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An arginine functionalized stationary phase for hydrophilic interaction liquid chromatography

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An arginine functionalized stationary phase for hydrophilic interaction liquid chromatography (HILIC) was firstly prepared by clicking arginine onto silica gel. It offers efficient separation of organic acids, nucleotides and sugars. More interesting, it exhibited excellent selectivity and enrichment toward acidic glycopeptides even at the ratio of 1/150 to non-glycopeptides.

As one of kinds of HILIC stationary phase (SP), zwitterionic SPs (ZIC-SPs) have found many applications for separation of highly polar compounds[1, 2] and enrichment of glycopeptides[2-6] recently. ZIC-SPs contain theoretically equal amount of positive (e.g. quaternary ammonium) and negative charge (e.g. sulfonate), then leading to a total neutral molecule.[7] They provide electrostatic attraction or repulsion interaction with target analytes besides hydrophilic interaction, rendering them highly selective. In addition, some novel ZIC-SPs containing weak acid (e.g. carboxylate) and weak base (e.g. amine) demonstrated larger flexibility relative to common ones since their easy modulation of ionization status of weak ion exchange groups.[2, 6, 8] In spite of these developments of ZIC-SPs, one potential problem is their less binding ability towards ionic analytes, which is probably caused by less electrostatic interaction due to their total neutral property. Although the use of charged HILIC SP is an option, it may lead to less selectivity relative to ZIC-SPs. A good alternative is to prepare a ZIC-SP containing residual charge, that is, two opposite charged groups associated with ZIC-SP has large charge difference in the solution at given pH value. Considering that guanidine is a strong base group in aqueous solution (pKa, ~13.6) and is easily protonated, while carboxylate is a weak acidic group (pKa, ~4.0), if a ZIC-SP contains these two groups, it can bear positive charge when immersed in typical solution (e.g. pH<6). Based on such consideration, we have made attempts to prepare an arginine-functionalized ZIC-SP via clicking arginine onto silica gel (termed as Click-Arginine-SP). The amine group of the amino end function of arginine can be transferred into azide, which can be clicked onto silica gel via copper-catalyzed cycloaddition of azides and alkynes. Since proposed in 2001,[9] click chemistry has become a modular synthetic approach towards the assembly of new molecule by efficiently coupling small units together owing to its high selectivity, high yield and mild reaction conditions. To the best of our knowledge, this is the first report about arginine-based HILIC SP via click chemistry.

The synthesis route of Click-Arginine-SP was shown in Fig. For comparison, a common way of amino acid bonded onto silica gel via open-ring reaction of amine with γ-glycidoxypropyl trimethoxysilane was used to prepare a arginine-based SP referring previous report [10] (details see support information, Fig.1).

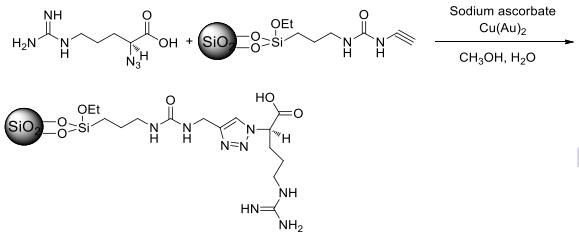

Fig. 1 Synthesis route of Click-Arginine-SP

The surface coverage of arginine was ~2.78mmol/g, which was calculated from the increase in nitrogen content measured by elemental analysis results. It was characterized by solid phase ^{13}C-CP/MAS NMR and the peak at 169.02 ppm and 154.73 ppm observed (see SI-Fig. 2) were attributed to the carbon atom of carboxylate and guanidine group, indicating the existence of arginine onto silica gel. In addition, the zeta-potential of Click-Arginine-SP at different pH value was measured, as shown in SI-Fig.3. Clearly, a positive zeta-potential at pH value up to 5.5 was observed for Click-Arginine-SP, indicating that the surface of Click-Arginine-SP was positively charged in this pH range. When pH value was >5.5, its zeta-potential was switched to negative. Such behavior was much different from obvious negative charge of bare silica and typical ZIC-SPs, for example, negative potential

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(-8.4 mV) at pH 5.0 was observed for a cysteine-based ZIC-SP described previously.[2]

Besides guanidine, triazole ring and carboxylate associated with ZIC-SP were proved to be good hydrophilic interaction groups previously.[11,12] The existence of these polar groups in Click-Arginine-SP determines its potentially good hydrophilicity. It was firstly evaluated by separation of several nucleosides and nucleotides, as shown in SI-Fig.4. Baseline separation was achieved. In contrast, bare silica demonstrated less retention for these analytes and relatively poor separation as well. Their elution order on both SPs are the same except uridine, which was strongly retained on Click-Arginine-SP. This indicated that hydrogen bonding interaction is probably involved. In addition, its good hydrophilicity was also demonstrated for separation of organic acids, as shown in Fig. 2. Six organic acids were well separated with good peak shape. In contrast, bare silica demonstrated much less retention and poor separation to these acids, which was due to electrostatic interaction between model organic acids and the positive charge of guanidine of Click-Arginine-SP (one exception for o-phthalic acid, probably due to adsorption dominating its retention over ion exchange). This was proved by the decrease of retention time of organic acids with the increase of electrolyte concentration (SI-Fig.5).

