Discriminated properties of PEI functionalized gold nanoparticles (Au-PEIs) predetermined by synthetic routes of ligand exchange and reduction processes: Paths and Fates

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ABSTRACT

Polyethyleneimine (PEI) functionalized AuNPs (Au-PEIs) have potential use as positively charged gold nanoparticles (AuNPs) for nano-medicinal applications, due to their cationic surfaces that promote cellular uptake and gene transfection.[1-6] Au-PEIs can be prepared by one of two methods: (1) ligand modification (exchange or co-attachment) of AuNPs and PEI, or (2) reduction of Au^{III} ions in the presence of PEI. Herein, the ligand exchanged Au-PEIs (Au-PEI[*LE*]s) and the materials by reduction (Au-PEI[*Red*]s) were prepared from citrate-stabilized AuNPs (10 nm, nominal) and $HAuCl_4$, respectively. We demonstrate differentiated product formation via each synthetic route and their discriminated physico-chemical properties by systematic examination. The physico-chemical properties and the conjugation mechanisms of Au-PEI[*LE*] and Au-PEI[*Red*] were characterized by orthogonal analyses system. Furthermore, the colloidal stabilities of all produced Au-PEIs were investigated under various physiologically relevant conditions and shown that the consequential fates – property and colloidal stability – of Au-PEIs were dependent on born pathways significantly. We found that some of Au-PEIs showed outstanding colloidal stability in certain circumstances which is very critical to be a drug carrier in nanomedicine.

Keywords: gold nanoparticles, polyethyleneimine, PEI, gold PEI conjugates, positively charged, synthesis, characterization, stability

1 INTRODUCTION

Polyethyleneimine (PEI) coated gold nanoparticles (AuNPs), hereafter Au-PEIs, have been studied due to several biologically relevant features, including interaction with anionic polyelectrolytes[7] and biological entities (cell surface),[8] cellular uptake,[9-11], gene delivery/transfection,[1-6, 12] hydrophilicity, and proton scavenging (pH tolerance).[6, 8] In general, Au-PEIs can be prepared by *ligand exchange* of surface modified AuNPs by PEI chains (Au-PEI[*LE*]), and *reduction method* started from $HAuCl_4$ in the presence of PEI (Au-PEI[*Red*]). Despite the breadth of the aforementioned work, it is still unclear how the specific properties of PEI and the methods utilized to conjugate it to AuNPs impact the final product size and stability (which presumably impact performance). Furthermore, knowledge regarding the stability of Au-PEIs under physiologically relevant conditions is critically important for biological applications, yet this has not generally been reported in prior studies. Colloidal stability can dramatically impact the findings for bio-relevant research, where performance can be impacted by one distinct population within a polydisperse material, agglomeration/aggregation effects, or surface modifications.[13] Here in, we explored a range of synthetic routes by systematic examination of variables, including reaction condition, the molar mass/backbone structure of PEIs, and molar mass ratio (PEI:Au). Au-PEI products were evaluated by a variety of approaches to evaluate the impact of synthetic route on physico-chemical properties. Orthogonal analytical methods were utilized including dynamic light scattering (DLS), ultraviolet-visible (UV-Vis) spectroscopy, transmission electron microscope (TEM), and attenuated total reflectance FT-IR (ATR-FTIR) measurements. Additionally, the colloidal stability for each produced Au-PEIs was investigated under physiologically relevant conditions including shelf-life (at least 6 months period), temperature variation, wide range of pH values, and behaviors in biological level media. We demonstrated that some of Au-PEIs showed remarkable colloidal stability which is a key requirement for drug carrier candidates in nanomedicine. In closing, comparing physico-chemical properties of Au-PEI produced between different synthetic approaches can provide the nanomedicine community useful information for better design of nano-vector development.

