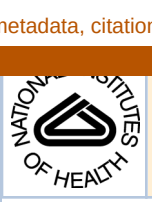

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Glycated AAV Vectors: Chemical Redirection of Viral Tissue Tropism

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Abstract

A chemical approach for selective masking of arginine residues on viral capsids, featuring an exogenous glycation reaction has been developed. Reaction of adeno-associated viral (AAV) capsids with the α-dicarbonyl compound, methylglyoxal resulted in formation of arginine adducts. Specifically, surface exposed guanidinium side chains were modified into charge neutral hydroimidazolones, thereby disrupting a continuous cluster of basic amino acid residues implicated in heparan sulfate binding. Consequent loss in heparin binding ability and decrease in infectivity were observed. Strikingly, glycated AAV retained ability to infect neurons in the mouse brain and were redirected from liver to skeletal and cardiac muscle following systemic administration in mice. Further, glycated AAV displayed altered antigenicity demonstrating potential for evading antibody neutralization. Generation of unnatural amino acid side chains through capsid glycation might serve as an orthogonal strategy to engineer AAV vectors displaying novel tissue tropisms for gene therapy applications.

Protein engineering tools have played a central role in understanding structure-function correlates of viruses (1, 2). The application of site directed mutagenesis to elucidate the structural basis of virus-receptor interactions is particularly noteworthy in this regard (3, 4). Altering virus-receptor interactions by selective incorporation of unnatural amino acids or chemical masking of amino acid residues, exposed on the viral capsid surface could greatly expand the ability to discover receptor footprints on viral capsid surfaces as well as design new viral vectors and vaccines.

Several studies exploring the genetic incorporation of unnatural amino acids and/or chemoselective ligation to viral capsids have been reported (5). For instance, viral capsids containing unnatural amino acids with alkyne (5), azide (5) or aminophenyl (6) side chains have been generated for coupling of drugs and/or targeting ligands. Synthetic strategies for chemoselective ligation to naturally occurring amino acid residues such as Lys, Glu, Cys, Trp and Tyr on the surface of viral capsids have also been developed (7–11). However, the application of such chemistries towards reengineering viruses for gene delivery remains unexplored thus far.

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Supporting Information Available

Experimental methods for virus production, reaction conditions, mass spectroscopic analysis, solid phase binding, in vitro transduction assays and live animal studies. This material is available free of charge via the Internet at http://pubs.acs.org.

In the current study, we focused on exploiting the naturally occurring chemical reaction involving α-dicarbonyl compounds with proteins, which leads to the formation of advanced glycation endproducts (AGE)(12). We hypothesized that methylglyoxal, an intermediate of the Maillard reaction, will selectively form adducts with guanidinium side chain of surface exposed Arg residues on viral capsids (Scheme 1). The reaction proceeds readily in aqueous solution and has been shown to predominantly modify Arg residues in various proteins with high efficiency. Proteomic analysis of Arg adducts has previously demonstrated the formation of dihydroxyimidazolidines followed by subsequent rearrangement into hydroimidazolones (13, 14).

AAV is a small non-enveloped ssDNA virus that is rapidly gaining momentum as a vector in human gene therapy applications (15). The AAV capsid is comprised of 60 capsid protein subunits assembled in icosahedral symmetry (T=1). Each capsid protein subunit contains a β-barrel core with long loops between the β-strands that create striking surface features. Surface spikes located at the three-fold symmetry axes are most prominent and have been implicated in receptor binding and cell entry. The remarkable plasticity of the AAV virion shell has been demonstrated through extensive mutagenesis studies (15).

Of several strains isolated to date, AAV serotype 2 is the most studied and binds to its primary receptor heparan sulfate using a basic cluster of amino acids (R484, R487, K532, R585, and R588) located at the three-fold axis of symmetry (16, 17). Previous site directed mutagenesis studies have demonstrated the critical role played by this continuous basic patch in cell surface recognition as well as liver tropism in mice (18, 19). In particular, mutation of Arg residues within this basic cluster has been shown to confer muscle tropism. However, the potential for generating atypical tissue tropisms through the latter approach is restricted by the ability to only incorporate naturally occurring amino acids with limited side chain diversity.

