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2,5-Deoxyfructosazine, a D-glucosamine derivative, inhibits T-cell interleukin-2 production better than D-glucosamine

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Abstract—D-Glucosamine has been widely reported to have immunosuppressive actions on neutrophils, lymphocytes, and other cells of the immune system. However, under conditions used in biological experiments (e.g., neutral pH, and phosphate buffers), we have found that D-glucosamine self-reacts to form 2,5-deoxyfructosazine [2-(D-*arabino*-tetrahydroxybutyl)-5-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine] (**1**) and 2,5-fructosazine [2,5-bis(D-*arabino*-tetrahydroxybutyl)pyrazine] (**2**). When tested for bioactivity at nontoxic concentrations, these D-glucosamine derivatives were more effective inhibitors of IL-2 release from PHA-activated T cells than D-glucosamine. Hence, fructosazines constitute a novel class of immunomodulators.

Keywords: Immune cell; Cytokine release; D-Glucosamine; Fructosazine

1. Introduction

D-Glucosamine, 2-amino-2-deoxy-D-glucose (D-GlcN), is an important multipurpose cell metabolite. This amino sugar is a precursor of glycolipids and glycoproteins, a regulator of glucose transport, and a substrate for the *O*-GlcNAc (*N*-acetyl-D-glucosamine) signaling pathway.[1,2] Recent studies have shown that D-GlcN has immunomodulatory properties. D-GlcN diminishes neutrophil oxidant production and migratory properties.[3] D-GlcN has also been shown to influence lymphocyte activation and proliferation.[4,5] Moreover, D-GlcN reduces IL-2 production by activated lymphocytes, apparently by diminishing the translocation of transcription factors to the nucleus.[6] In vivo, D-GlcN has been reported to diminish allograft rejection and the acute phase of an animal model of multiple sclerosis.[4,7] Hence, D-GlcN and its biological mechanisms of action are of considerable importance.

Under certain conditions such as neutral pH, D-GlcN can react under surprisingly mild conditions. Moreover, due to the presence of both amino and aldehyde groups, D-GlcN can undergo a variety of decomposition reactions. Consequently, it is unclear if the reported bioeffects of D-GlcN are due solely to D-GlcN's effect on cells, or if a reaction product also possess biological activity. In the present study we find that cyclocondensation products of D-GlcN have greater biological activity than native D-GlcN.

2. Results and discussion

2.1. Chemistry

In aqueous solution above pH 7.0, D-GlcN undergoes a cyclocondensation reaction that results in complex mixtures of reaction products. It has been reported that 2,5-deoxyfructosazine [2-(D-*arabino*-tetrahydroxybutyl)-5-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine] (**1**) and 2,5-fructosazine [2,5-bis(D-*arabino*-tetrahydroxybutyl)-

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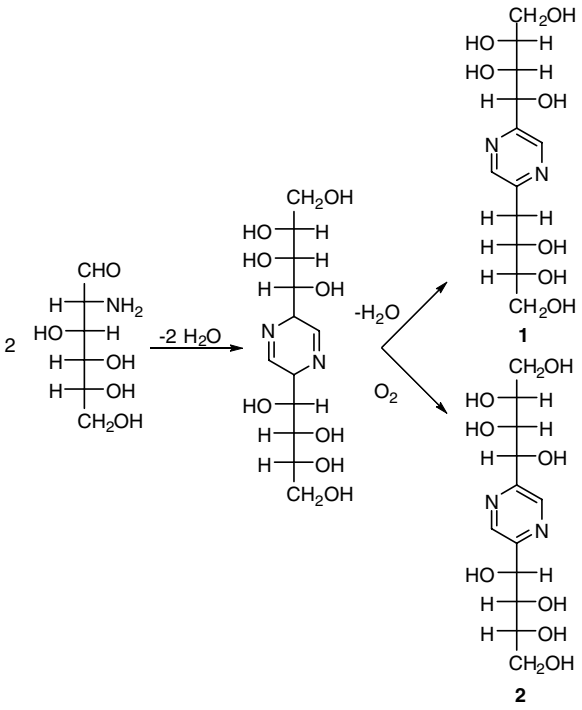
pyrazine] (**2**) are major components (Scheme 1).[8] As compounds **1** and **2** are closely related, conventional reversed-phase HPLC columns do not provide a satisfactory separation. Consequently, we developed a simple LC–ESIMS method using a porous graphitic carbon column for separation and identification of reaction products.

