Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 30-36

Protein Biomass as New Adsorbent for Precious Metal Ions

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Abstract

Use of biomass as alternatives to petrochemicals has attracted a broad range of scientific and industrial interests. We propose here a novel environmentally-friendly recycle system for precious metal ions such as gold and platinum ions using protein-rich biomass. My keynote lecture is composed of three pieces; (1) selective adsorption of precious metal ions onto proteins, (2) the mechanism study of the metal ion adsorption, and (3) demonstration of selective recovery of precious metal ions from actual copper refining solution. A biomass was found to have an extremely high loading potential 250g/kg-biomass for gold ions. This article describes not only scientific investigations but also practicality of the strategy. The recycle system proposed here will enable selective recovery of precious metal ions from industrial wastewater without extra emissions of CO_2.

Keywords: Biomass, Metal recycle, Bioadsorption, Protein reuse, Precious metal group, PGM.

1.0 Introduction

In the last decade, carbon dioxide emission has become one of the major global concerns and petrochemical-independent products have attracted much attention. Biomass, such as agricultural products and aquatic resources, does not emit extra carbon dioxide when burned. Reproducible biomass is therefore expected to be carbon-neutral and petrochemical-independent [1]. Several research groups reported that various types of biomass (e.g. microorganisms, polyphenols and polysaccharides) facilitate adsorption of several metal ions in aqueous solution [2-4]. However, selective recovery of targeted metal ions from the aqueous solution, and study of the adsorption mechanism have been challenging tasks, because biomass is an unpurified product composed of a wide variety of substances. This drawback also prevents any practical demonstration of using biomass for metal recycling from industrial waste containing various metal ions. Protein-rich biomass is produced as a byproduct in the food and agricultural industries, and is usually inexpensive (far less than 100 US$/kg). Owing to the high protein content (~90%), biomass is an attractive source of peptides and amino acids [5].

Proteins have various functional groups in their own structures. Some of them have specific interaction with metal ions and the interaction often plays an important role in a living cell and in enzymatic reaction. The specific interaction of proteins with metal ions has potential to serve for a selective adsorption of a targeted metal ion. In this study, we take proteins as a noble adsorbent material and investigated the interaction between proteins and noble metal ions (Au(III) and Pd(II)).

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In the present study, we investigated and revealed strong and selective interactions between proteins and precious metal ions. Studies on synthesized peptides identified one of the adsorption sites of precious metal ions in proteins. Finally, we demonstrated that protein-rich biomass acts as an adsorbent selective for Au^{3+} and Pd^{2+} in industrial wastes.

2.0 Materials and Methods

2.1 Au adsorption test by peptides and proteins

Ovalbumin, lysozyme and bovine serum albumin (BSA) were used as an adsorbent. A typical adsorption experiment was conducted as follows: a protein (1 g/l) was dissolved in an Au(III) aqueous solution (10 ppm) of which pH was adjusted to 4.0 by NaOH. The protein-Au solution was mixed for 1 hour at room temperature. The solution was then filtered by a ultrafiltration membrane (MWCO 10,000 Da). The Au concentration in the filtrate was determined by ICP-atomic emission spectroscopy (Perkin Elmer Optima 3000) and atomic absorption spectroscopy (Shimadzu AA-600). Various tripeptides (N-Ac-X-Gly-Leu) were synthesized on Merrifield resin based on the well-established 9-fluorenylmethoxycarbonyl chemistry (Bodanszky *et al.*)[6]. The N termini of the tripeptides were acetylated using acetic anhydride. Cys-containing peptide was reduced by dithiothreitol (10 mM) overnight and washed with 0.1 N HCl. The N-acetyl tripeptides immobilized on the resin were subjected to adsorption. The N-acetyl tripeptides (0.7 mol) immobilized on the resin were added to 10 ppm metal ion solutions (5 ml, pH 4.0) containing 0.5 M $NaClO_4$. After gentle stirring for 1 h, the solutions were centrifuged. Supernatants were subjected to atomic absorbance spectrophotometry to determine free metal ions in solution.