2 EXPERIMENTS

2.1 Materials and Instruments[ξ]

$Gold^{III}$ chloride hydrate ($HAuCl_4 \cdot 3H_2O$, ACS reagent) was purchased from Sigma-Aldrich (St. Louis, MO). Branched 2 kDa PEI (PEI2kB, 50 % mass fraction in water)

[ξ] The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

and 25 kDa PEI (PEI25kB) were obtained from Sigma-Aldrich (St. Louis, MO). 10 kDa branched PEI (PEI10kB) and 25 kDa linear PEI (PEI25kL) were obtained from Polysciences Inc. (Warrington, PA). Other specific reagents used in this study are identified in the references.[14, 15] All chemicals were used without further purification. Deionized water (18.2 MΩ·cm) was produced by an Aqua Solutions (Jasper, GA) Type I biological grade water purification system. Details regarding other instruments (DLS, UV-Vis, TEM, and ATR-FTIR) and methodology are also provided in references.[14, 15] The uncertainty of size and zeta potential represent the mean and one standard deviation of at least three measurements.

2.2 Preparation of Au-PEIs

General method for **Au-PEI*[LE]*s**

One mL of each PEI solution (1.0 w.% in DI water) was added dropwise to 10 mL of citrate-stabilized gold colloid suspension, which was then stirred at room temperature for 5 h and purified using centrifugal filtration (Amicon Ultra, Millipore; regenerated cellulose membrane, MWCO = 100 kDa). The filtrate was removed and re-diluted with DI water to the starting concentration, and then purified Au-PEIs were passed through a 0.2 µm nylon filter to remove any large impurities such as dust. It is important to note that the purification step was not applied to Au-PEI2kB and Au-PEI25kL due to their rapid aggregation during the conjugation process.

General method for **Au-PEI[*Red*]s**

One ml of each PEI solution (PEI25kL; 1.0 w.%, branched PEIs; 10 w.% in DI water) was added to a 10 mL of 2.5 mmol/L of aqueous $HAuCl_4$ solution in a borosilicate glass vial covered with a cap (EPA vials, Thermo Scientific, Waltham, MA) at room temperature and heated up to 80 °C using a magnetic hot plate with stirring. In these experiments, the temperature was increased from room temperature at a rate of about 5 °C/min. After reaching 80 °C, the reaction mixture was stirred for 1.5 h, then cooled down (removal from hotplate) to the ambient temperature. Resulting products were purified by dialysis (molecular weight cut off 100 kDa) against DI water for 2 days.

3 RESULTS AND DISCUSSIONS

3.1 Synthesis and Characterization

When preparing Au-PEI[*LE*]s, PEI stocks are introduced to the citrate AuNP suspension, yielding a slight color change (ruby red to purplish red) after reaction, indicative of the ligand exchange from citrate to PEI. However, in case of linear PEI (PEI25kL), the reaction mixture was precipitated within 5 min during the reaction (aggregation). The final product by reduction method yielded a ruby red suspension of Au-PEI[*Red*]s when using branched PEIs. In contrast, employing linear PEI yielded a cloudy pale red violet color suspension. The physico-chemical properties of Au-PEIs were determined by orthogonal measurement techniques including DLS (z-average hydrodynamic diameter (D_H), zeta potential (ZP)), TEM (D_{TEM}), and UV-Vis (surface plasmon resonance (SPR) band). Representative data of each Au-PEIs were presented in Table 1.