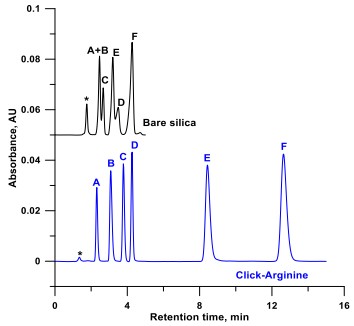

Fig.2 Separation of organic acids on bare silica and Click-Arginine-SP. Conditions: mobile phase: A:H_2O; B:ACN;C: NH_4FA (250 mM NH_4FA, pH, 3.52).2% A/90%B/8%C; detection wavelength,254 nm; flow rate, 1.0 mL/min; column temperature, 30 °C. Peak identification: A: cinnamic acid, B: benzoic acid, C: p-hydroxybenzoic acid, D: acetylsalicylic acid, E: 2,5-dihydroxybenzoic acid, F: o-phthalic acid.

To highlight the advantage of Click-Arginine-SP, a comparison was made to an arginine-based SP prepared via common way, as illustrated in Fig.3. Except co-elution of benzoic acid and p-hydroxybenzoic acid, effective separation for other analytes was obtained on the SP prepared in common way. In comparison, Click-Arginine-SP demonstrated stronger retention and better separation for all analytes. The existence of triazole produced in the click chemistry process was probably responsible for this, which was proved previously to play a role in the separation anions.[11-13] The difference was also indicated by much higher nitrogen content of Click-Arginine-SP (net increase, ~4.12 mmol/g) relative to common way.

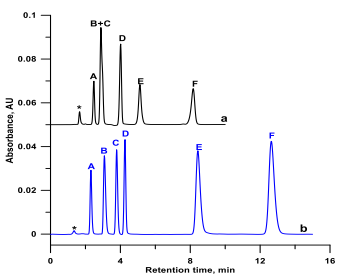

Fig.3 Comparison of arginine stationary phase prepared by traditional way (a) and click manner (b). Conditions same as Fig. 2. Peak identification: A, cinnamic acid, B, benzoic acid, C, p-Hydroxybenzoic acid, D, acetylsalicylic acid, E, [illegible] dihydroxybenzoic acid, f, o-phthalic acid.

The retention mechanism of Click-Arginine-SP for polar analytes was found be matched well with typical HILIC mode as indicated by the behavior of good linear decrease of the capacity factors of analytes (lnk) with the increase of water content ($\ln\varphi_{H2O}$), as shown in SI-Fig. 6, indicating that adsorption mechanism is probably dominant for model nucleosides and nucleotides, as proposed previously.[15]

The potential of Click-Arginine-SP for separation of carbohydrate was demonstrated for separation mono-, di-, trisaccharides and oligosaccharides, as shown in Fig.4. Clearly, six sugars could be well separated with good peak shape and high efficiency. Take melezitose as an example, its peak symmetry factor was 1.04 and theoretical plate number was ~98500/m. Their elution order was according to their polarity order (from weak to strong), which was consistent with typical HILIC characteristic. Click-Arginine-SP showed much stronger retention for these model sugars by a direct comparison with an ionic-bonded cellulose SP described recently.[16] Take the last peak (raffinose) as example, under the same elution condition, the retention time of raffinose on Click-Arginine-SP was ~4 fold longer than that of cellulose SP (figure not provided). This will be significantly helpful to enrichment of sulars or similar compounds.

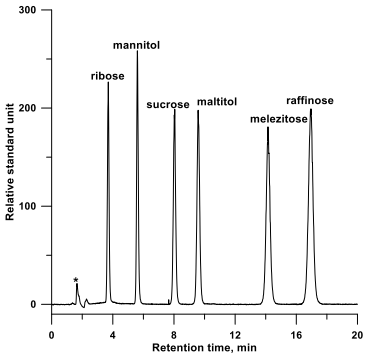

Fig. 4 Separation of six sugars on Click-L-Arginine-SP. Conditions: mobile phase, A, H_2O; B, ACN. 25%A/75%B; evaporative light scattering detection (ELSD); nitrogen nebulizer gas 30 psi, tube temperature 50°C; gain 50. Peak identification: A: ribose, B: mannitol, C: sucrose, D: maltitol, E: melezitose, F: raffinose