Porous graphitic carbon (PGC) exhibits exceptional physical and chemical stability, the ability to purify both hydrophobic and polar compounds, and the capacity to resolve isomers. PGC columns have been successfully applied to the separation polar phenolic compounds,[9] polyethoxylated alcohols,[10] lipids,[11] and carbohydrates.[12] The overall retention on PGC columns is a combination of multiple mechanisms including molecular shape, dispersive interactions with the matrix and charge-induced interactions with PCG's polarizable surface.[13] As demonstrated in Figure 1, the PGC column provides good separation and resolution for D-GlcN products (panel B). As illustrated in panel B, there are three major products in the solution. Unreacted D-GlcN is not observed as it has no absorption at 290–310 nm. The ratio of compound **1** and **2** (Scheme 1) is estimated to be 3:1 from *m*/*z* intensity and HPLC



Scheme 1. Structures of D-GlcN reaction products.

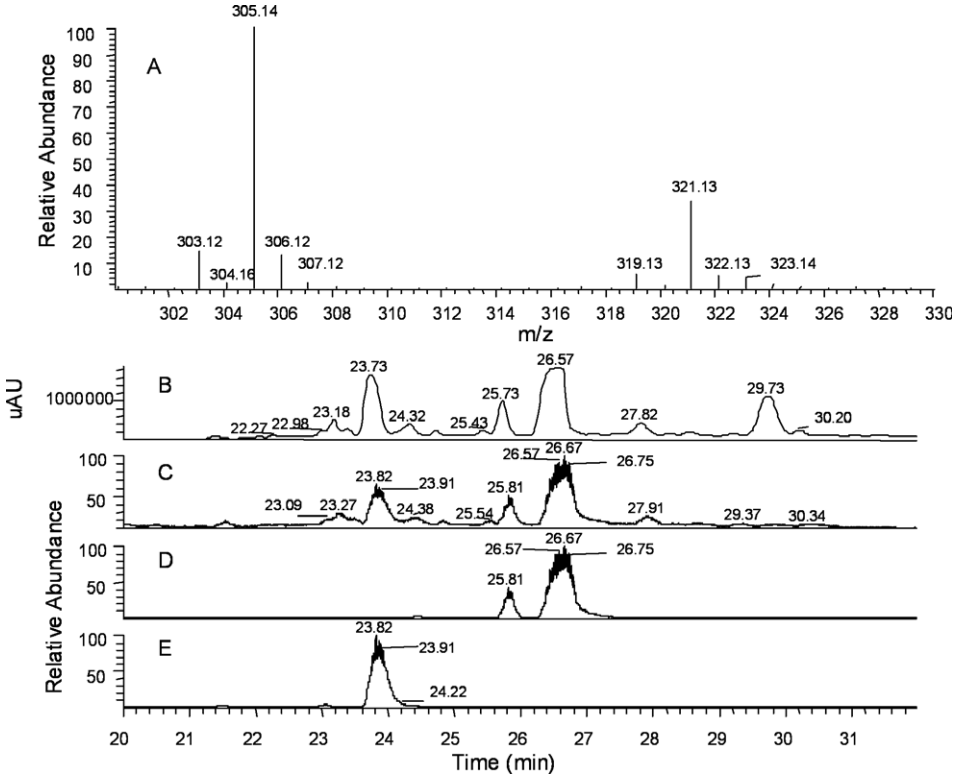


Figure 1. Chemical analysis of D-GlcN reaction products. (A) Porous graphitic carbon-LCMS spectrum of D-GlcN reaction products in PBS using positive-ion mode analysis. Mass spectrum of major total ion current (TIC) peaks (23.75–27.94 min); (B) elution profile from the PGC column (absorption at 300 nm); (C) TIC trace (15–45 min) showing that the peak at 29.73 min in panel B did not ionize well; (D) extracted base peak of *m*/*z* 305.1 showing its correspondence to the major peak at 26.67 min; (E) extracted base peak of *m*/*z* 321.1 showing its correspondence to the major peak at 23.82 min.