We hypothesized that an orthogonal approach based on selective chemical masking of Arg residues with α-dicarbonyl compounds would enable display of unnatural amino acid residues with diverse functional groups on the AAV capsid surface. Such modification of amino acid side chains might enable design of viral vectors displaying atypical tissue tropisms. As proof of principle, we chose methylglyoxal (MGO), a highly reactive compound derived from glycolysis and known for its propensity to form Arg and Lys adducts (14). It should be noted that chemical conjugation to Lys residues on AAV2 capsids are routinely carried out and demonstrate no adverse effect on capsid infectivity (20).

Overnight reaction of AAV2 with methylglyoxal at neutral pH produced glycated AAV vectors. Characterization of the glycated vectors was carried out using MALDI-TOF/MS of trypsin-treated capsid proteins. Rationale for such analysis is based on earlier observations that altered tryptic digestion of glycated proteins enables proteomic analysis of MGO adducts (13). Tryptic fragments obtained from the major AAV capsid protein subunit (VP3; Genbank# AAC03779.1) were subject to MALDI-TOF/MS analysis. Peak signals were normalized to that of a peptide fragment located within the capsid interior (16) containing residues L299 through K309 (m/Z = 1375.7), which are expected to remain unmodified when subject to glycation. Analysis of mass spectra using the MASCOT search engine revealed a decrease in peak signal intensities corresponding to peptide fragments containing Arg residues and, to a lesser extent, Lys residues (Supporting Information). Specifically, the MGO-derived hydroimidazolone adduct is expected to add 54 m/Z units to the modified peptide fragment. New peak signals, observed between m/Z 2400 and 2600 were specifically assigned to peptide fragments containing hydroimidazolone adducts at R585 and R459 positions (Figure 1a). Computation of the ratio of peak signal intensities pertaining to the latter residues before and after glycation indicates that 29.5% of R585 and 58.9% of

R459 residues are glycated. Although the latter results indicate the potential formation of mixtures of variably glycated capsids and are semi-quantitative in nature, we have optimized chemical modification of AAV vectors to consistently generate the same phenotype (Supporting Information). Moreover, the aforementioned results are in agreement with earlier studies and corroborate the notion that surface-exposed Arg residues on proteins are preferred targets for glycation (13, 14).

The ability of glycated AAV2 to bind heparin was determined using a streptavidin-coated 96-well assay plate coated with biotinylated heparin. Binding was determined using Q-PCR and binding to biotinylated dextran was utilized as a control. Glycated AAV2 capsids display a significant loss ($p < 0.05$) in heparin binding ability in comparison with unmodified AAV2 capsids (Figure 1b). Further, MGO-modified AAV2 vectors demonstrate an approximately 1000-fold decrease in gene transfer efficiency in HEK293 cells (Figure 1c). These results corroborate the notion that glycated AAV2 is unable to bind its cognate attachment receptor, heparan sulfate (21). Of the five residues (R484, R487, K532, R585, and R588) located within the heparan sulfate receptor footprint, R585 or R588 have been shown to be indispensable for heparin binding and infectivity (18, 19). Taken together with MS data, the aforementioned results suggest that conversion of the guanidinium side chain of R585 into the hydroimidazolone derivative likely disrupts the heparan sulfate footprint on the AAV2 capsid surface. It should also be noted that although glycation of R459 was observed, this residue has not been implicated in heparin binding or infectivity (18, 19).

Another observation supporting the chemical masking of arginine residues within surface-exposed domains is the altered antigenicity of glycated AAV2 vectors. Using a solid phase binding assay, we determined that MGO-modified AAV2 vectors showed a > 2-fold decrease in binding to a prototypical monoclonal antibody (A20), which recognizes intact AAV2 capsids (Figure 1d). An isotype matched antibody served as control. These results indicate the potential for glycated AAV vectors to evade pre-existing neutralizing antibodies in the human population (22).

