, the optimum reaction temperature was 40 ℃, the optimum reaction time was 15-20min. The gold nanoparticles are extremely stable and can resist aggregation, even after several months.

Introduction

Methanotrophs are a group of Gram-negative eubacteria that utilize methane as the sole energy and carbon source [1]. In the first step of carbon assimilation by methanotrophs, methane is oxidized to methanol by Methane Monooxygenase (MMO). MMO exists in both the iron-containing soluble form (soluble methane monooxygenase, sMMO) and the copper-containing membrane-bound particulate form (particulate methane monooxygenase, pMMO) [2]. Copper requirements are higher for methanotrophic bacteria. Since the expression of copper containing particulate MMO（pMMO） depends on copper availability [3]. One way that methanotrophic bacteria meet their high copper requirement is by the biosynthesis and release of high affinity copper binding compounds called Methanobactin (Mb). Methanobactin (Mb) is a copper-binding small peptide that appears to function as an agent for copper detoxification, sequestration and uptake in methanotrophs. Mb has been identified in the extracellular fractions of both Methylosinus trichosporium OB3b [4,5] and Methylococcus capsulatus Bath [6]. Mb is composed of a tetrapeptide, a tripeptide, and several unusual moieties. The copper-loaded Mb from Methylosinus trichosporium OB3b is a 1217 Da peptide that binds and coordinates copper by two thionyl imidazolate moieties in a N2S2 distorted tetrahedral arrangement [4]. Mb can also bind and reduce Au (III) to Au (0) [5]. In the previous paper, we have demonstrated a facile Mb-mediated synthetic route to prepare gold nanoparticles [7]. In this paper, the effect of pH, reaction temperature and reaction time on the formation of gold nanoparticles were further studied.

Materials and methods

Culture of methanotrophs. Methylosinus trichosporium 3011 was obtained from the Institute of Microbiology and Virology. Methanotrophs were cultivated with mineral salts medium[7]. Cultivation was carried out in a 250 mL closed vial with 50 mL mineral salts medium on a shaker at 150 rpm. and

30 ℃. Methane was added periodically by establishing a partial vacuum in the flask and backfilling with methane and air（1:10）. The gas phase methane level was maintained at about 10% (V/V) by exchanging the headspace volume three times per day.

Culture conditions for Mb production. A volume (5 mL) of Methylosinus trichosporium 3011 cultures from mid exponential growth phase was used to inoculate mineral salts medium (50 mL) with various concentration of methanol in a 250 ml closed conical flask. Methanol was added after sterilization of the mineral salts medium. Cultures were grown on air or mixture of methane and air (1:10, V/V) at 30℃ with shaking (150 rpm). The cultures were grown to stationary phase for Mb production. Copper (Ⅱ) was omitted from the medium.

Preparation of Mb. Mb from the spent medium of Methylosinus trichosporium 3011 was isolated as previously described for Methylococcus capsulatus Bath by Choi et al. [6] and Methylosinus trichosporium OB3b by Kim et al. [10]. The cells were removed by centrifugation at 10,000×g for 30 min. The supernate was loaded onto a 2.5 × 20-cm Diaion HP-20 column (Mitsubishi Chemical Holdings, Tokyo, Japan). The bound Mb was washed with two column volumes of H_2O and eluted with 40% methanol: 60% H_2O. The eluant was lyophilized for concentration and storage. The freeze-dried samples following chromatography on Diaion HP-20 columns were the source of Mb used in this study.

Results and discussion. According to Yoon et al [8], we have developed a method to determine Mb quantitatively by using its high affinity for copper based on the comparative exchange of copper (II) from an indicator dye CAS. The combination of CAS, HDTMA and copper ratio of 1.0:8.0:0.7 proved to be most advantageous for Mb detection [9]. In this paper, this method has been used to determine Mb concentration. As shown in Figure 1, the changes in the absorbance spectra of the CAS after the release of copper were detected with EDTA and Mb. The spectrum of the CAS-Cu-HDTMA complex clearly showed a sharp absorbance peaks at 605 nm as a function of copper removal from CAS. Both Mb and EDTA exhibit a linear dependence of the absorbance at 605 nm versus concentration of the chelator. So the EDTA concentration was chosen as an equivalent of Mb content for detection of Mb concentration in solution. A working curve between the change of absorbance of CAS-Cu and the EDTA concentration was established (data not shown).

