Overview

Purpose
To develop new chemical methods/reactants for protein footprinting that enable studies aimed at uncovering biochemical function.

Results
Several new chemical reagents were synthesized and tested. Diazonium salts and diaryl ketones can generate carbocation and diradicals respectively in an FPOP platform. Notably, Calmodulin can be modified as a model protein at the global level. Trifluoromethyl radicals can be produced using a benchtop-stable trifluoromethyl radical source and can effectively label humanNeuregulin Y Fragment 18-36.

Introduction

- Protein footprinting provides direct assessment of structure and conformational change whereby chemical probes solvate the accessible surface of a protein. An effective footprinting approach is FPOP which provides a "snapshot" of protein conformation when protein is exposed to "OH from H\(_2\)O\(\cdot\) photolysis. However, several challenges remain: 1) some residues are unreactive (e.g., A, D, N, G, S, T), and 2) even IPPOF silent. 2) "OH reactivity is hard to tune because its structure is simple, and 3) the diffusion of "OH in a lipid membrane may give nonspecific labeling. Therefore, we recognize ‘a call to develop new labeling agents’ as a significant goal.

- Strategy

- Chemical agents
  - 1) AcOH
  - 2) Na\(_2\)CO\(_3\)
  - 3) Cu (3aminoethyl)phosphonate
  - 4) CaCO\(_3\)
  - 5) Liposomes

- Trifluoromethyl ketones

Figure 1 Proposed chemical agents and corresponding modification reactions


d) - 3H-Arg-Tyr-Tyr-Ser-Ala-Leu
  e) - Arg-His-Tyr-Ile-Ala-Leu-Arg-Tyr-Gln-Arg-Tyr-NH\(_2\)

Calmodulin (CaM) is a ubiquitous, calcium-binding protein that can bind to and regulate a multitude of different protein targets. CaM mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth and the immune response.

Figure 2 (a) One frame of the solution NMR structure of apo-CaM (PDB 1FC6); (b) sequence of Neuregulin Y (18-36).

Methods

- Protein Footprinting: FPOP Platform

The FPOP platform is identical to that reported recently. The protein solutions were diluted with buffer and labeling reagents added to 10 μM protein, 10:100 mM chemical reagents. The sample 50 μL was introduced to capillary with a syringe pump and passed a laser water where it was irradiated (248 nm or 355 nm). A flow rate (~15-20 μL/min) was calculated to ensure a 25% exclusion volume, based on laser spot size (~2 mm) and laser frequency. The 50 μL sample solution was subjected to 900 to 1200 laser shots in total. After labeling, an aliquot representing 0.3 μg of protein was analyzed by using a Bruker Maxis Q TOF mass spectrometer (Billerica, MA, USA) under denaturing conditions to provide protein-level information. The NPY peptides were separated by reversed-phase HPLC by an Eksigent NanoLC Ultra (Dublin, CA, USA) and introduced to a Thermo LTQ-FIT mass spectrometer (Walther, MA, USA) via nano-ESI. Peptide ions were measured and multiple charge states were identified CID.

- Chemical Synthesis

New reagents were synthesized based on the following schemes and cited references.1-3

Figure 3 Synthesis of isotope-encoded, photoactivatable reagents

Results and Discussion

- Calmodulin and Neuregulin Y Fragment

CaM is a ubiquitous, calcium-binding protein that can bind to and regulate a multitude of different protein targets. CaM mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth and the immune response.

Figure 4 Neuregulin Y (NPY) 18-36

- Trifluoromethyl radical mapping of NPY(18-36)

NPY(18-36) is a potent agonist of NPY Y1 receptors. NPY(18-36) has potential clinical application because NPY is implicated in the pathophysiology of congestive heart failure.

Figure 5 Calmodulin (CaM)

- Discovery of a mild method for trifluoromethyl radical mapping

The FPOP platform is identical to that reported previously.4 The protein solutions were diluted with buffer and labeling reagents added to 10 μM protein, 10:100 mM chemical reagents. The sample 50 μL was introduced to capillary with a syringe pump and passed a laser water where it was irradiated (248 nm or 355 nm). A flow rate (~15-20 μL/min) was calculated to ensure a 25% exclusion volume, based on laser spot size (~2 mm) and laser frequency. The 50 μL sample solution was subjected to 900 to 1200 laser shots in total. After labeling, an aliquot representing 0.3 μg of protein was analyzed by using a Bruker Maxis Q TOF mass spectrometer (Billerica, MA, USA) under denaturing conditions to provide protein-level information. The NPY peptides were separated by reversed-phase HPLC by an Eksigent NanoLC Ultra (Dublin, CA, USA) and introduced to a Thermo LTQ-FIT mass spectrometer (Walther, MA, USA) via nano-ESI. Peptide ions were measured and multiple charge states were identified CID.

Figure 6 Mass spectra of modified calmodulin. (a) Mass spectra confirm diradical labeling. (b) Putative mechanism for the footprinting reaction

Conclusions

Several practical methods for new protein footprinting are demonstrated. The isotope-encoded photoactivatable reagents (azides, diaryl ketones) can be readily synthesized using commercial available methyl-3-iodide. Preliminary results demonstrate that calmodulin can be modified by carbocation and diradicals by using the FPOP platform. In addition, a highly efficient method of trifluoromethyl modification, using NPY(18-36), is demonstrated with a benchtop-stable trifluoromethyl radical source. The operational ease of this transformation coupled with its predictable reactivity in complex settings bodes well for its widespread use.

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References