2.2 Competitive adsorption of metal ions to proteins

Metal-ion standard solutions (1000 ppm) were mixed and diluted with 0.1 M HCl to prepare an aqueous solution containing five different metal ions (Au^{3+}, Pd^{2+}, Pt^{2+}, Cu^{2+} and Zn^{2+}). Proteins (2 mg) were dissolved in the solution (10 ml) containing the metal ions, followed by gentle stirring for 1 h at room temperature. The solutions were filtered through an ultrafiltration membrane with a molecular cutoff of 10 kDa (Amicon Ultra-4, Millipore). The filtrate was subjected to inductively coupled plasma (ICP) atomic emission spectrometry (Optima 3100 RL; Perkin Elmer) to determine the concentration of free metal ions. The adsorption percentage (R) of metal ions was calculated by the following equation:
R = 100 × [(Initial metal ion concentration) – (Metal ion concentration in the filtrate)]/ [Initial metal ion concentration] A solution containing five different metal ions without proteins was filtered through the ultrafiltration membrane and no adsorption of metal ions to the filtration membrane was verified.

2.3 Adsorption of metal ions to protein-rich biomass

Protein-rich biomass tested here was soybean protein (Fuji Oil Co, Japan), chicken egg-shell membrane (Q.P. Corporation, Japan) and seasoning Pollack-roe membrane (Yamaya Communications, Japan). Each biomass (10 mg) was added to 5 ml 0.1 M HCl solution containing five different metal ions (Au^{3+}, Pd^{2+}, Pt^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+}; 10 ppm each). After gentle stirring for 1 h at room temperature, the solution was filtered and the filtrate was subjected to ICP-atomic emission spectrometry to determine free metal ions in solution. In the case of soybean protein, prior to filtration, 0.1 M sodium acetate solution was added to

adjust the pH to 4. Soybean protein was insoluble around pH 4. The precipitated soybean protein was removed by filtration and the filtrate was subjected to ICP-atomic emission spectrometry.

3.0 Results and Discussion

3.1 Au adsorption by proteins

Figure 1 depicts the adsorption isotherms for Au(III) ion at 298 K. The Au adsorption onto proteins increased with increasing the protein concentration. All the tested proteins could adsorb Au ions. The adsorbed Au ions were easily detached from proteins by the addition of 2 mM thiourea (data not shown).

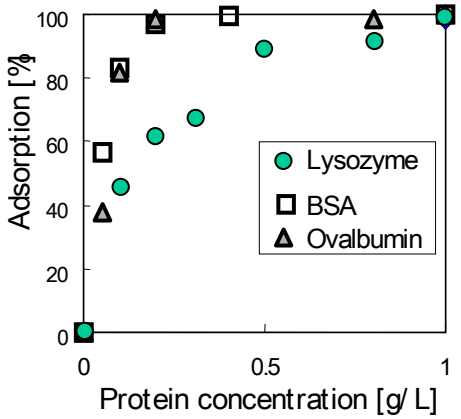

Fig. 1 Adsorption isotherm for Au ions at 298 K.

The number of adsorbed Au ions at 95% adsorption varied with the proteins (Table 1). Interestingly, the total numbers of a thiol group and a histidine residue in proteins was in a good agreement with the numbers of adsorbed Au ions. A thiol group is generally known to have a strong interaction with Au. And Best et al. demonstrated that tripeptide containing histidine has also a strong interaction with Au(III) ion [7]. These interactions can account for the Au adsorption on the proteins.

Table 1. The numbers of adsorbed Au(III) ion, a histidine residue and a thiol group in a protein molecule

Proteins	Au(III) ion adsorbed	Histidine	Thiol group
Ovalbumin	11-12	7	4
BSA	17-18	17	0.7
Lysozyme	1-2	1	0

The competitive adsorption of several metal ions (Au, Pd, Pt, Cu, and Zn, 10 ppm each) on the proteins (1 g/l) revealed that all the proteins tested exhibited strong affinity with Pd(II) ion (Fig. 2). This result agrees with the previous report that the interaction of the tripeptide with Pd(II) ion was stronger than that with Au(III) ion [7]. The present study suggests that protein itself can be utilized as an adsorbent for precious metal ions such as Au(III) and Pd(II).