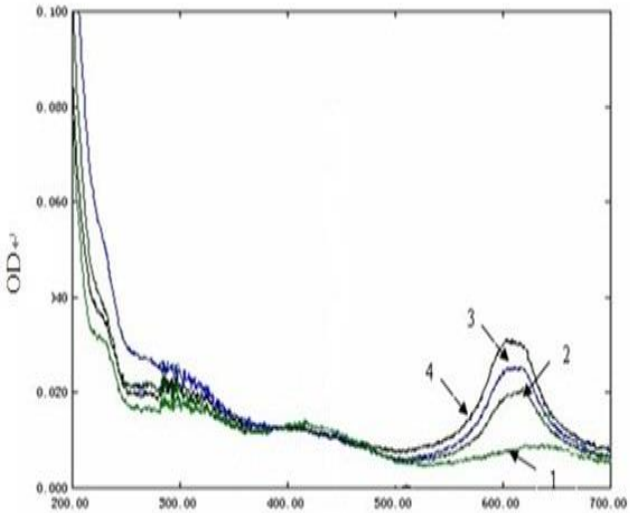

Figure. 1 The absorbance spectra of the CAS reagent
1: CAS+HDTMA；
2: CAS+HDTMA+ $CuSO_4$+EDTA；
3: CAS+HDTMA+ $CuSO_4$+mb；
4: CAS+HDTMA+ $CuSO_4$

Mb can bind Cu (II) ions, and reduce them to Cu (I) ions and stabilizes the resulting Cu (I) ions using a mechanism that is not well understood. It has been reported that Mb from Methylosinus trichosporium OB3b contains two oxazolone rings and two thiol groups that are directly involved in binding Cu (I) ions. Mb can also be able to bind and reduce a number of other metals, including gold. Researchers have determined that gold in the 3+ oxidation state, Au (III), can be reduced to its zero oxidation state, Au (0) at or below ratios of one Au (III) per Mb molecule. At ratios of Au (III) to Mb above one to one, Mb binds and catalytically reduces Au(III) to Au(0) with the concomitant production of gold nanoparticles [5]. We

have reported a facile Mb-mediated one-step synthetic route to prepare monodispersed gold nanoparticles. Continuous reduction of Au (III) by Mb can be achieved by using hydroquinone as the reducing agent[7].

In this paper, this approach that Mb binds and catalytically reduces Au (III) to Au (0) has been used for the synthesis of gold nanoparticles. Continuous reduction of gold by Mb was achieved with hydroquinone as electron donor. As been shown in Figure 2, Figure 3 and Figure 4, it has been found

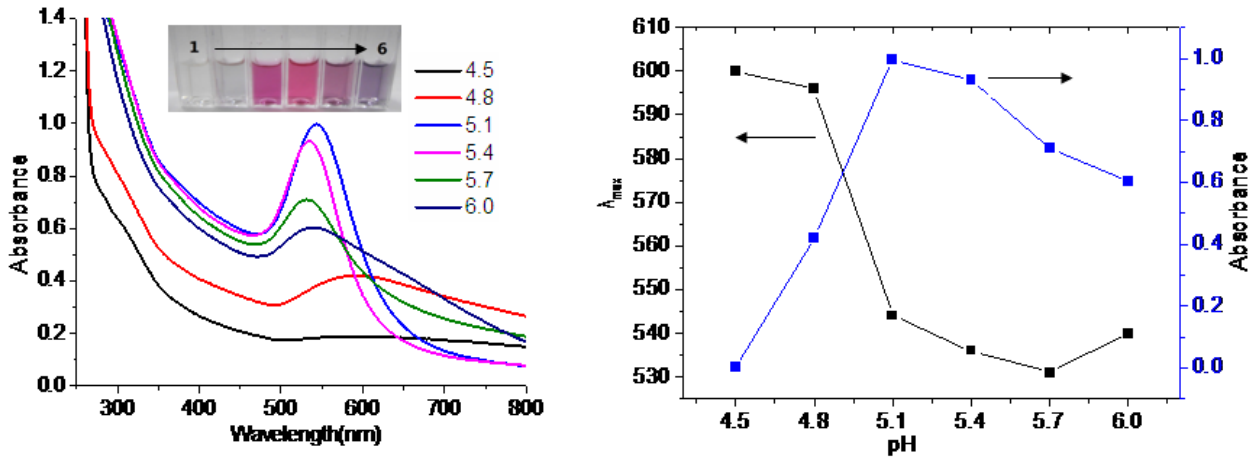

Figure. 2 The effect of pH on the formation of gold nanoparticles.

