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Isotopically-coded short-range hetero-bifunctional photo-reactive crosslinkers for studying protein structure☆

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ABSTRACT

The resolution and the fidelity of a protein structural model, constructed using crosslinking data, is dependent on the crosslinking distance constraints. Most of the popular amine-reactive NHS-ester crosslinkers are limited in their capacity to provide short distance constraints because of the rarity of lysine residues occurring in close proximity in the protein structure. To solve this problem, hetero-bifunctional crosslinkers containing both a photo-reactive functional group and an NHS-ester group can be used to enable non-specific crosslinking within the proximity of these lysine residues. Here we develop three such isotopically-coded hetero-bifunctional photo-reactive crosslinkers, bearing azido, diazirine or benzophenone photo-reactive groups (azido-benzoic-acid-succinimide (ABAS)-$^{12}C_6/^{13}C_6$, succinimidyl-diazirine (SDA)-$^{12}C_5/^{13}C_5$, and carboxy-benzophenone-succinimide (CBS)-$^{12}C_6/^{13}C_6$, respectively). These crosslinkers were validated using several model proteins/peptides and were then applied to study the structure of the native α-synuclein protein. In that case the ABAS crosslinker proved to be the most suitable, with 10 crosslinks being found in the native α-synuclein structure.

Biological significance

Structural proteomics can be used for studying protein structures which may be difficult to examine by traditional structural biology methods such as NMR or X-ray crystallography. Crosslinking in particular is used to provide distance constraints for molecular modeling of individual proteins and protein complexes. The shortest distance constraints are most valuable for the modeling process. To be able to provide such short distance constraints, non-specific photo-reactive chemistry can be used for crosslinking reactions. However, detection of such non-specific crosslinks is difficult because the signal from any particular crosslink is low due to the broad reactivity of the crosslinking reagents. To overcome this problem, we have employed isotopic labeling of these crosslinkers. In this paper, we have demonstrated their effectiveness for studying the native α-synuclein protein structure. The non-specific reactivity, in combination with isotopic coding of these crosslinkers, allowed

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for the formation and detection of short-range crosslinks, targeting a variety of amino acids. These reagents may prove useful for future applications to a variety of protein structural problems. This article is part of a Special Issue entitled: Protein dynamics in health and disease. Guest Editors: Pierre Thibault and Anne-Claude Gingras.

1. Introduction

Chemical crosslinking is a useful technique for obtaining structural information on proteins and protein complexes which are difficult to characterize by other methods. The crosslinking reaction creates covalent linkages between two amino acid residues, and subsequent enzymatic digestion and mass spectrometric analysis allows identification of the crosslinked sites. These crosslinks provide distance constraints which can be used in molecular modeling of protein structures [1,2]. In order to increase the resolution of crosslinking data for this purpose, short-distance crosslinks are required; the shorter the crosslink, the more "tight" the model, and the more confident the structure. Crosslinkers traditionally use NHS-ester chemistry, which is specific for primary amino groups. The availability of two lysines that can be crosslinked diminishes as the distance is reduced. To mitigate this problem, non-specific crosslinking chemistry can be used.

Photo-reactive groups, such as phenyl azide, benzophenone, and diazirines, generate radicals upon stimulation by UV light, and these radicals react in a non-specific manner with amino acid residues (Fig. 1), typically by insertion of the radical into a methyl or methylene group [3]. By combining these groups with NHS-ester chemistry, heterobifunctional crosslinkers can be produced, which offer short-range crosslinking capabilities not available with homo-bifunctional NHS-ester reagents. Three such crosslinkers are azido-benzoic-acid-succinimide (ABAS), carboxy-benzophenone-succinimide (CBS), and succinimidyl-diazirine (SDA) which utilize phenyl azide, benzophenone, and diazirine photo-reactive groups, respectively. These reagents have been a common strategy in protein crosslinking for many years [3–7], primarily for the purpose of conjugating a protein or peptide to another molecule in a photo-activated manner. These three crosslinking reagents result in short-distance crosslinks of ~7 Å or 5 Å, as measured from the nitrogen atom of the newly formed amide from the NHS-ester reaction to the atom of the target modified by the photo-reactive group (Fig. 1).

