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Immunological Consequences of Methamphetamine Protein Glycation

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Methamphetamine (**1**), commonly known as "speed", "ice", or "crank", is a potent psychostimulant that has recently seen extensive growth in its use and production in clandestine laboratories. Although numerous detrimental consequences of **1** use are documented,[1] the molecular mechanisms underlying these physiological effects remain unknown. In our laboratory, we have initiated a research program into the chemical reactivity of long-lived drugs of abuse and their respective metabolites. Nornicotine, a long-lived metabolite of nicotine ($t_{1/2} \approx 8$ h), is capable of catalyzing aldol reactions *in aqua*[2] and contributing to aberrant protein glycation in vitro and in vivo.[3] Methamphetamine has an extended half-life ($t_{1/2} \approx 12$ h), a potentially reactive secondary amine, and as such, we felt it was a good candidate to participate in previously uncharacterized biochemical reactions. Given the extended half-life of **1**, coupled with the apparent structural similarities to nornicotine (Figure 1), we considered that **1**-based protein glycation could have profound biological consequences in the context of addiction. Herein, we report our findings into the immunological effects of this process.

The initial stage of protein glycation is accepted to proceed via the Amadori rearrangement (Scheme 1), although the specific mechanism varies widely based upon factors such as pH, ionic strength, and temperature.[4] As such, we analyzed the reaction of methamphetamine with glucose in buffer so as to detect the corresponding Amadori rearrangment product under physiological conditions. Incubation of glucose (200 mM) and (+)-**1** (10 mM) for 12 h at 37 °C in phosphate buffer (pH 7.4) led to detectable quantities (~0.2 mM) of the Amadori rearrangement product **2** by LC/MS. Encouraged by these results, an advanced glycation endproduct (AGE) derived from **1** and BSA was prepared by incubation of **1**, glucose, and BSA at 37 °C for two weeks (Scheme 1). Using commercially available polyclonal antibody (pAb) serum elicited against a methamphetamine−protein conjugate (U. S. Biological), we observed specific covalent modification of BSA by ELISA and dot blot after extended incubation periods (Figure 2). In essence, the biochemical formation of a **1**-derived AGE has numerous similarities to traditional hapten preparation,[5] in which a nonimmunogenic molecule is covalently conjugated to a carrier protein via a linker using a chemical coupling reagent prior to immunization.[6] In light of this parallel, we postulated that proteins glycated by **1** could evoke an abnormal immune response.

It has been reported that protein glycation can lead to increased immunogenicity of endogenous proteins[7] and, furthermore, that autoantibodies against AGEs can be detected in the serum of diabetic patients.[8] However, increased immunogenicity has not been demonstrated in the context of glycation with an exogenous small molecule. Our rationale was that if the **1**-AGE increases the immunogenicity of mouse serum albumin (MSA), a rodent would mount an immune response against the covalently bound methamphetamine, thereby generating serum antibodies that could remove the drug from the bloodstream.

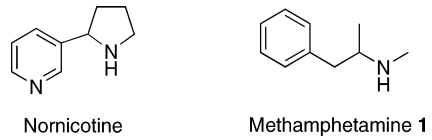


Figure 1. Structures of nornicotine and methamphetamine **1**.

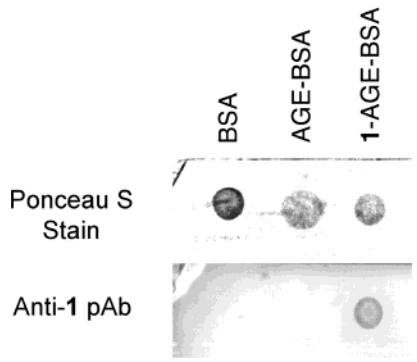
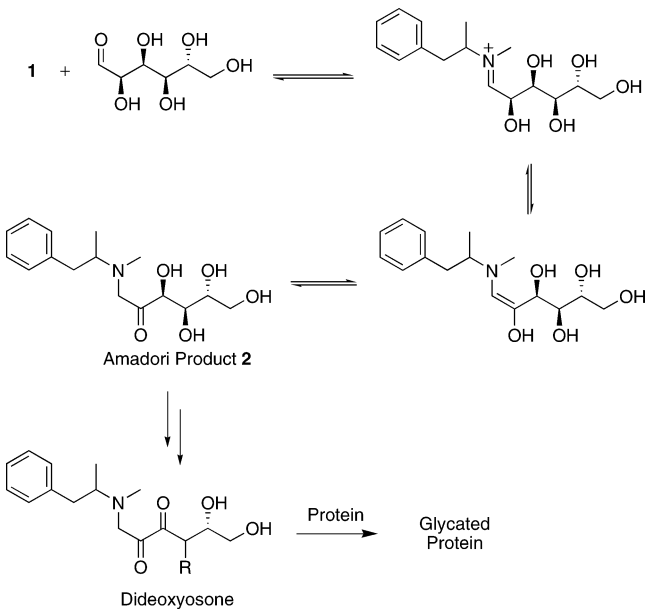


Figure 2. Dot blot detection of methamphetamine-derived covalent modification of BSA.

