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Protein haptenation by amoxicillin: High resolution mass spectrometry analysis and identification of target proteins in serum

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ABSTRACT

Allergy towards wide spectrum antibiotics such as amoxicillin (AX) is a major health problem. Protein haptenation by covalent conjugation of AX is considered a key process for the allergic response. However, the nature of the proteins involved has not been completely elucidated. Human serum albumin (HSA) is the most abundant protein in plasma and is considered a major target for haptenation by drugs, including β-lactam antibiotics. Here we report a procedure for immunological detection of AX–protein adducts with antibodies recognizing the lateral chain of the AX molecule. With this approach we detected human serum proteins modified by AX in vitro and identified HSA, transferrin and immunoglobulins heavy and light chains as prominent AX-modified proteins. Since HSA was the major AX target, we characterized AX–HSA interaction using high resolution LTQ orbitrap MS. At 0.5 mg/mL AX, we detected one main AX–HSA adduct involving residues Lys 190, 199 or 541, whereas higher AX concentrations elicited a more extensive modification. In molecular modeling studies Lys190 and Lys 199 were found the most reactive residues towards AX, with surrounding residues favoring adduct formation. These findings provide novel tools and insight for the study of protein haptenation and the mechanisms involved in AX-elicited allergic reactions.

1. Introduction

The widely prescribed β-lactam antibiotics are among the drugs most frequently eliciting allergic reactions, thus posing an important clinical problem. In the most severe cases allergic reactions may be life-threatening and reduce the therapeutic options against infections. Protein haptenation plays a key role in immunological reactions to β-lactams. This process occurs through the nucleophilic opening of the β-lactam ring, generally by the attack of free amino groups in proteins, and gives rise to a penicilloyl–protein adduct which is able to elicit an immune response (reviewed in [1]). See Fig. 1A for a schematic representation of the formation of an amoxicilloyl–protein adduct.

Abbreviations: AX, amoxicillin; HSA, human serum albumin; ECL, enhanced chemiluminiscence.

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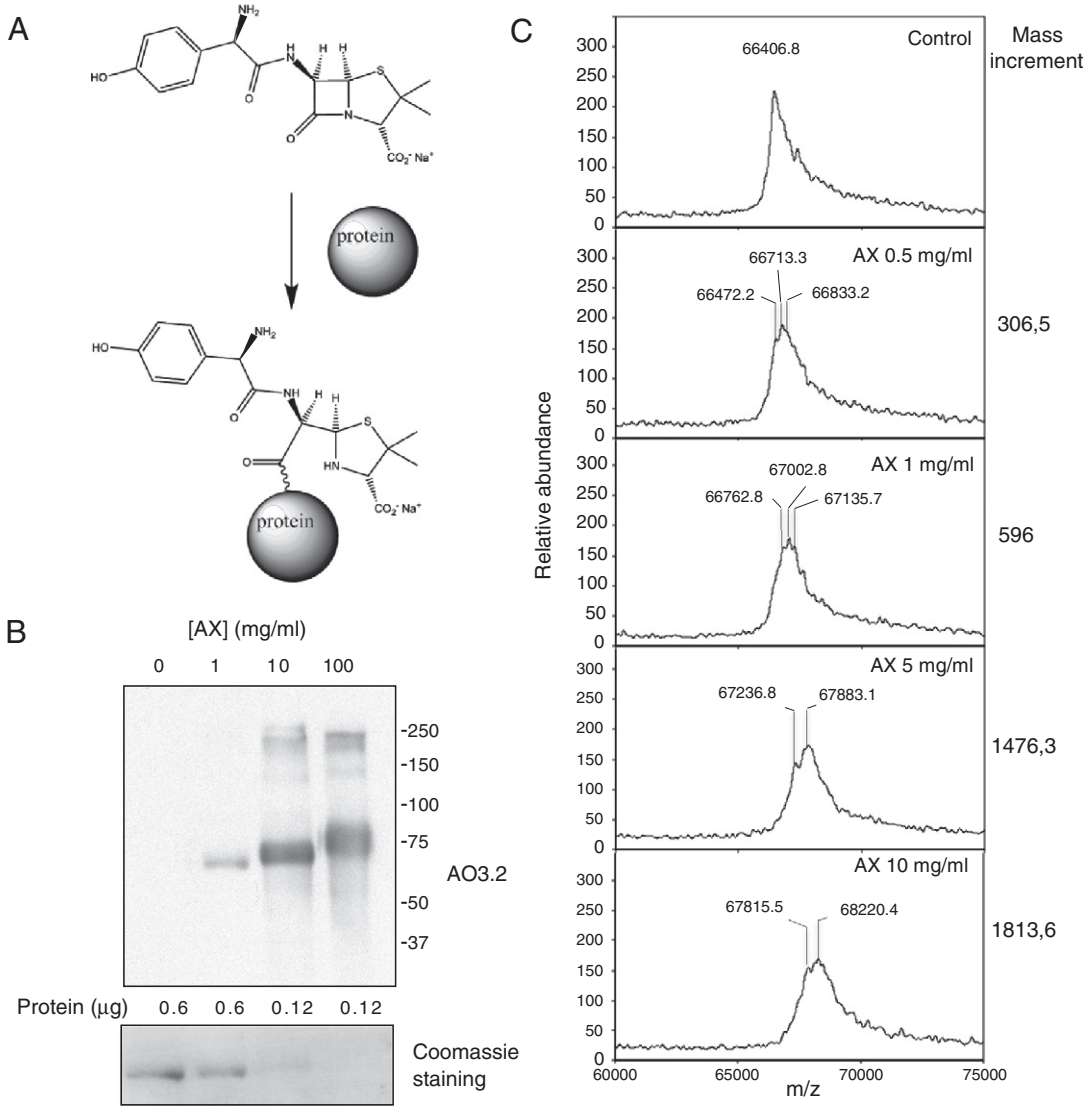

