Chiral Self Assembled Monolayers as Resolving Auxiliaries in the Crystallization of Valine

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ABSTRACT: Chiral drugs are a subgroup of drug substances that contain one or more chiral centers. For reasons of safety and efficacy, the pure enantiomer is usually preferred over the racemate in many marketed dosage forms. Thus, resolution of racemic mixtures is an active area of research. In this work, chiral self assembled monolayers (SAMs) on gold were employed as resolving auxiliaries in the crystallization of the amino acid valine. Results showed the ability to obtain one enantiomer in excess on the crystals grown on the chiral SAMs when starting with racemic solutions. The enantiomer obtained in excess was the one having opposite chirality to the monolayer being used. In addition, it was possible to obtain crystals of the pure enantiomer when starting with a solution having an enantiomeric excess value of 50%. Control experiments carried out without chiral SAMs showed that at equilibrium, mixtures of the pure enantiomer and racemic compound were obtained under these conditions. The enantiomer obtained on the chiral SAMs was the one that was initially present in excess regardless of the chirality of the monolayer being used. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3931–3940, 2010

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INTRODUCTION

Chirality is defined as the geometric property responsible for nonidentity of an object with its mirror image. A *chiral* object may exist in two *enantiomorphic* forms which are mirror images of each other. Objects that are super imposable on their mirror images are *achiral*. At the molecular level, there are achiral as well as chiral molecules. Chiral molecules exist in two *enantiomeric* forms; the term enantiomorphic is generally applied to macroscopic objects. The oldest known manifestation of molecular chirality is the *optical activity*, the property, that is, exhibited by the rotation of the plane of polarization of light. The two enantiomers of a given compound have opposite optical activity. One is positive or *dextrorotatory*, while the other is negative or *levorotatory*.[1]

An equimolar mixture of two enantiomers whose physical state is unspecified or unknown is called a *racemate*. The separation of the two enantiomers that constitute a racemate is called a *resolution* or an *optical resolution*. Crystalline racemates may belong to one of three different classes, namely, *conglomerate*, *racemic compound*, or *pseudoracemate*. A conglomerate is a mechanical mixture of the crystals of the two pure enantiomers. The most common type of crystalline racemate is that in which the two enantiomers are present in equal quantities in a well-defined arrangement within the crystal lattice. The resultant homogeneous solid phase is called a racemic compound. The third possibility corresponds to the formation of a solid solution between the two enantiomers coexisting in an unordered manner in the crystal. This solid solution is known as a pseudoracemate.

Chiral drugs are a subgroup of drug substances that contain one or more chiral centers. More than one-half of marketed drugs are chiral.[2] In some cases, chiral drugs are marketed as racemates as it is frequently costly to resolve a racemic mixture into pure enantiomers. Opposite enantiomers often differ significantly in their pharmacological,[3] toxicological,[4] pharmacodynamic, and pharmacokinetic[5,6] properties. From the points of view of safety and efficacy, the pure enantiomer is preferred over the racemate in many marketed dosage forms.[7] Thus, resolution of enantiomer mixtures is an active area of research.

Racemic compound systems account for more than 90% of all racemic mixtures[8] and cannot be resolved by direct crystallization. A common route to resolution is to react the racemic mixture with an optically

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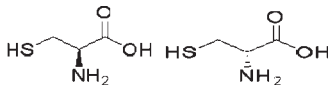
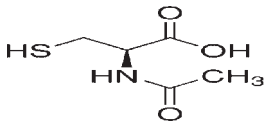
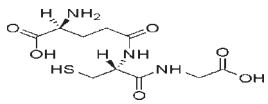
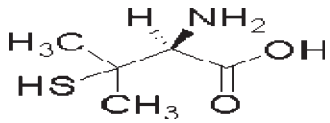
pure resolving agent, such as an acid or a base, to give a mixture of diastereomers which are nonenantiomeric and hence have different physical properties. The phase diagram of such a mixture is generally asymmetric and crystallization will yield crystals enriched in one of the isomers.