Scheme 1. Methamphetamine Protein Glycation as Initiated by Glucose and **1**[a]



[a] R = H or OH depending on the nature of the oxidation.

To begin to examine the validity of this hypothesis, methamphetamine-glycated MSA was prepared and exhaustively dialyzed against phosphate buffer to ensure the absence of any free glucose or **1**. Mice were immunized with this modified protein, and serum samples were analyzed by ELISA to ascertain if either an anti-**1**-

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AGE or anti-MSA response was present. All initial immunizations were performed using an alternative injection regimen that used frequent doses in small amounts to more closely simulate the "binge and crash" pattern of methamphetamine abuse. Indeed, in these binging periods, chronic users are known to participate in "runs" in which as much as a gram of the drug is injected every 2 to 3 h to maintain a suitable response.[9]

After the preliminary series of injections, an appreciable titer[10] was discovered against **1**-AGE (1:1000−1:2000), while no significant titer was observed against the MSA carrier protein or in control injections of MSA alone. This finding was particularly surprising as aberrant covalent protein modification has been shown to yield a detectable titer against the carrier protein.[11] Presumably, autoreactive B cells were selected against through various mechanisms (e.g., anergy, clonal deletion) prior to affinity maturation and subsequent secretion of IgG. Furthermore, it is noteworthy that no adjuvant was required to achieve significant titers against **1**-modified MSA, suggesting the immune system needs little priming to recognize foreign glycation motifs. The obtained titer against the **1**-AGE-MSA was long-lasting and could be increased to 1:4000−1:32 000 by standard booster injections. Interestingly, these titers against **1**-AGE-MSA are 1 order of magnitude larger than those obtained against a methamphetamine−KLH conjugate used in the development of an active vaccine for methamphetamine addiction.[12]

The presence of antibodies against proteins that have been covalently modified by a drug of abuse has been described in the context of chronic cocaine addicts.[13] As such, we assessed if **1**-based glycation gives rise to antibodies capable of binding methamphetamine, leading to an unrecognized mechanism of tolerance. Competition studies were performed by ELISA using the obtained anti-**1**-AGE pAb serum. Gratifyingly, negative control competitions with MSA, AGE-MSA prepared from glucose and MSA, a small molecule AGE-MSA analogue prepared from N^{α}-acetyllysine (Ac-Lys-OH) and glucose, and synthetically prepared **1**-derived Amadori rearrangement product[14] all showed no appreciable competition with **1**-glycated RNase A for antibody binding.

Although competition with free **1** was poor ($K_{d,app} \approx 10$ mM), a small molecule analogue of the **1**-AGE-MSA prepared from Ac-Lys-OH did effectively compete ($K_{d,app} \approx 10\ \mu$M) with **1**-AGE-RNase A. This compound was prepared such that the lysine side chain that becomes glycated in MSA is mimicked, thereby providing a similar reaction environment to **1**-AGE formation reactions. Glycation inherently generates a complex heterogeneous mixture of products, and as such, exact quantitation of the extent of binding was not possible. However, our results do provide clear evidence that the primary epitope of the anti-**1**-AGE serum incorporates the methamphetamine moiety as well as the glucose-derived cross-linking region and, furthermore, that detectable methamphetamine binding does occur.

While **1** did not strongly compete for binding to our pAbs, the discovery that these antibodies bind to any extent is of potential significance in the context of addiction. Once methamphetamine is administered, a user could develop an immune response against a **1**-AGE-modified protein, and the produced antibodies could bind some proportion of the serum methamphetamine, thereby reducing the available concentration of the drug and ensuing high. Furthermore, autoantibodies against methamphetamine-modified proteins could have undesirable consequences such as the misregulated activation of inflammatory pathways, leading to extensive tissue damage.

In total, our results provide an intriguing possibility for an unrecognized mechanism underlying methamphetamine addiction and the associated health consequences. Further studies into the importance of this process in vivo are underway and will be reported in due course.

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Supporting Information Available: Experimental procedures for immunogen preparation, immunization conditions, and ELISA experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Titer is defined as a measure of the presence and amount of antibody in blood against a particular type of tissue, cell, or substance. In this case, the titer is the observed binding to **1**-AGE-RNase A.
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