Mapping the Disulfide Bonds in Nurse Shark J Chain

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Introduction

The mucous membranes in our gastro-intestinal and respiratory tracts are highly susceptible to invasion by pathogens. Our immune system secretes antibodies (Abs) to the outside of the mucous membranes to fight these pathogens. In mammals, the secreted Abs are polymers with two or more identical Ab monomers bound together (Fig.1). They also contain joining or J chain. J chain in mammals aids in more efficient Ab polymerization and enhances Ab secretion across mucous membranes by helping the Abs bind the polymeric immunoglobulin receptor (pIgR) on mucosal epithelial cells [1,2]. The mammalian J chain has 8 cysteines (Cys) that form three intrachain disulfide bonds and two interchain disulfide bonds that attach to two of the Abs of the polymer (Fig.1 and 2).

Little is known about J chain in elasmobranchs (sharks, skates and rays). All known elasmobranch sequences are missing conserved Cys which could lead to structural differences compared to mammalian J chain (Fig.2); [4]. If mammals are missing these Cys, attachment to pIgR is reduced and the Ab secretion is diminished [1]. This calls into question the function of elasmobranch J chain. My project is aimed at mapping the disulfide bond pattern in the nurse shark J chain and ultimately comparing it to that of mammalian J chain. We chose to work with nurse sharks because their serum (containing the Abs) was available, and more is known about their Ab and J chain gene sequences than other elasmobranchs. This study will shed light onto the evolution of the function of J chain.

Methodology and Results

Purified bovine IgM was used as a control because the human pattern is known, and it is thought that all mammalian disulfide bond patterns are conserved.

Nurse shark pentameric IgM was successfully isolated from whole serum using a GE Healthcare Superose 6 size exclusion column.

The protein was digested with trypsin for 16 hrs at 37°C, followed by an increase of temperature to 80°C for ten minutes and a second digest with more trypsin for 16 hrs. This ideal digest protocol was achieved after many trials of temperatures and lengths. A partial theoretical peptide yield for nurse shark IgM using trypsin is pictured to the left.

Pepitides were analyzed by HPLC/Mass Spectrometry. From the sharp peaks on the mass spectrometer profile, it was seen that trypsin effectively cut the pentameric IgM into peptides.

Mass spec data was inputted into the MassMatrix software designed to identify peptide cross-links from tandem mass spectrometry data [5,6,7,8].

Analysis and Discussion

Despite having good digestion patterns, only one of the expected disulfide bridges (C7-C8) was identified in the control bovine IgM J chain (Fig.4); [5,6,7,8].

Due to the lack of accurate results, any disulfide bonds present in the nurse shark joining chain detected by the MassMatrix software cannot be considered significant. Continued effort is needed to optimize the experimental protocol in order to obtain more accurate results from the computer program. A conference call with the co-creator of the program helped identify some common pitfalls [Dr. Hua Xu, MassMatrix, personal communication]. This highlighted the need to use reagents that will not cause the disulfide bonds to mix and scramble and will result in effective protein cleavage. Capping the free cysteines with a reagent will prevent the bond scrambling. In order to have no more than one cysteine in a peptide, trypsin plus an additional enzyme or chemical will be used in a double digestion.

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References