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Synthesis of isoprenoid chain-contained chemical probes for an investigation of molecular interactions by using quartz crystal microbalance

Wujun Liu, Yixin Zhang, Shuhua Hou, Zongbao Kent Zhao *

Division of Biotechnology and Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian 116023, PR China

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ABSTRACT

Five probes including four that contained isoprenoid chain were synthesized. These probes were assembled onto the gold-coated quartz crystal chips for analysis of their interactions with four yeast proteins by using the quartz crystal microbalance technology. Results showed that 3-phosphoglycerate phosphokinase and triosephosphate isomerase had clear interactions with certain probes, while glutathione reductase and phosphoglucose isomerase gave much lower interaction signals. It also suggested that 3-phosphoglycerate phosphokinase had two sites interacting with the probe attached with a geranyl moiety. Further molecule simulation experiments provided supportive information on these intermolecular interactions. Together, our data suggested that there are hydrophobic interactions, with relatively good selectivity, between isoprenoid chain and proteins.

Isoprenoid chains such as isopentenyl, geranyl, farnesyl, and geranylgeranyl, are building blocks for many natural molecules,[1] terpenoids, sterols, carotenoids, and dolichols.[2] They are also present as intact tails in ubiquitous biologically active molecules such as vitamin K, coenzyme Q, tocotrienols, and flavonoids.[3] Furthermore, they can be attached to a protein through a process called protein post-translational modification.

The isoprenoid chain *per se* may have interactions with different acceptors in the biological systems, such that the presence of the isoprenoid chain concerned many processes, for example, natural product biosynthesis and some metabolic diseases.[4] However, these interactions have been poorly documented; partially because the isoprenoid chain is chemically unreactive in the intracellular environment. In an early study, we prepared photoaffinity probes that contained the biotinyl group and benzophenonyl moiety linked to the terminal carbon of the geranyl group, and performed a chemical proteomic analysis of the interactions between those probes and the proteome of the yeast *Saccharomyces cerevisiae*.[5] There were 30 proteins with a variety of biological functions being identified. These proteins could be classified as a kinase, ATP related enzyme, dehydrogenase, and endonuclease. To improve the efficacy of the synthetic probe for discovering and profiling the elusive isoprenoid chain interactome, we also synthesized a number of structurally different probes and demonstrated their capability in terms of capturing proteins from the yeast proteome.[6]

Similar to long chain fatty acids interacting with their target proteins,[7] isoprenoid chain may also follow hydrophobic interactions with proteins. In biology, hydrophobic interactions are the most important non-bonded interactions which determine protein folding[8] and ligand-receptor binding.[9] While ionic interaction and hydrogen bonding can be determined relatively easily according to structural information, it is more difficult to use a quantitative method to find the hydrophobic interaction site.[10] Moreover, hydrophobic interactions are expected to be much weaker than other interactions, which furnish intrinsic difficulty for detection and quantification. Both surface plasmon resonance[11] and quartz crystal microbalance (QCM)[12] are surface-sensitive, label-free techniques for real-time monitoring of small mass changes to reveal useful information about the binding event, for which they have been widely used in analysis of molecular interactions involving small organic molecule,[11,13] biomolecules,[14] DNA,[15] and peptides.[16]

Although the benzophenone moiety could partially mimic isoprenoid chain, benzophenone-based compounds were weak competitive inhibitors to PPTases,[17] suggesting that the structure of probe should be redesigned. In this Letter, we reported our results on the synthesis of five new probes (Scheme 1) and their interactions with four yeast proteins by using the QCM technology. Further molecule simulation experiments provided supportive information on these molecular interactions. Our data suggested that there are hydrophobic interactions, with relatively good selectivity, between isoprenoid chain and proteins.

Probes **4a**–**4d** and **6** were designed, which contained a lipoic acid moiety as the anchor for assembling the probe on the gold-

* Corresponding author. Tel./fax: +86 041 84379211.
E-mail address: zhaozb@dicp.ac.cn (Z.K. Zhao).

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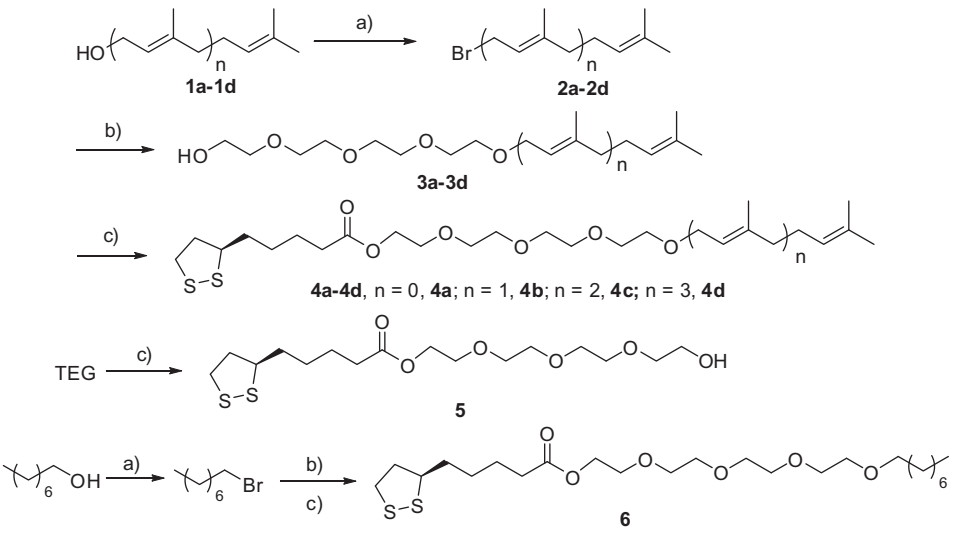

