Poly(β-aminosulfonamides) as gene delivery vectors: synthesis and *in vitro* screening†

Lin Gan,[a] Jennifer L. Olson,[b] Clifton W. Ragsdale*[b] and Luping Yu*[a]

Received (in Cambridge, UK) 17th September 2007, Accepted 13th November 2007
First published as an Advance Article on the web 22nd November 2007
DOI: 10.1039/b714278a

A series of poly(β-aminosulfonamides) was synthesized and demonstrated to be efficient *in vitro* transfection reagents.

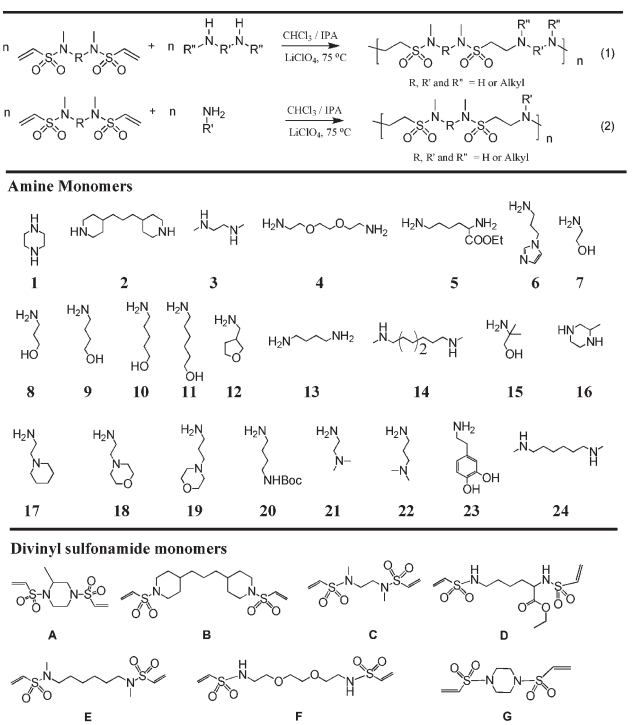
A major challenge in gene therapy is the development of safe and efficient methods of gene delivery to target cells. Viral carriers have attracted a great deal of attention due to their high efficiencies, but these vectors present many challenges including adverse immunological responses, limitations on DNA encapsulation size and high production costs.[1–6] Synthetic transfer vehicles, by contrast, offer the advantages of potential immunological neutrality, greater flexibility in structural modification and reduced costs of synthesis. The major handicaps for synthetic vectors have been toxicity and low efficiency.[1,3,5]

In this paper, we report a series of poly(β-aminosulfonamides) (PBASs) for gene delivery. We chose to investigate sulfonamide-containing vectors because sulfonamide moieties are extensively used in human pharmaceuticals (sulfa drugs, sulfonylureas, thiazides) and are predicted to be well tolerated. A specific advantage to PBASs is that the polymerization process employs simple Michael addition reactions and can be used to generate a series of polymers for screening. Previous work by R. Langer and P. Ferruti has shown the value of a large screening library when identifying novel transfectants.[7–15] Finally, the sulfonamide group confers a stability that should be advantageous for any further polymer modifications.

The series of poly(β-aminosulfonamides) was generated by polymerization of divinylsulfonamides with bis(secondary amines), bis(primary amines) and mono(primary amine) utilizing the Michael addition reaction (Scheme 1). Amine monomers **1**–**24** are commercially available. The divinylsulfonamide monomers **A**–**G** were derived from certain of the amine monomers and were designed to have a range of rigidities, hydrophobicities and linker lengths.

Polymerization was carried out in vials with Teflon lined screw caps at 75 °C in the dark for one week. Chloroform proved to be a good solvent for both monomers and the resulting polymers. Isopropanol (IPA) was added to accelerate the reaction by assisting proton transfer. No adduct product of IPA with divinylsulfonamides was detected. For sluggish reactions caused by steric hindrance, different Lewis acid catalysts were tested and $LiClO_4$ was found to accelerate the reaction effectively. An advantage of using $LiClO_4$ is its solubility in diethyl ether (114 g per 100 mL ether), which facilitated the purification process. Both bis (secondary amines) and mono (primary amine) monomers polymerized well under standard conditions. Gelation due to cross-linking was observed with bis (primary amines), which could be countered by carrying out the reaction at room temperature for 3 days. When polymerization was complete, the polymers were precipitated repeatedly in diethyl ether and dried under vacuum at 60 °C for 2 days or, for the bis(primary amines), at room temperature for 5 days.

From paired combinations of the two monomer groups, 55 polymers were synthesized and characterized. MALDI-TOF MS was used to verify structure and purity and to determine the molecular weights of the resulting polymers, which ranged from 1200 to 8300 Da. 47 polymers were soluble in water or DMSO. Different monomeric components of the poly (β-aminosulfonamides) impart pH-sensitive characteristics to the polymers. The



Scheme 1 Polymerization of amine monomers **1**–**24** with divinylsulfonamide monomers **A**–**G** *via* Michael addition reactions (top) afforded a series of PBAS polymers.