The separation of two oligosaccharide mixtures were also explored on Click-Arginine-SP, as shown in SI-Fig.7 and 8, respectively. Galactooligosaccharide is neutral while sodium alginate is acidic. As illustrated, both oligosaccharides were well separated. In comparison, acidic oligosaccharide had much stronger retention, which can be explained by the electrostatic attraction interactions between cationic Click-Arginine-SP and anionic sodium alginate and also hydrophilic adsorption of the analytes between the mobile phase and the aqueous layer on top of the stationary phase interaction.[17]

In light of good hydrophilicity of Click-Arginine-SP, it was explored as solid phase extraction sorbent to enrich

glycopeptides prior to MS detection. Herein tryptic fetuin digest was chosen as model sample and enrichment procedures were listed in SI. The achieved mass spectra of fetuin digests were provided in Fig.5. Signals of glycopeptides without enrichment were severely suppressed by much non-glycopeptides and only 4 glycopeptide signals were detected (A). After enrichment via Click-Arginine-SP, abundant non-glycopeptides were almostly eliminated and signals of glycopeptides were markedly improved, resulting in 30 detected fetuin glycopeptide (B). Such selectivity was further evaluated to capture glycopeptides from digests of fetuin and bovine serum albumin (BSA) at molar ratio of 1:10, 1:50 and 1:100. As shown in SI-Fig. 9 (1:10, 1:50) and Fig. 5, the selectivity of Click-Arginine-SP was almost not affected for the cases of fetuin/BSA at molar ratio of 1:10, 1:50 and 1:100. Even up to 1:150, Click-Arginine-SP still displayed high selectivity toward glycopeptides (SI-Fig.10). A direct comparison with an arginine stationary phase prepared by traditonal way was made (see Fig.5D) and it showed that a majority of non-glycopeptides contaminate the glycopeptide fraction for molar ratio of 1:100. These results clearly exhibited excellent selectivity of Click-Arginine-SP towards glycopeptides.[6,18] More important, Click-Arginine-SP also had high adsorption capacity, ~300 mg/g (e.g. 60 mg/g in a recent report[19]), which is also greatly helpful for enrichment of glycopeptides. To explore the possible adsorption mechanism, quantum chemistry calculation was used by choosing a similar simple molecule, sialic acid, as given in SI-Fig.11. It showed that arginine adopts a face-to-face mode when binding sialic acid through multiple hydrogen bonding interactions, in which arginine acts as both a donor and an acceptor of hydrogen. In addition, triazole and hydroxyl groups of Arg-ZI-SP also contribute to these interactions.

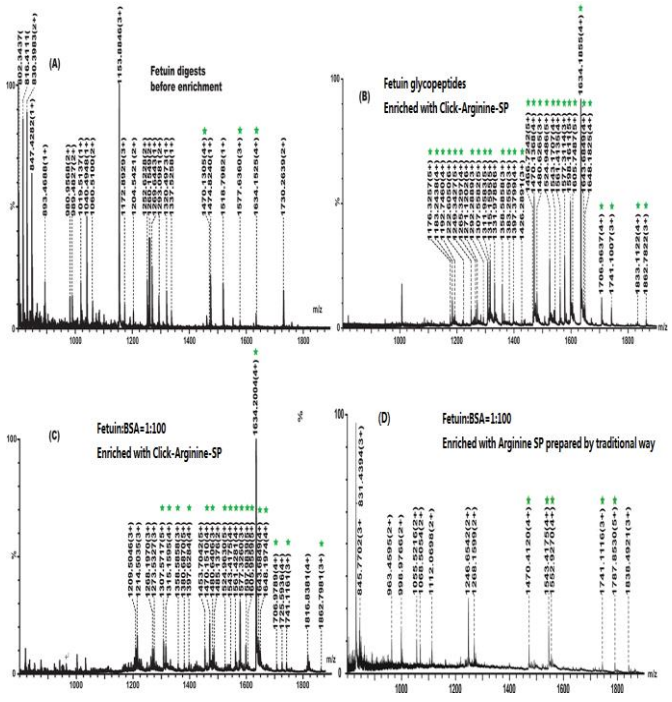

Fig. 5 Mass spectra of tryptic digests of bovine fetuin and bovine serum albumin (BSA) before and after enrichment with Click-Arginine-SP and Arginine-SP prepared by traditional way. Tryptic digests of fetuin before (A) and after enrichment with Click Arg (B)；Enrichment of glycopeptides from fetuin/BSA at molar ratio of 1:100 with Click-Arginine-SP (C) and Arginine-SP prepared by traditional way (D).

In summary, a novel arginine functionalized HILIC ZIC SP was synthesized by clicking arginine onto silica gel. It exhibited good hydrophilic property. More important, Click-Arginine-SP demonstrated excellent selectivity and high capacity towards glycopeptides and could become a promising material in glycomics and glycoproteomics.

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