Table 1. Fundamental properties of Au-PEIs

Au-PEIs	D_H[a] (nm)	D_{TEM}[b] (nm)	SPR[c] (nm)	ZP[d] (mV)
Citrate AuNPs	12.1 ± 0.1	8.3 ± 1.3	518	-37.1 ± 1.4
Au-PEI2kB[*LE*]	21.8 ± 0.7	8.3 ± 0.8	524	+24.6 ± 3.0
Au-PEI10kB[*LE*]	62.9 ± 0.3	8.4 ± 0.7	530	+25.8 ± 0.7
Au-PEI25kB[*LE*]	78.8 ± 0.6	8.4 ± 0.9	534	+29.4 ± 1.3
Au-PEI25kL[*LE*]	N/A[e]	N/A[e]	N/A[e]	N/A[e]
Au-PEI2kB[*Red*]	21.5 ± 0.5	11.3 ± 2.0	522	+12.7 ± 1.4
Au-PEI10kB[*Red*]	22.0 ± 0.3	8.1 ± 2.6	523	+21.2 ± 1.2
Au-PEI25kB[*Red*]	22.2 ± 0.4	8.6 ± 2.3	524	+22.5 ± 1.7
Au-PEI25kL[*Red*]	88.2 ± 0.7	30.3 ± 7.0	545	+40.0 ± 0.4

a: D_H obtained by DLS, *b*: Au core size obtained by TEM; more than 150 particles were measured for each Au-PEIs and size reported is the population mean with one standard deviation about the mean., *c*: surface plasmon resonance band by UV-Vis measurements, *d*: zeta potential by DLS, and *e*: not available due to the precipitation during the reaction.

As shown in Table 1, D_H and SPR bands (by UV-Vis) of the Au-PEI[*LE*]s showed significant changes when compared to the starting citrate AuNP, increasing in value with the molar mass of associated PEIs (branched), while the core sizes (D_{TEM}) were almost identical regardless of PEIs. On the other hand, synthesizing Au-PEI[*Red*]s using branched PEI yield nearly identical values of D_H, D_{TEM}, and SPR for each Au-PEIs. These results clearly demonstrate that the application of different synthetic pathways result in Au-PEIs with varying properties. ZPs of Au-PEIs indicate the successful creation of positively charged AuNPs in comparison of negative surface of citrate AuNPs.

3.2 Reaction mechanism of Au-PEIs

ATR-FTIR studies have been conducted to determine if the synthetic mechanism of Au-PEI[*LE*]s follows a direct ligand exchange or layer *by* layer (L-*b*-L) process. To examine the interaction between PEI and citrate moiety on AuNPs, we conducted flow cell experiments with ATR-FTIR (Figure 1).[15] The ATR-FTIR spectra for the addition of PEI2kB to the citrate-stabilized AuNP film is shown in spectrum (i). The spectrum after the addition of three 1 mL aliquots of 1.0 % mass fraction solution of PEI over a similar mixing time for the general method is shown in spectrum (iii).

Absorbance has been normalized and spectra offset on

the vertical axis for ease of viewing. The data clearly shows the loss of each characteristic citrate vibrational mode after the addition of the PEI2kB. Although the amount of citrate remaining was not quantitatively determined, the resulting spectrum for the Au-PEI2kB film after flushing and drying

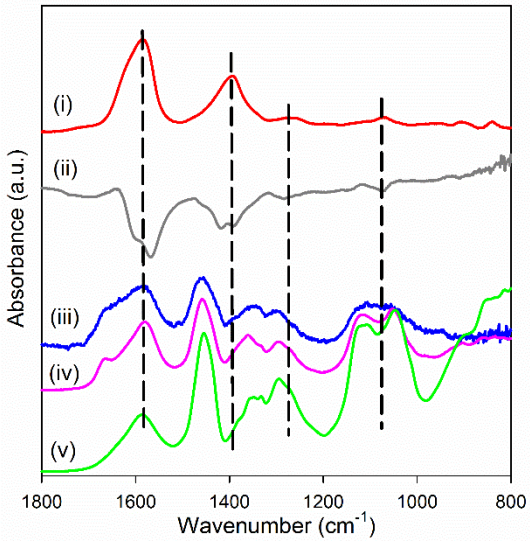
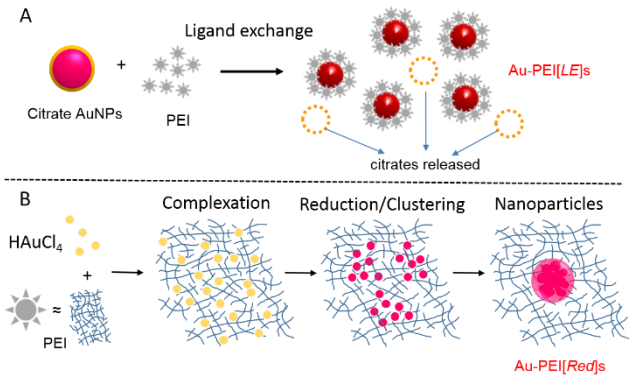