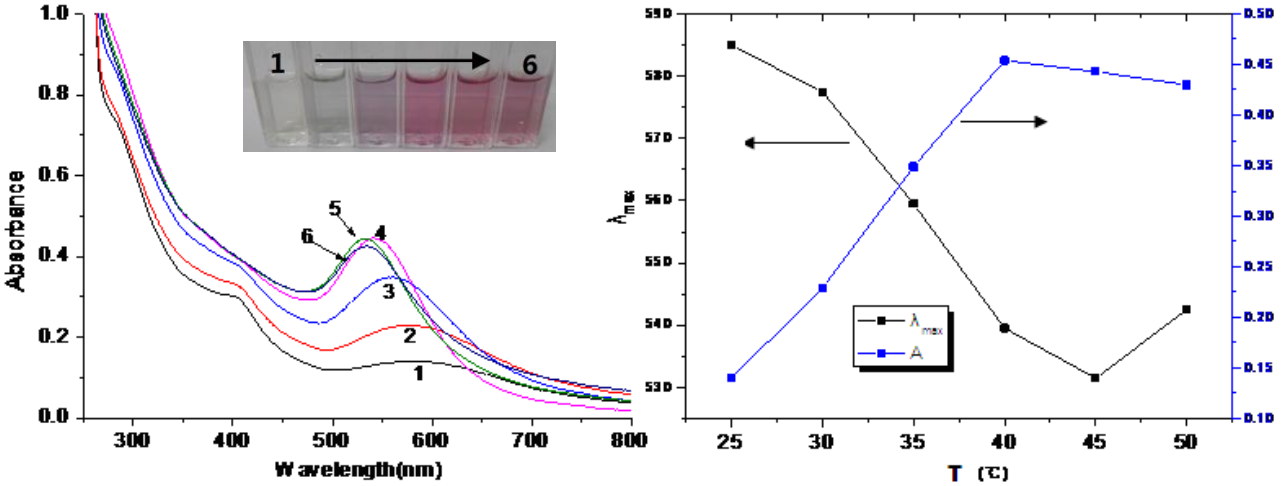

Figure. 3 The effect of reaction temperature on the formation of gold nanoparticles.

1: 25℃; 2:30℃; 3:35℃; 4: 40℃; 5:45℃; 6: 50℃

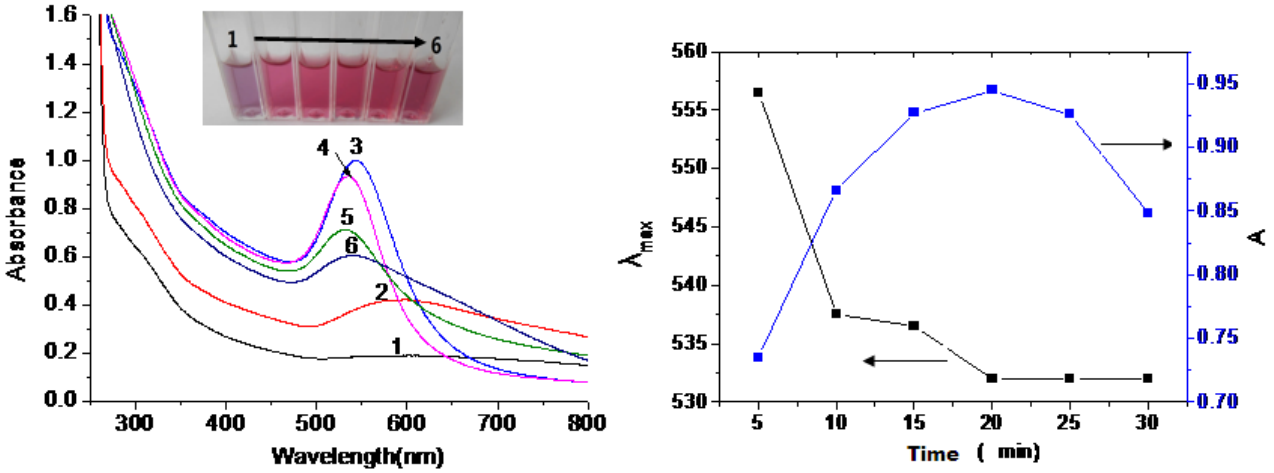

Figure. 4 The effect of reaction time on the formation of gold nanoparticles.

1:5min; 2:10min; 3:15min; 4: 20min; 5:25min; 6: 30min

that the optimum pH value was 5.1; the optimum reaction temperature was 40 ℃; the optimum reaction time was 15-20min. Gold nanoparticles can be seen with the naked eye, as they turn a gold solution from yellow/gold to a deep cranberry/red. The gold nanoparticles also display a characteristic absorbance of approximately 540 nm-550 nm on absorption spectra. Also, the gold nanoparticles are extremely stable and can resist aggregation, even after several months.

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