Supramolecular asymmetric photochirogenesis mediated by synthetic antibody: asymmetric photocyclodimerization of 2-anthracenecarboxylate mediated by synthetic scFv antibody

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Asymmetric photochirogenesis has been one of the major interest in recent years because of several unique advantages over the thermal counter, since the photochemical process proceeding through the electronically excited state then often provided strained and/or thermally difficult-to-accessible products of unique structures in a single step. However, some drawbacks, such as the weak and short excited-state interactions, cause the difficulty to control the reaction.

Recently, supramolecular approach for asymmetric photochirogenesis has received much attention, causing the possibility of improving these issues. Due to the chirality properties, the bio- and biorelated molecules, such as cyclodextrins and proteins, have been widely used as the chiral reaction media for asymmetric synthesis. Previously, we have reported the supramolecular enantiodifferentiating photocyclodimerization of 2-anthracenecarboxylate (AC) mediated by mammalian serum albumins as a chiral reaction media in detail to render the cyclodimers with high enantioselectivities of up to 97 % enantiomeric excess (ee) (Fig. 1).

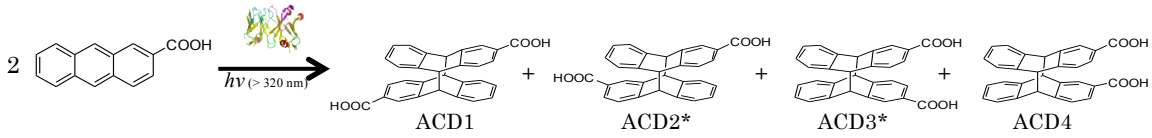

Figure 1. Supramolecular Photocyclodimerization of 2-Anthracenecarboxylate.

In this work, we reported the first successful attempt of utilizing synthesized single-chain antibody (scFv) using a conventional phage display technique to SMAP photocyclodimerization of 2-anthracenecarboxylate as a chiral media. We were also interested in the ground- and excited-state interaction between AC and synthetic scFv antibody, and also the effects of external factors to the photochirogenesis of AC mediated by scFv antibody, including temperature, pH and ionic strength.

First, the ligand consisted with AC dimer (ACD) 3 as a target product, polyethylene glycol (PEG) spacer, and biotin (Fig. 2) was synthesized by solid phase Fmoc-peptide synthesis strategy, and purified by HPLC. The ligand was applied as a hapten in a phage display technique from human

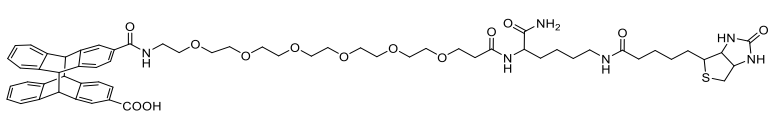

Figure 2. Structure of ACD3-PEG5-K(Bio) ligand.

single fold scFv libraries Tomlinson J. After three rounds of selection, we successfully isolated several candidates showing high affinity to ACD3 based on the results of the enzyme-linked immunosorbent assay (ELISA) technique. The candidate antibodies were selected again by examining the selectivity of ACD3 from dimer mixture. The sequence of candidate synthetic antibodies was determined by DNA sequencer and subcloned into an expression vector. The synthetic scFv antibody (Fig. 3) was expressed in *E.coli*, and purified with Ni-NTA column and gel permeation chromatography with a high purity.

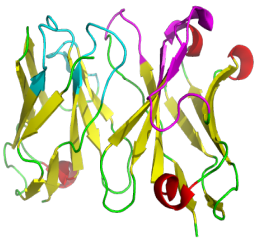

Figure 3. Structure of synthetic scFv antibody

Supramolecular enantiodifferentiating [4+4] photodimerization of AC mediated by the synthetic antibody was conducted in different AC/antibody ratios in the Ar bubbled Tris-HCl buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.5). The reaction solution was added to 1.0 cm × 1.0 cm quartz cell, and the cell was incubated 30 min at room temperature in the dark. The solution was irradiated by Xe lamp with the glass filter for the output of >320 nm wavelength for 60 min at 20 °C. The UV-spectrum of the solution was recorded before and after irradiation in order to confirm the conversion. Then, the synthetic antibody in the mixture was denatured by addition of acetonitrile and incubation overnight. The solution was then filtrated to remove the denatured protein, and the filtrate was injected into the ODS - OJ-RH columns tandemly connected with the fluorescence-detector-equipped HPLC.

As shown in Fig. 4, mediated synthetic scFv antibody significantly induced %ee of ACD 3 up to 37.5%. Also, the product distribution of ACD3 and ACD4, which is another *head-to-head* product, that usually be suppressed in the aqueous solution due to the ionic repulsion between carboxylate groups. Contrarily, the *head-to-tail* products, which are more favorable in the aqueous solution, were diminished.

[AC]/ [scFv]	%Product Distribution				%ee		HH/HT
	ACD1	ACD2	ACD3	ACD4	ACD2	ACD3	
∞	41.4	34.0	14.5	10.1	−0.3	+0.4	0.3
4	20.2	15.5	27.1	37.2	−6.0	+31.6	2.0
3	18.4	13.8	27.5	40.3	−9.6	+35.6	2.1
2	13.8	12.5	30.0	43.7	−8.4	+37.5	2.8
2[a]	5.3	4.4	32.7	57.6	−26.9	+42.9	9.3

Figure 4. The result of photodimerization of AC mediated by synthetic antibody ([a]the experiment was conducted at 4 °C)

Even though we could obtain only qualitative results, the antibody and AC established the concrete ground-state interaction. Fortunately, we found that the contribution is higher when the temperature was lowered from 20 to 10 °C. The fluorescence lifetime at 10 °C of the AC in free state and inside the antibody cavity were estimated to be 17.9 and 1.60 ns, respectively.

From this temperature effect, we decided to study the effects of external factors upon photochirogenesis. First, the temperature during incubation and irradiation was set to 4 °C. Then, the pH of Tris-HCl buffer was varied among 7.5, 8.0 and 8.5. Finally, the NaCl concentration was varied from 75 mM to 600 mM to study the effect of ionic strength to the reaction.

As also shown in Fig. 4, the results of photodimerization at 4 °C showed slightly increase of %ee, but significant improvement in HH/HT ratios up to 3 times. The ionic strength and the pH also have moderate effects to the product distribution and ee.

This conclusion is highly encouraging in expanding the range of substrates and the scope of supramolecular photochirogenesis with biomolecules. However, the main problem with imperfect asymmetric reaction is that due to the nature of hapten, the binding site is located outer space of the antibody, causing high flexibility of AC monomers, so the future work of this project is to design more appropriate hapten for the phage-display selections.