Scheme 1. Synthesis of probes. Reagents and conditions: (a) CBr_4, PPh_3, DCM, 60–89%; (b) TEG, NaH, DMF, 0 °C, 45–71%; (c) DMAP/EDC, lipoic acid, DMF, 30 °C, 35–92%.

coated quartz crystal chips via strong interactions between the bisthiol unit and gold.[18] To increase the hydrophilic interactions with polar solvent and the biocompatibility of isoprenoid chain, these probes had a tetraethyleneglycol (TEG) linker[19] to link isoprenoid tails and the lipoic acid moiety by an ether and ester bond, respectively (Scheme 1). Alcohols **1a**–**1d** were treated with CBr_4/PPh_3 to give bromides **2a**–**2d**, which were converted to **3a**–**3d** via Williamson etherification in good yields. The coupling reactions between lipoic acid and **3** were performed in DMF in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) to give probes **4a**–**4d** in up to 92% yield (Supplementary data, Fig. S1).[20] We also prepared compound **5** as a hydrophilic component for the purpose of establishing the mixed monolayers with probes **4a**–**4d** for QCM binding measurements. In addition, compound **6** was prepared to demonstrate interaction differences between isoprenoid chain and alkyl chain.

To investigate the usefulness of these probes to estimate molecular interactions between isoprenoid chain and proteins by the QCM technology, four commercially available yeast proteins, namely, triosephosphate isomerase (TPI), 3-phosphoglycerate phosphokinase (3PGK), glutathione reductase (GR), and phosphoglucose isomerase (PGI), were selected. Among them, TPI and 3PGK have been found to be interacting with isoprenoid chain-containing photoaffinity probes in our early proteomic study.[5]

Probes **4a**–**4d** and compounds **5** and **6** were assembled onto the gold-coated quartz crystal chips at different molar ratio by a typical procedure (Supplementary data). The probe modified chip was directly interrogated with the injection of sample protein at its saturating concentration. Frequency shift was detected by the ADS Plus type QCM instrument (ANT Technology Co., Ltd, Taipei, Republic of China) connected to a personal computer. For example, when 3PGK flowed through the quartz chip modified with **4b**, a typical sensorgram was recorded (Fig. 1A). The frequency shift (ΔF) reached 45 Hz. In the literature, mixed monolayers have been demonstrated as optimal surfaces with minimal nonspecific adsorptions that were well suited to study specific binding events.[21] Therefore, the chips were loaded probes with different molar ratios between **4** and **5**, and a complete frequency shift dataset was obtained (Fig. S2). It was found that frequency shifts were larger when the molar ratio between **4** and **5** was 2:1. Therefore, a number of probe-protein pairs were assayed, and results are shown in Figure 1B. It was clear that large frequency shifts were induced by 3PGK for those chips modified by **4**, indicating that strong molecular interactions

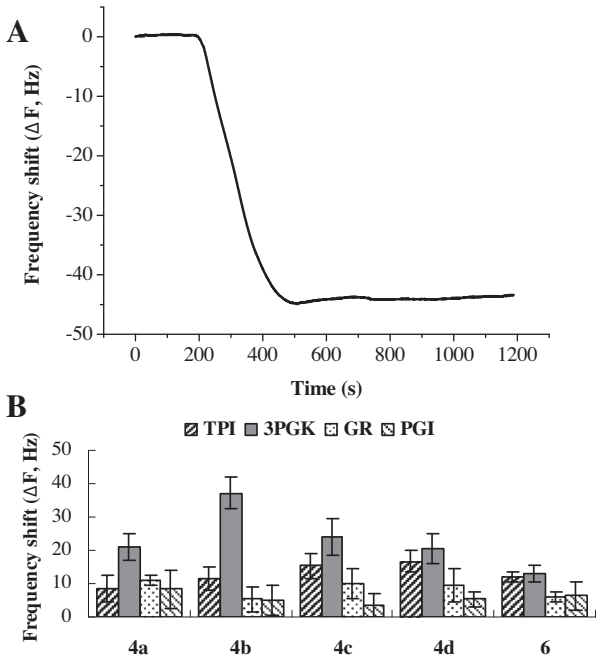

Figure 1. QCM analysis molecular interactions between synthetic probes and proteins. (A) The saturation binding curve over time of 3PGK interacting with **4b**. (B) Frequency shifts of interaction of probes **4a**–**d** and **6** with proteins. All quartz chips were modified by a mixture of indicated probe and **5** (mol/mol, 2:1). Concentrations of 3PGK, TPI, GR, and PGI were 200, 100, 54, and 120 μg/ml, respectively.