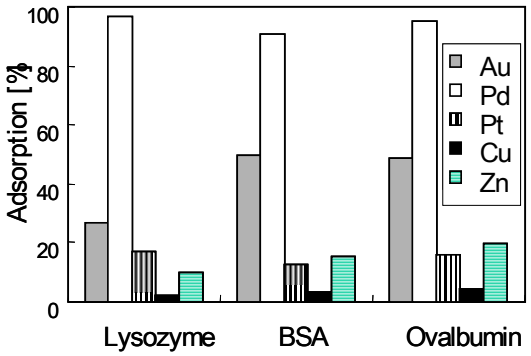

Figure 2 Adsorption of various metal ions onto proteins. The protein concentrations were 1 g/L and the concentrations of metal ions were 10 ppm.

3.2 Competitive adsorption of metal ions to proteins

There have been many studies on adsorption of metal ions using biomaterials but very few have reported selective adsorption of precious metal ions in the presence of different metal ions [8,9]. Fig. 2 shows competitive adsorption of metal ions using 1.0 g/l proteins. Interestingly, all the proteins tested selectively adsorbed Pd^{2+} and Au^{3+} to varying degrees, while little adsorption of Pt^{2+}, Cu^{2+} and Zn^{2+} was observed. Although many papers have revealed interactions between amino acid residues (e.g. His) and various transition metal ions (e.g. Cu^{2+} and Zn^{2+}), there is, so far, no report on the selective adsorption of precious metal ions to proteins. We believe that the present study describes for the first time that Pd^{2+} and Au^{3+} were selectively adsorbed to proteins. It should be noted that proteins selectively adsorbed Pd^{2+} and Au^{3+} in an acidic aqueous solution containing 0.1 M HCl, because in many cases metal ion wastes are in acidic conditions.

3.3 Adsorption isotherms of precious metal ions to proteins

The adsorption isotherms of precious metal ions (Au^{3+} and Pd^{2+}, 10 ppm each) to the purified proteins [lysozyme, bovine serum albumin (BSA) and ovalbumin] were examined. The adsorption of Au^{3+} and Pd^{2+} to the proteins increased with protein concentration. The adsorption of Au^{3+} and Pd^{2+} reached ~100% at a protein concentration of 0.6 g/l. All the proteins tested here showed high adsorption capacity for Au^{3+} and Pd^{2+}. For example, BSA adsorbed 24.3 Au ions and 65.7 Pd ions per protein molecule, and lysozyme adsorbed 2.7 Au ions and 11 Pd ions per protein molecule. The adsorption capacity varied greatly with the type of protein.

In general, electrostatic adsorption of metal ions to adsorbents is influenced by pH because forms of metal ions and their ionic charges depend on pH. The effect of pH on the adsorption of Au^{3+} and Pd^{2+} to proteins was studied. Both Au^{3+} and Pd^{2+} adsorption was affected by pH. Au^{3+} adsorption decreased above pH 6 but Pd^{2+} adsorption decreased below pH 3. There was little difference in the pH effect among the proteins. Taking account of the pI diversity of proteins tested, the surface charge of the proteins was not so important for the adsorption of these precious metal ions. The present study employed $[AuCl_4]^-$ and $[PdCl_4]^{2-}$ as Au and Pd ions. The Cl ligand in these complexes can be replaced by OH [10]. The stability of these tetrachloro complexes is influenced by pH and Cl^- concentration. The pH effects on

adsorption were probably attributed to the stability of the metal complexes. Ionic strength of solution is also a significant factor for electrostatic adsorption. Varying the concentration of sodium perchlorate from 0 to 1 M to control the ionic strength of the solution, we did not observe any notable difference in the Au^{3+} and Pd^{2+} adsorption to the proteins. The effects of pH and ionic strength suggest that there was a major mechanism for the adsorption of these precious metal ions to proteins other than electrostatic interaction.

3.4 Investigations on the precious metal ion–protein adsorption mechanism

Au^{3+} and Pd^{2+} are likely to be reduced to produce nanoparticles. It is possible that the interaction between the proteins and precious metal ions produced the nanoparticles. A gold or a palladium nanoparticle solution generally has a visible color depending on the nanoparticle size [11,12]. In the present study, we did not observe any change in color of the solution when mixing precious metal ions and proteins, indicating that there was no production of nanoparticles.

