Demonstration of a Highly Purified Type-A Hydrolyzed Collagen Product as an Excellent Protein Stabilization Matrix

Brian R. Fernández: Los Angeles, California

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ABSTRACT

Protein stabilization is critical to the IVD industry, particularly for QC materials used by clinical laboratories to verify the performance of assay systems.

Prionex® (DSM Pentapharm, Switzerland) is a unique preparation of porcine derived type-A hydrolyzed collagen that is shown here to be an excellent stabilizing excipient in a cardiac marker control formulation containing Troponin I-C complex and BNP-32, two inherently unstable polypeptides with vastly different structures and properties.

Proposed here are several likely mechanisms of stabilization with Prionex preparations, which include the lack of proteolytic activity, inert structure, and highly charged amino acid content.

INTRODUCTION

The preservation of biological activity of protein preparations is critical for applications in the IVD industry. Protein standards, including quality controls (QC), calibrators, are used in clinical diagnostic settings to verify the performance of assay systems and establish accurate calibration curves. Stable protein preparations ultimately serve to ensure accurate patient diagnoses and outcomes.

Protein/peptide biomarkers are formulated to help maintain their native three-dimensional structure in-vitro to ensure accuracy and reproducibility on assay systems. Human and Bovine Serum Albumins (HSA/BSA) have both been used as protein stabilizers. The mechanisms of stabilization may be due to a combination non-covalent electrostatic interactions and competitive protection from adsorption loss and proteolytic degradation.

Gelatin, a byproduct of partially hydrolyzed collagen (HC), is also used as a protein stabilizer and excipient. Prionex® (DSM Pentapharm, Switzerland) is a unique preparation of porcine derived type-A HC that is marketed as an alternative to serum albumin for various applications, including in-vitro protein stabilization.

OBJECTIVE: To demonstrate the ability of Prionex® to stabilize a cardiac marker QC formulation containing Tropnin I-C complex and BNP-32, prepared with and without additional HSA.

MATERIALS & METHODS

Prionex is produced according to a procedure wherein porcine skin is treated with pepsin, purified, and subjected to mild acidic hydrolysis at elevated temperature to yield a non-gelling hydrolyzed collagen (HC) peptide product with an average MW of 20 kDa.