Fig. 1 – Interaction of AX with HSA as detected by immunological and MALDI-TOF MS approaches. (A) Structure of AX and the adduct formed with a protein (amoxicilloyl–protein adduct) through a non-specified residue. The main nucleophilic sites from the protein potentially able to react with the β-lactam ring of AX include lysine, histidine, cysteine and the amino terminal group. (B) Immunological detection of AX-modified HSA by Western blot with the AO3.2 monoclonal antibody. HSA was incubated in the presence of increasing concentrations of AX under the conditions used for antibody generation (see the Materials and methods section) and analyzed by SDS-PAGE followed by Western blot and ECL detection. Aliquots of the incubations containing decreasing amounts of protein (indicated at the bottom) were loaded in each lane to avoid saturation of the signal. The Coomassie staining of the blot is shown in the lower panel. The protein amount of the two lanes on the right was close to the detection level. (C) MALDI-TOF MS analysis of HSA incubated with the indicated concentrations of AX for 16 h at 37 °C. The mass increment observed in every condition is indicated on the right. Results are representative of at least 4 assays with similar results.

Important efforts have been devoted towards the understanding of the pathogenic role of protein haptenation by β-lactams, the identification of the adducts formed and their ability to activate the immune system. Because human serum albumin (HSA) is the most abundant protein in serum, most works have attempted the characterization of penicilloyl–HSA adducts. The first pioneering studies on these aspects, employing HPLC separation of tryptic peptides and EDMAN degradation sequencing, were published by Yvon et al. [2,3] and reported the sites of adduct formation in albumin obtained from a penicillin-treated patient or prepared by in vitro conjugation. More recently, mass spectrometry (MS) has been applied to the study of adducts of HSA with various β-lactam antibiotics, including benzylpenicillin [4], flucloxacillin [5] and piperacillin [6], formed either in vitro or in the serum of patients. Noteworthy, there seems to exist a high degree of variability among the adducts detected and the factors that influence this process are not completely understood.

Unlike the other investigated penicillins, amoxicillin (AX) is a zwitterionic molecule which possesses a primary amino