New Reactive Organic Reagents for Footprinting Proteins

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Overview

Purpose

To develop new chemical methods/reagents 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 humanNeuropeptide Y fragment 18-36.

Introduction

❑ Protein Footprinting

➢ Protein footprinting provides direct assessment of structure and conformational change whereby chemical reagents probe the solvent-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_2O_2 photolysis. However , several challenges remains: 1) some residues are unreactive (e.g., A, D, N, G, S, T) and even FPOP 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 reagents" as a significant goal.

❑ Strategy

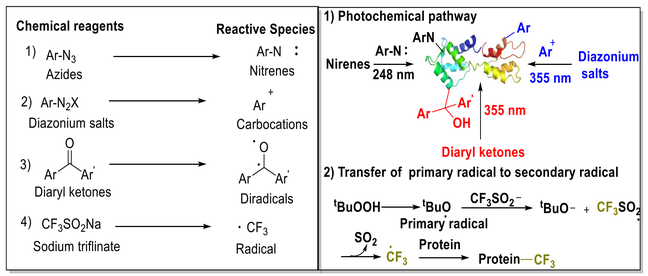
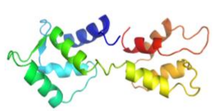


Figure 1 Proposed chemical reagents and corresponding modification reactions

❑ Calmodulin and Neuropeptide Y Fragment

(a)



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.

(b)

H-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu
Arg-His-Tyr-Ile-Asn-Leu-Ile
Thr-Arg-Gln-Arg-Tyr-NH_2

Neuropeptide Y (NPY) is present in high concentrations in the mammalian heart. Structure-activity studies with selected partial sequences of NPY reveal that NPY(18-36) is a competitive antagonist of NPY in rat cardiac ventricular membranes. NPY cardiac receptor antagonist, NPY(18-36), has potential clinical application because NPY is implicated in the pathophysiology of congestive heart failure.

Figure 2 (a) One frame of the solution NMR structure of apo-CaM (PDB 1CFC), (b) sequence of Neuropeptide Y (18-36) .

Methods

❑ Protein Footprinting: FPOP Platform

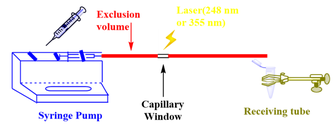


Figure 3. The FPOP Platform

The FPOP platform is identical to that reported recently[1-3]. 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 window 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-FT mass spectrometer(Waltham, MA, USA) via nano-ESI. Peptide ions were sequenced and modifications located CID.

❑ Chemical Synthesis

New reagents were synthesized based on the following schemes and cited references[4-5].

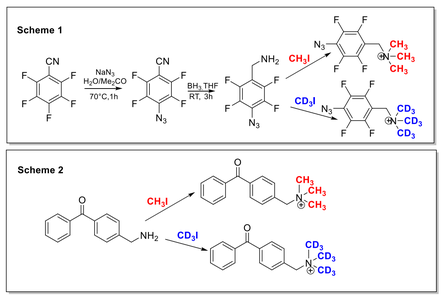


Figure 4. Synthesis of isotope-encoded, photoactivatable reagents

Results and Discussion

❑ Trifluoromethyl radical mapping of NPY(18-36)

➢ Discovery of a mild method for trifluoromethyl radical mapping

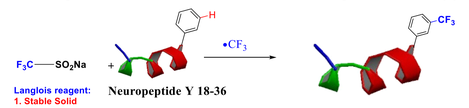
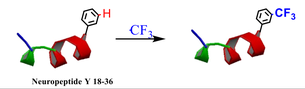


Figure 5. Langlois reagent[6]: an old reagent with a new use.