Despite demonstrating decreased infectivity *in vitro*, glycated AAV2 capsids infect neurons as efficiently as untreated AAV2 following intracranial injection into the mouse brain (Figure 2). Taken together with the altered antigenicity described earlier; these results suggest that glycated AAV2 vectors might be well suited for CNS gene transfer applications. Further, bioluminescent images of mice obtained following intravenous (tail vein) administration of glycated AAV2 show expansion of viral tissue tropism beyond the liver to other systemic organs (Figure 2c). Quantitation of luciferase expression in tissue lysates reveals approximately 100-fold higher transgene expression levels in cardiac and skeletal muscle with MGO-treated AAV2 in comparison with untreated AAV2 vectors (Figure 2d). In addition, a concomitant decrease in transgene expression within the liver (over 10-fold) is observed. These results demonstrate the feasibility of chemical redirection of AAV2 capsids from liver to muscle tissue and corroborate a similar transduction profile obtained by mutagenesis of R585/R588 residues within the heparan sulfate footprint (19, 23).

In conclusion, the aforementioned studies validate the notion that selective chemical masking of Arg residues with α-dicarbonyl compounds can serve as an orthogonal approach to reengineer viral surface topology and consequently, antigenicity, receptor usage and tissue tropism. This initial study is one of the first to demonstrate feasibility of displaying unnatural hydroimidazolone side chains on viral capsids. A broad range of α-dicarbonyl compounds ranging from glyoxal to oxo-(7-oxoacetyl-9H-fluoren-2-yl)-acetaldehyde are currently being explored for formation of diverse Arg adducts on AAV capsids. An increase in complexity of the α-dicarbonyl side chain may allow generation of novel tissue tropisms,

while simultaneously enabling design of AAV vectors with capable of evading neutralizing antibodies. This chemical approach represents a new dimension in viral capsid engineering and is likely to impact viral vector design for human gene therapy applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kwon I, Schaffer DV. Designer gene delivery vectors: Molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. Pharm Res. 2008; 25:489–499. [PubMed: 17763830]
2. Law LK, Davidson BL. What does it take to bind CAR? Mol Ther. 2005; 12:599–609. [PubMed: 16109509]
3. Racaniello VR. Early events in poliovirus infection: Virus-receptor interactions. Proc Natl Acad Sci USA. 1996; 93:11378–11381. [PubMed: 8876143]
4. Cotmore SF, Tattersall P. Parvoviral host range and cell entry mechanisms. Adv Virus Res. 2007; 70:183–232. [PubMed: 17765706]
5. Strable E, Finn MG. Chemical modification of viruses and virus-like particles. Curr Top Microbiol Immunol. 2009; 327:1–21. [PubMed: 19198568]
6. Carrico ZM, Romanini DW, Mehl RA, Francis MB. Oxidative coupling of peptides to a virus capsid containing unnatural amino acids. Chem Commun(Camb). 2008; (10):1205–1207. [PubMed: 18309418]
7. Wang Q, Lin T, Johnson JE, Finn MG. Natural supramolecular building blocks. cysteine-added mutants of cowpea mosaic virus. Chem Biol. 2002; 9:813–819. [PubMed: 12144925]
8. Hooker JM, Kovacs EW, Francis MB. Interior surface modification of bacteriophage MS2. J Am Chem Soc. 2004; 126:3718–3719. [PubMed: 15038717]
9. Antos JM, Francis MB. Selective tryptophan modification with rhodium carbenoids in aqueous solution. J Am Chem Soc. 2004; 126:10256–10257. [PubMed: 15315433]
10. Meunier S, Strable E, Finn MG. Crosslinking of and coupling to viral capsid proteins by tyrosine oxidation. Chem Biol. 2004; 11:319–326. [PubMed: 15123261]
11. Barnhill HN, Reuther R, Ferguson PL, Dreher T, Wang Q. Turnip yellow mosaic virus as a chemoaddressable bionanoparticle. Bioconjug Chem. 2007; 18:852–859. [PubMed: 17428027]
12. Rabbani N, Thornalley PJ. The dicarbonyl proteome: Proteins susceptible to dicarbonyl glycation at functional sites in health, aging, and disease. Ann NY Acad Sci. 2008; 1126:124–127. [PubMed: 18448805]
13. Brock JW, Cotham WE, Thorpe SR, Baynes JW, Ames JM. Detection and identification of arginine modifications on methylglyoxal-modified ribonuclease by mass spectrometric analysis. J Mass Spectrom. 2007; 42:89–100. [PubMed: 17143934]
14. Lo TW, Westwood ME, McLellan AC, Selwood T, Thornalley PJ. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. J Biol Chem. 1994; 269:32299–32305. [PubMed: 7798230]
15. Mitchell AM, Nicolson SC, Warischalk JK, Samulski RJ. AAV's anatomy: Roadmap for optimizing vectors for translational success. Curr Gene Ther. 2010; 10:319–340. [PubMed: 20712583]