One of the major limiting factors in a crosslinking experiment is the small number of crosslinked peptides compared to their non-crosslinked counterparts [8]. This problem is worsened in the case of non-specific crosslinkers, which may form multiple products, each of which further dilutes the signal of any one crosslinked species. The low signal intensity of these crosslinks makes them difficult to detect using traditional intensity based data-dependent MS/MS acquisition methods. Isotopic coding of the crosslinking reagents, however, generates a distinctive doublet signature in the mass spectra which can be utilized to ensure the acquisition of MS/MS spectra for those peptides which have been modified by the crosslinker [9]. By ensuring that both the heavy and light precursor ions are fragmented, the MS/MS fragment ions also show doublet signatures which can also be utilized for increased confidence in the crosslink assignment [10]. In order to ini-

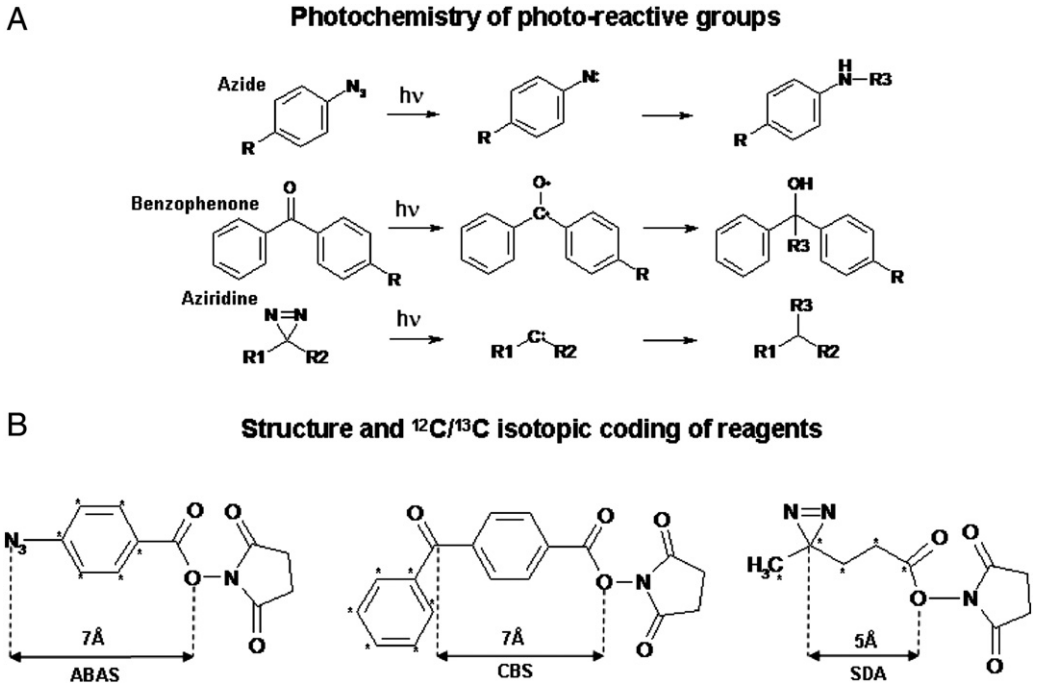

Fig. 1 – A. Photochemistry of three photo-reactive functional groups [3]. B. Structure, isotopic coding, and spacer length of the three photo-reactive, hetero-bifunctional, isotopically-coded crosslinkers ABAS-$^{12}C_6/^{13}C_6$, CBS-$^{12}C_6/^{13}C_6$ and SDA-$^{12}C_5/^{13}C_5$.