Another possibility to be clarified is the production of fine particles that were larger than nanoparticles and were precipitated. We tried desorption of the precious metal ions from proteins using thiourea. Thiourea reduces Au^{3+} to Au^{+} and forms a stable 2:1 complex in aqueous solution [13]. Addition of excess thiourea (2 mM) to the Au^{3+}–protein complex resulted in >95% desorption of Au ions from proteins. Au ions desorbed from proteins could be passed through an ultrafiltration membrane with a molecular cutoff of 10 kDa. This result means that Au^{3+} was not reduced to form fine particles or precipitates by its interaction with proteins. Using the N-acetyl-His-Gly-Leu peptide, competitive adsorption of metal ions was investigated in the presence of various metal ions (Au^{3+}, Pd^{2+}, Pt^{2+}, Cu^{2+} and Zn^{2+}). We observed selective binding of Au^{3+} and Pd^{2+} to the tripeptide. Most of the Au^{3+} and Pd^{2+} bound to the tripeptide were readily desorbed from the tripeptide by the addition of an excess amount of thiourea (data not shown). These results with the tripeptides agree well with those of the proteins, strongly suggesting that the His-containing peptide was one of the binding sites of Au^{3+} and Pd^{2+}in proteins.

However, lysozyme, BSA and ovalbumin have one, 17 and seven His residues, respectively. The number of His residues does not account for the adsorbed precious metal ions listed in Table 1. Moreover, more Pd^{2+} than Au^{3+} ions were bound to proteins. There may be another adsorption mechanism for Pd^{2+} adsorption different from that of Au^{3+}. Further investigations on other binding sites are required.

3.5 Protein-rich biomass as adsorbents selective for precious metal ions

From a practical viewpoint, an adsorbent for metal ions should be inexpensive. We adopted protein-rich biomass as an adsorbent instead of the purified proteins tested in the above investigations. Since protein-rich biomass has many attractive features, e.g., its abundance, low cost, reproducibility and zero-emission of CO_2, the efficient use of biomass in various industries is desired throughout the world. Here, we employed soybean protein, chicken egg-shell membrane, and seasoning Pollack-roe membrane as protein-rich biomass, which are produced in huge amounts as by-products in the Japanese food industry. The protein contents of soybean protein, chicken egg-shell membrane and seasoning Pollack-roe membrane are approx. 91, 90 and 85%, repsectively.

We studied the competitive adsorption of metal ions to the protein-rich biomass. All the biomass tested adsorbed Au^{3+} and Pd^{2+}, but did not adsorb Cu^{2+}, Ni^{2+} to such an extent. In the case of Pt^{2+} adsorption, there were differences among the types of biomass. Egg-shell membrane effectively adsorbed Pt^{2+}, while soybean protein adsorbed only a small amount of Pt^{2+}. Pt^{2+} adsorption to the egg-shell membrane was probably due to electrostatic interaction, because varying the ionic strength using $NaClO_4$ reduced adsorption (supporting information, S2). The adsorption selectivity of protein-rich biomass was very similar to that of purified proteins (Fig. 1). These results allow us to conclude that protein-rich biomass, as well as purified proteins, works as an adsorbent selective for precious metal ions.

4.0 Conclusion

Proteins exhibit specific interactions with various metal ions, which play important roles in a living cell. Here, we found that various proteins selectively adsorbed precious metal ions at a wide range of pH values. Studies on protein sequences and on synthesized peptides revealed that a histidine-containing sequence had specific interactions with precious metal ions (Au^{3+} and Pd^{2+}). We then investigated a few types of protein-rich biomass as adsorbents for precious metal ions. In the presence of various transition metal ions, Au^{3+} and Pd^{2+} were also selectively adsorbed onto the biomass tested. The bound precious metal ions were recovered by nitrohydrochloric acid after charring the metal-bound biomass. Finally, the successful recovery of Au^{3+} and Pd^{2+} from a metal refining solution and a metal plating waste was demonstrated using the biomass. An environmentally-friendly recycling system was proposed for precious metal ions using protein-rich biomass.

Acknowledgements

We thank Q.P. Corporation, Fuji Oil and Yamaya Communications for providing protein-rich biomass. We thank Dr. M. Waki for his valuable advice on peptide synthesis. We thank Muromachi Technos for providing metal plating waste containing precious metal ions.

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