16. Xie Q, Bu W, Bhatia S, Hare J, Somasundaram T, Azzi A, Chapman MS. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. Proc Natl Acad Sci USA. 2002; 99:10405–10410. [PubMed: 12136130]

17. Levy HC, Bowman VD, Govindasamy L, McKenna R, Nash K, Warrington K, Chen W, Muzyczka N, Yan X, Baker TS, Agbandje-McKenna M. Heparin binding induces conformational changes in adeno-associated virus serotype 2. J Struct Biol. 2009; 165:146–156. [PubMed: 19121398]

18. Opie SR, Warrington KH Jr, Agbandje-McKenna M, Zolotukhin S, Muzyczka N. Identification of amino acid residues in the capsid proteins of adeno-associated virus type 2 that contribute to heparan sulfate proteoglycan binding. J Virol. 2003; 77:6995–7006. [PubMed: 12768018]

19. Kern A, Schmidt K, Leder C, Muller OJ, Wobus CE, Bettinger K, Von der Lieth CW, King JA, Kleinschmidt JA. Identification of a heparin-binding motif on adeno-associated virus type 2 capsids. J Virol. 2003; 77:11072–11081. [PubMed: 14512555]

20. Lee GK, Maheshri N, Kaspar B, Schaffer DV. PEG conjugation moderately protects adeno-associated viral vectors against antibody neutralization. Biotechnol Bioeng. 2005; 92:24–34. [PubMed: 15937953]

21. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. J Virol. 1998; 72:1438–1445. [PubMed: 9445046]

22. Calcedo R, Vandenberghe LH, Gao G, Lin J, Wilson JM. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J Infect Dis. 2009; 199:381–390. [PubMed: 19133809]

23. Asokan A, Conway JC, Phillips JL, Li C, Hegge J, Sinnott R, Yadav S, DiPrimio N, Nam HJ, Agbandje-McKenna M, McPhee S, Wolff J, Samulski RJ. Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. Nat Biotechnol. 2010; 28:79–82. [PubMed: 20037580]

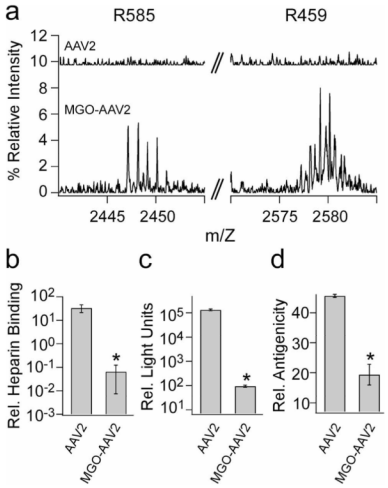


Figure 1.