[a]*Department of Chemistry and James Franck Institute, University of Chicago, CIS E419A, 929 E. 57th St., Chicago, IL 60637, USA. E-mail: lupingyu@uchicago.edu; Fax: +1 773-702-0805; Tel: +1 773-702-8698*
[b]*Department of Neurobiology, University of Chicago, 947 E. 58th St., Chicago, IL 60637, USA. E-mail: cragsdale@uchicago.edu; Fax: +1 773-702-1216; Tel: +1 773-702-9609*

† Electronic supplementary information (ESI) available: synthesis and physical characterization details and cytotoxicity data. See DOI: 10.1039/b714278a

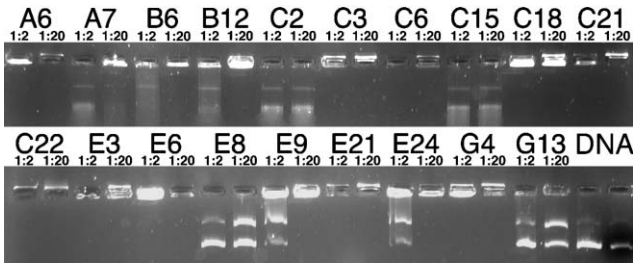


Fig. 1 Gel retardation assays of PBAS binding to pDNA. 1 : 2 and 1 : 20 identify the v/v ratios employed for the pDNA (2 mg mL^{-1}) and polymer (6.3 mM) mixtures.

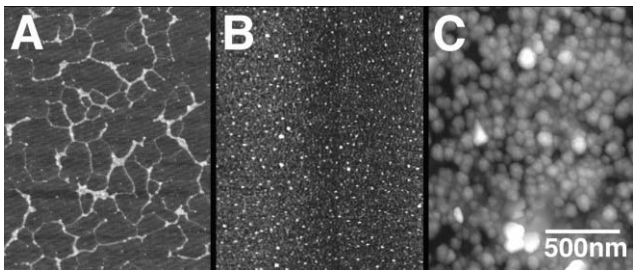


Fig. 2 AFM images of (A) pDNA alone, (B) polymer E24 alone and (C) pDNA incubated with E24 at 1 : 2 v/v ratio.

majority of the polymers showed buffering effects between pH values of 5.0 and 7.1 (see Table S1 in the Supporting Information†).

PBAS/pDNA polyplexes were characterized using standard biophysical methods. Competency to bind DNA was assessed in agarose gel electrophoresis experiments (Fig. 1). 34 of the 47 soluble PBAS polymers formed complexes with pDNA at v/v ratios greater than 1 : 2. Atomic force microscopy studies of the PBAS polymer E24 complexed with pDNA demonstrated the formation of spherical nanoparticles with diameters ranging from 60 to 100 nm (Fig. 2).

Transfection efficacy was measured in COS-7 cells with the Tropix luciferase assay. The transfections were performed in triplicate in 96-well plates. The polymers (1.6 mM) and the pRSV-Luc plasmid DNA (12 ng ul^{-1}) were combined at different volume ratios and incubated for 30 min. The resulting polyplex solutions were added to COS-7 cell cultures for 4 h and then replaced with growth medium. Luciferase activity was quantified after 72 h with a bioluminescence plate reader. The most effective *in vitro* transfectants were C6, C18, E21 and E24 (Fig. 3). The transfection levels of E24 proved to be 10-fold greater than those of the polyethyleneimine reagent JetPEI (Polyplus) and 4-fold greater than those of the proprietary cationic polymer OmniPORTER (MP Biomedicals). Cell toxicity was measured by MTT assay. Over half of the soluble polymers showed greater than 80% viability (see the Supporting Information†).

We tested the ability of E24 to deliver fluorescent protein-encoding plasmids to COS-7 cells (Fig. 4). These experiments demonstrated that at least part of E24's greater efficacy over OmniPORTER is due to greater numbers of transfected cells. These reporter experiments confirmed the low toxicity of E24 and demonstrated E24's ability to transfect cells in serum-containing media (not illustrated).

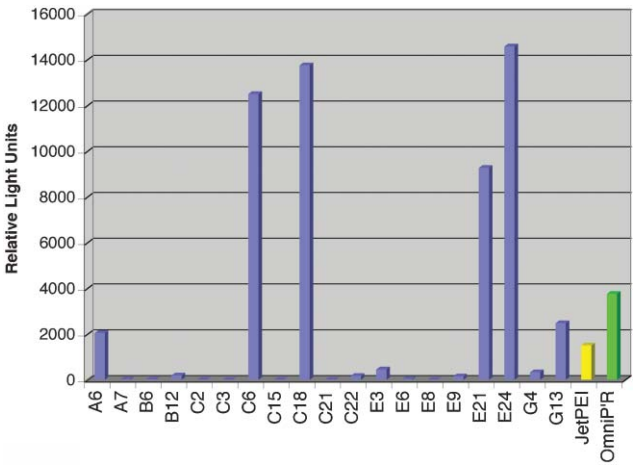


Fig. 3 Luciferase *in vitro* transfection results comparing the most effective PBAS polymers (blue) with two of the leading commercial cationic transfectants (yellow, green).