Chiral surfaces and interfaces have received considerable interest in recent years because of their importance in separation of enantiomers.[9] Cleavage of quartz or calcite, materials with chiral bulk structures, leads to surfaces that are naturally chiral. Chiral surfaces can also be created by anchoring or adsorption of a chiral molecule on a nonchiral surface.[10–12] The ability to differentiate between enantiomers of a chiral molecule is one of the most interesting properties of a chiral surface. The use of both naturally chiral surfaces and chiral self-assembled monolayers as resolving auxiliaries in the crystallization of racemates has been previously reported. Naturally chiral bulk crystalline surfaces have been shown to display chiral selectivity when immersed in a racemic solution.[13] Self-assembled monolayers of (+)-L and (−)-D cysteine on gold substrates were used as resolving auxiliaries in the crystallization of rac-glutamic acid (a conglomerate) and rac-histidine (a racemic compound).[14,15] Similarly, chiral discrimination between (*R*)- and (*S*)-thalidomide enantiomers was achieved using SAMs of (*R*)- and (*S*)-1,1′-binaphthalene-2, 2′-dithiol (BNSH).[16] Chiral discrimination between (D)- and (L)-phenylalanine enantiomers was achieved using SAMs of (BNSH).[17]

In this work, we used self assembled monolayers (SAMs) of L-cysteine, D-cysteine, *N*-acetyl-L-cysteine, L-glutathione, and D-penicillamine on gold as resolving auxiliaries in the crystallization of the amino acid valine which crystallizes as a racemic compound.

Table 1. Chemical Structures of the Chiral Self-Assembled Monolayers

Monolayer	Chemical Structure
L and D-cysteine	
N-Acetyl-L-cysteine	
L-Glutathione	
D-Penicillamine	

MATERIALS AND METHODS

Materials

L-Cysteine (LC) was obtained from Acros Organics, Morris Plains, NJ. D-Cysteine (DC), L-glutathione (LG), D-penicillamine (DP), *N*-acetyl-L-cysteine (NALC), DL-valine, d-valine and L-valine were purchased from Sigma–Aldrich Corp., St Louis, MO. Deionized water was obtained from a Barnstead Nanopure Infinity water purification system. Titanium (99.995%) and gold pellets (99.999%) were purchased from Kurt J. Lesker Company, Clairton, PA. Table 1 shows the chemical structures of the chiral SAMs used in this work.

Gold Surfaces and SAMs Preparation

Microscope glass slides were immersed in "piranha solution" (3:1 concentrated H_2SO_4/30% H_2O_2) for 30 min. *Caution*: Piranha solution reacts violently with organic materials and should be handled with extreme care. The glass slides were then thoroughly washed with water and rinsed with copious amounts of ethanol and blown dry with nitrogen. Gold surfaces were prepared by evaporation of titanium onto glass slides, followed by evaporation of gold. The deposition of metals on the glass slides was carried out using an electron beam evaporator (Thermionics Vacuum Products, Clawiter Hayward, CA, base vacuum of 10^{-7} torr). The slides were first coated with a thin layer of titanium (~100 Å) to promote adhesion followed by a layer of gold (~500 to 1000 Å). The SAMs were formed on the gold surfaces by immersing the substrates overnight in 10 mM solutions of L-cysteine, D-cysteine, *N*-acetyl-L-cysteine, L-glutathione, and D-Penicillamine in deionized water. After removal from solution, the substrates were rinsed with copious amounts of deionized water and blow dried with nitrogen. Self assembled monolayers of cysteine on gold have been previously studied using X-ray photoelectron spectroscopy (XPS) and Scanning Tunneling Microscopy (STM).[14,18] It has been reported that cysteine molecules are absorbed onto gold through the sulfur atom, while leaving the other two groups (COOH and NH_2) free. STM images showed that LC chemisorption induces the lifting of the $(22 \times \sqrt{3})$ reconstruction of the Au(111) surface and that the LC adlayer adopts a hexagonal $(\sqrt{3} \times \sqrt{3})R30^\circ$ structure.[18] The self-assembly of NALC on gold has been studied by *in situ* attenuated total reflection infrared (ATR-IR) spectroscopy, polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS), and a quartz crystal microbalance (QCM)[19] and it has been reported that

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