a) Tryptic digestion of chemically modified AAV2 VP3 capsid proteins demonstrates two preferred sites for Arg adduct formation. The increase in signal at m/Z 2446.1 and 2577.2 corresponds to the hydroimidazolone adduct at R585 and R459 positions within the peptides 566-TTNPVATEQYGSVSTNLQRGNR-588 (expected adduct m/Z = 2446.173) and 448-TNTPSGTTTQSRLQFSQAGASDIR-471 (expected adduct m/Z = 2577.231), respectively. b) Solid phase binding assay of unmodified and glycated AAV2 capsids to heparin-coated plates. Binding is relative to the charge neutral dextran. c) In vitro infectivity assay of unmodified and glycated AAV2 in HEK293 cells (10,000 viral genomes/cell). d) Solid phase binding assay of unmodified and glycated AAV2 capsids to plates coated with the monoclonal antibody A20. Binding is relative to an isotype-matched antibody control. Error bars represent standard deviation (n = 3). Asterisks represent statistical significance ($p < 0.05$). Additional experimental details available in Supporting Information.

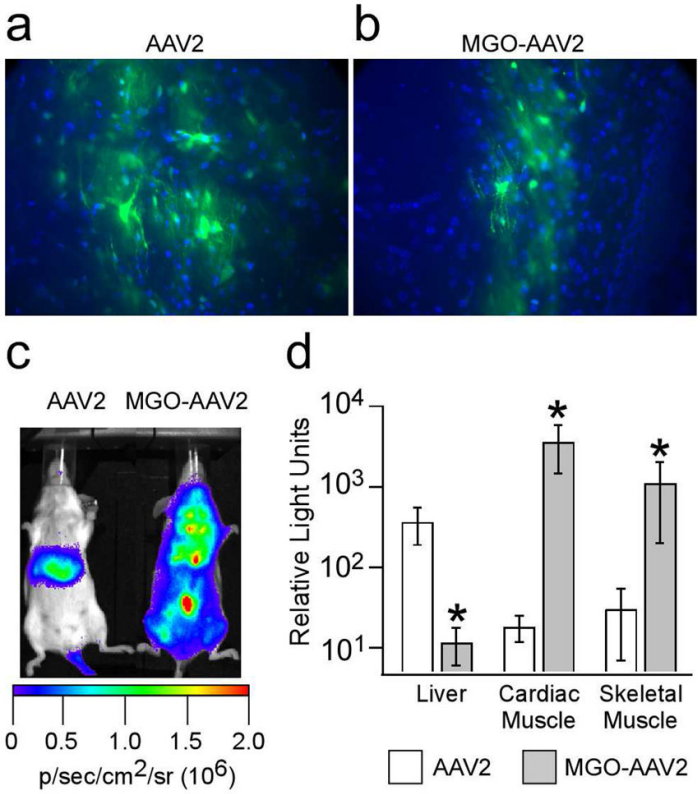
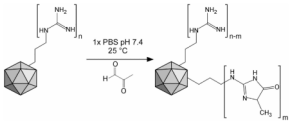


Figure 2.

Epifluorescence images of neuronal GFP expression in 40 μm brain sections obtained from BALB/c mice injected intracranially with 7.5×10^8 a) untreated AAV2 and b) MGO-treated AAV2 vectors. Brain sections were taken 2 weeks post-injection and stained with DAPI prior to imaging. c) Live animal bioluminescent images of BALB/c mice (n = 3) injected with untreated and MGO-treated AAV2 vectors. Mice were injected via tail vein at a dose of 5×10^{10} viral particles. Mice were imaged 4 weeks post-injection. Changes in luciferase expression patterns indicate altered tissue tropism profiles and are represented on a rainbow scale. d) Luciferase expression from untreated and MGO-treated AAV2 vectors in different tissue lysates. Relative light units represent luciferase transgene expression and levels are normalized to tissue mass. Error bars represent standard deviation (n = 3). Asterisks represent statistical significance ($p < 0.05$). Additional experimental details available in Supporting Information.



Scheme 1.
Glycation of AAV vectors with methylglyoxal