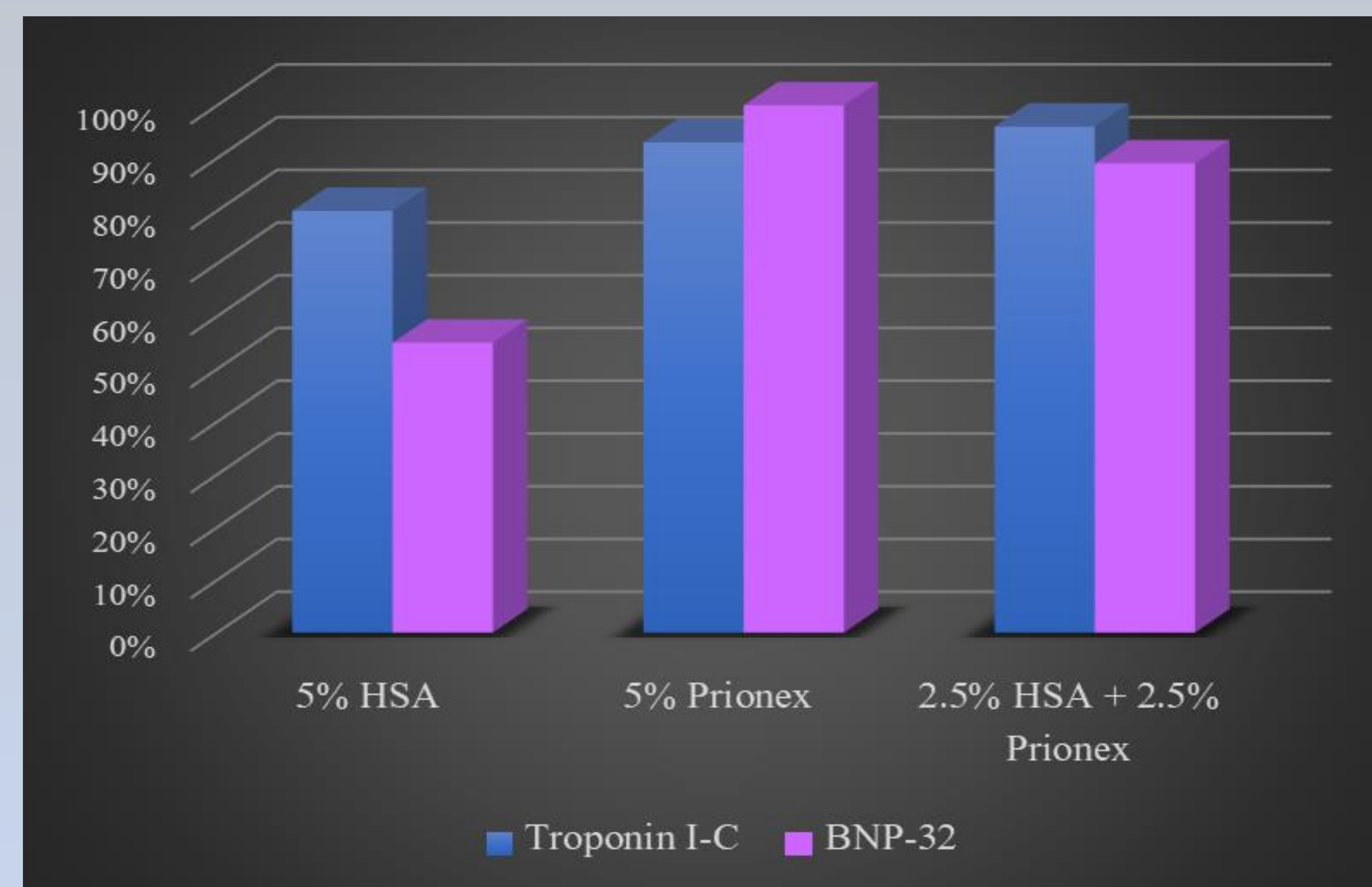
A proprietary cardiac marker control formulation adjusted to 10 ng/mL with Troponin I-C and 200 pg/mL BNP were prepared in HSA, Prionex, and an equal blend, each to a 5% total protein content.

Samples were incubated for 6 days at 25˚C and were assayed for Troponin recovery on the Siemens Dimension RXL and for BNP recovery on the Abbott Axsym.

RESULTS

[Figure 1] After 6 days at 25˚C, Troponin I-C and BNP-32 recovered 80% and 55% in 5% HSA; 93% and 100% in 5% Prionex; 96% and 89% in the 2.5% HSA + 2.5% Prionex blend. Values are expressed as a percentage of initial Day 0 recoveries.

Figure 1: Percent Recovery after 6 days at 25°C



DISCUSSION

The results from **Figure 1** confirm findings published by Gaffney[1] that demonstrate the effectiveness of HC to preserve the biological activity of two very different proteins: a large multimeric protein of 42.3 kDa (Troponin I-C) and a short 32-amino acid peptide hormone (BNP-32).

Prionex dramatically improved the recovery of BNP, which is known to be very susceptible to endogenous proteases as part of its in-vivo clearance mechanisms.[2] The thermal processing of Prionex inactivates viruses, prions, and contaminant proteolytic enzymes.

Albumin has several ligand-binding pockets which can reduce the bioavailability of peptides. HC has been shown to remain structurally inert, lacking major secondary or tertiary structures that fold into binding pockets. The highly charged nature of the amino acid content may explain stabilization from electrostatic interactions.[3]

Prionex also improved the recovery of Troponin I-C, particularly when blended with HSA. One study showed that collagen peptides of 8.5 to 20 kDa promoted HSA stability by retarding the thermal unfolding of tertiary structure and delaying aggregation.[4] The blended matrix may function synergistically to preserve the confirmations of both HSA and Troponin complex.

CONCLUSION: Prionex® has been shown to be an effective stabilizer of two cardiac markers with vastly different properties. The lack of enzymatic activity, inert structure, and charged amino acid content are likely mechanisms of stabilization. More than a pathogen-free alternative to albumin, Prionex is attractive as an exceptional protein stabilizer for many potential applications.

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