occurred between 3PGK and **4**. For TPI, larger frequency shifts were found for **4c** and **4d**, but those for **4a** and **4b** were smaller than that of compound **6**. These results suggested that TPI had stronger interactions with **4c** and **4d**. Except for those mentioned above, others gave comparable or smaller frequency shifts compared with those involving compound **6**. For example, frequency shifts were less than 8 Hz for interactions where PGI was involved. Therefore, these results agreed with our previous proteomic study, in which TPI and 3PGK were found interacting with isoprenoid chain-containing photoaffinity probes.[5]

According to the Sauerbrey equation,[22] ΔF is determined by the following equation:

$$\Delta F = (-2.26 * 10^6\ \mathrm{cm^2/Hz\ g}) F_0^2 \Delta m$$

where F_0 and Δm represent the inherent frequency of the quartz chip and the mass change per square centimeter, respectively. According to the materials provided by ANT Company, the sensitivity of the chip used in this study was 0.5 ng/Hz. The frequency changes were converted to interaction molecular ratio (Fig. S2). Theoretically, the molecule density on the chip was 10^{-12} mol/cm^2. Thus, it was estimated that each 3PGK interacted with two **4b**.

When protein samples flowed through the modified chip, ΔF signal reduced in response to a reduced protein concentration if the protein had affinity binding to ligand on the chip. Experimental data could be processed according to the Scatchard plot to obtain kinetic parameters for affinity binding events.[23] Figure 2 shows the Scatchard plot line for 3PGK binding to **4b**, from which the association constant (K_a) and the molecular binding number were determined as 3.17 μM^{-1} and 1.89, respectively. These data indicated that the affinity of 3PGK to **4b** was low, and that the surface of 3PGK may have two sites capable of interacting with **4b**. This kind of binding was not observed for compound **6** where the octanyl group was present in lieu of the geranyl group. It should be noted that yeast 3PGK have two different binding sites for ATP and 3-phosphoglycerate.[24] Interestingly, this result was consistent with our previous chemical proteomics data and streptavidin blot analysis result.[5]

To get more insights into molecular interactions between these synthetic probes and proteins, we also performed molecular simulation experiments using the AutoDock Vina program. The charges on both the protein and small molecule fragment in addition to a systematic search of conformational space is used to find the geometries of the molecule and locations on the protein surface that give the lowest interaction energy. Molecular fragments of **4**, namely, the isoprenoid chain, were used to estimate interactions with these yeast proteins (Fig. S3). Results showed that geranyl, farnesyl, and geranylgeranyl moiety could interact with these proteins, and that the affinity energy was about 4.0 kcal/mol. Thus, it was suggested that the attachment of isoprenoid chain enhanced molecular interactions to 3PGK. On the other hand, affinity energy values were less than 3.5 kcal/mol for 3PGK interacting with fragments of **5** and **6**, the TEG moiety and the octanyl group, respectively.

In conclusion, we successfully prepared isoprenoid chain-contained probes and performed the QCM analysis and molecular docking experiment using these probes. Results indicated that relatively strong intermolecular interactions occurred in some protein-probe pairs, suggesting that hydrophobic interactions involving isoprenoid chain may be an interaction mode for those natural isoprenoid chain-contained compounds in the biological system. We are now using a similar strategy to study intermolecular interactions where isoprenoid chain and other bioorganic molecules are involved.

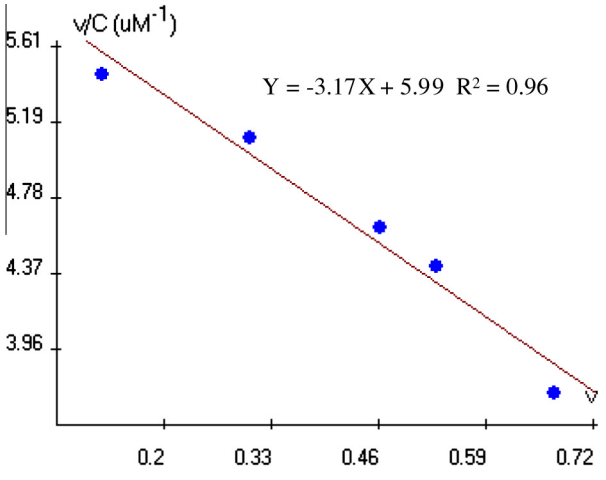

Figure 2. Scatchard plot of 3PGK affinity binding to **4b**. The quartz chip was modified by a **4b** and **5** mixture in a molar ratio of 2:1. *V*: $\Delta F/\Delta F_{max}$, C: concentration of 3PGK.

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Supplementary data

Supplementary data (procedures for probe synthesis, QCM analysis and molecular simulation, Figs. S1–S3) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.09.004.

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