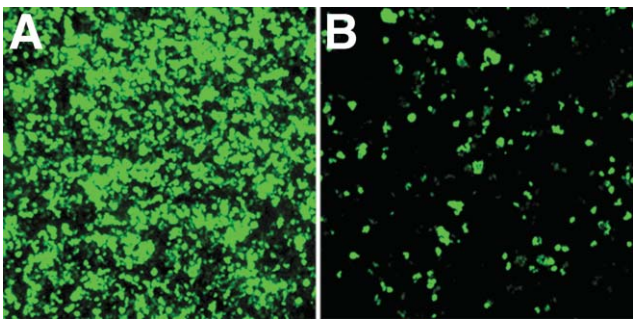


Fig. 4 Transcription efficiency of the PBAS polymer E24 (A) is greater than that of the commercial compound OmniPORTER (B). Illustrated are confluent fields of COS-7 cells transfected with the 3 × Venus[16] fluorescent protein reporter plasmid.

The four best PBAS transfectants complex with DNA, but do not appear to share other common properties that distinguish them from other soluble PBASs. Three of the four best PBASs have molecular weights between 1600 and 1700 Da, but the fourth, C18, weighs 5200 Da. Three of the best polymers packed DNA well at even a low ratio (1 : 2 v/v), as tested by gel electrophoresis, but polymer E24 did not. The buffering ranges of the four best compounds also varied. Interestingly, the pH buffer ranges provide some evidence against a "proton sponge"[17] mechanism for PBAS-mediated plasmid delivery as the leading reagent, E24, has a narrow buffering range and many ineffective PBAS polymers fall within the predicted ideal range. Detailed biological studies on cellular delivery pathways will be important in elucidating the mechanisms of gene transfer by PBASs.

This investigation was supported with funds provided by the Branfman Family Foundation. We thank Dr Miriam Domowicz for help with the Tropix luciferase assay.

Notes and references

1 D. W. Pack, A. S. Hoffman, S. Pun and P. S. Stayton, *Nat. Rev. Drug Discovery*, 2005, **4**, 581.
2 A. L. Parker, C. Newman, S. Briggs, L. Seymore and P. J. Sheridan, *Expert Rev. Mol. Med.*, 2003, **5**, 1–14.

3 I. M. Verma and N. Somia, *Nature*, 1997, **389**, 239–242.
4 R. G. Vile, S. J. Russell and N. R. Lemoine, *Gene Ther.*, 2000, **7**, 2–8.
5 P. L. Felgner and G. Rhodes, *Nature*, 1991, **349**, 351–352.
6 W. J. Moree, G. A. van der Marel and R. J. Liskamp, *J. Org. Chem.*, 1995, **60**, 5157.
7 D. G. Anderson, D. M. Lynn and R. Langer, *Angew. Chem., Int. Ed.*, 2003, **42**, 3153–3158.
8 D. M. Lynn and R. Langer, *J. Am. Chem. Soc.*, 2000, **122**, 10761.
9 D. M. Lynn, D. G. Anderson, D. Putnam and R. Langer, *J. Am. Chem. Soc.*, 2001, **123**, 8155.
10 A. Akinc, D. M. Lynn, D. G. Anderson and R. Langer, *J. Am. Chem. Soc.*, 2003, **125**, 5316–5323.
11 J. J. Green, J. Shi, E. Chiu, E. S. Leshchiner, R. Langer and D. G. Anderson, *Bioconjugate Chem.*, 2006, **17**(5), 1162–1169.
12 G. T. Zugates, D. G. Anderson, S. R. Little, I. E. B. Lawhorn and R. Langer, *J. Am. Chem. Soc.*, 2006, **128**(39), 12726–12734.
13 F. Danusso and P. Ferruti, *Polymer*, 1970, **11**, 88–113.
14 S. C. W. Richardson, N. G. Pattrick, Y. K. S. Man, P. Ferruti and R. Duncan, *Biomacromolecules*, 2001, **2**, 1023–1028.
15 P. Ferruti, J. Franchini, M. Bencini, E. Ranucci, G. P. Zara, L. Serpe, L. Primo and R. Cavalli, *Biomacromolecules*, 2007, **8**(5), 1498–1504.
16 G. Genove, B. S. Glick and A. L. Barth, *Biotechniques*, 2005, **39**, 814–818.
17 O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J. P. Behr, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 7297–7301.