➢ Initial investigation into footprinting reaction parameters



Entry	Peptide/Reagent	Temperature/°C	Solvent	Time/min	Modified Ratio/%
1	1:1000	25	PBS buffer PH=7.5	10	0
2	1:1000	25	Tri buffer PH=7.0	10	0
3	1:1000	25	MOPS buffer PH=7.0	10	0
4	**1:1000**	**25**	**PBS Buffer PH=6.5**	**10**	**45**
5	**1:1000**	**25**	**PBS Buffer PH=6.5**	**5**	**40**
6	**1:1000**	**0**	**PBS Buffer PH=6.5**	**10**	**30**
7	1:10000	25	PBS Buffer PH=6.5	10	Messy
8	1:10000	0	PBS Buffer PH=6.5	10	Messy

➢ Experimental results

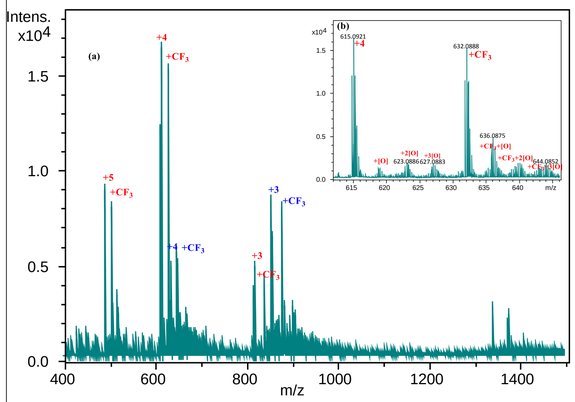


Figure 6. (a) Spectrum of modified NPY(18-36). (b) Mass spectra (+4 charge state) after modification of NPY(18-36). (c) The sample includes two different peptides. The NPY(18-36) is peptide 1 marked with red color. Peptide 2 is a [NPY+Asn] mutant marked with blue color. Both peptides are modified.

❑ Carbocation mapping of Calmodulin

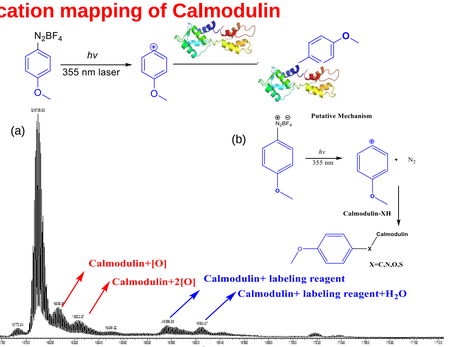


Figure 7 . Mass spectra of modified calmodulin. (a) Deconvolution confirms carbocation labeling of calmodulin. (b) Putative mechanism for footprinting.

❑ Diradical mapping of Calmodulin

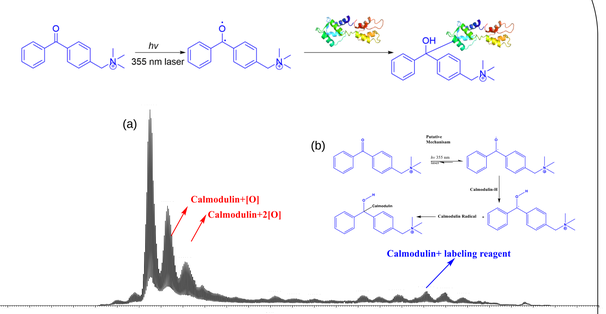


Figure 8 . Mass spectra of modified calmodulin. (a) Mass spectrum confirms diradical labeling. (b) Putative mechanism for the footprinting reaction

Conclusions

Several practical methods for new protein footprinting are demonstrated. The isotope-encoded photoactivable reagents (azides, diaryl ketones) can be readily synthesized using commercial available methyl-d_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.

References

[1] Hambly, D. M.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2005**, 16, 2057.
[2] Gau, Brian C.; Chen, H.;Zhang, Y.; Gross, M. L. *Anal. Chem*. **2010**, 82, 7821
[3] Zhang, B.; Rempel, D.L.; Gross, M. L. *J. Am. Soc. Mass Spectrom*. **2016**,27,552
[4] Leyva,E.; *J. Fluor. Chem*. **2013**, 56, 164.
[5] Keana, J.F. *J.Org. Chem*.**1990**,55 , 3640.
[6] Baran, P. S. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, 108, 14411

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