Figure 1. In-situ (in the flow cell) ATR-FTIR spectra for (i) citrate-stabilized AuNPs, (ii) loss spectrum after the addition of PEI2kB in the flow cell, (iii) dried and washed product after PEI2kB addition, and reference spectrum of (iv) dried Au-PEI2kB conjugate (separate batch, purified) and (v) dried PEI2kB (only) for comparison.

exhibits predominantly bands associated with PEI. The removal of citrate is further supported by the comparison of the dried film after rinsing (spectrum iii) and the purified separate batch sample (spectrum iv) that exhibit very similar signatures with predominantly PEI vibrations. These serial results obtained in situ support the replacement of citrate moieties by PEI chains. Consequently, the ATR spectra provide evidence that conjugation is principally accomplished by a ligand exchange reaction rather than an electrostatic-induced L-*b*-L process between negative surface (citrate) and positively charged ligand (PEI).



Scheme 1. Illustrative presentation of synthetic process of Au-PEIs depends on preparation route; a) by ligand exchange, and b) reduction method.

Based on the results as described above, we proposed the synthetic mechanisms of Au-PEIs in Scheme 1. Panel A illustrates the ligand exchange process that citrate moieties are replaced with PEI chains on gold core surface and panel B demonstrates reduction process of Au^{III} ions with PEIs and formation of the final Au^0 nanoparticles.

3.3 Stability study

Colloidal stability is an important issue for any application of AuNPs. We evaluated the stability of the Au-PEIs over a range of relevant conditions utilizing previously established protocols[16] and compared those of as functions of molar mass of PEI and synthetic pathways in both. For the study of long-term stability, Au-PEIs aged for 6 months under ambient laboratory conditions yielded a size distribution and SPR band (Figure 2).

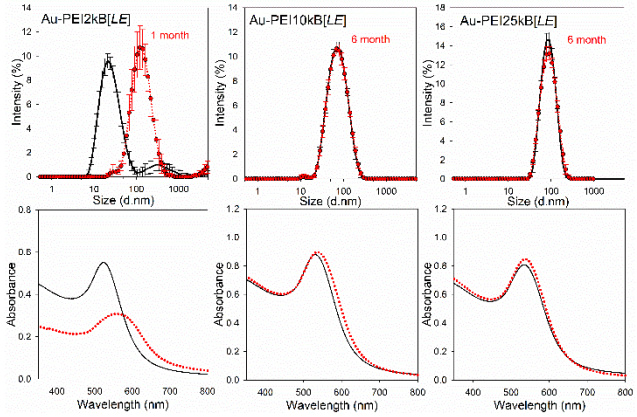


Figure 2. Shelf-life test of Au-PEI[*LE*]s; (upper) DLS size distributions for the initial product (black line) and after aging (red line), (bottom) UV-Vis absorbance showing SPR band for the initial product (black line) and after aging (red line). Error bars represent standard deviation.

The aged samples of Au-PEI10kB and -25kB yielded nearly identical DLS mean size and size distributions compared with the initial, freshly prepared and purified products (Figure 2, top). In contrast, those for Au-PEI2kB significantly increased within one month, indicating reduced colloidal stability compared with the higher molar mass PEIs. The associated SPR band intensities and peak positions (Figure 2, bottom) provide a more sensitive probe to changes in the near field environment of the AuNPs.[15] Notably, the degree of long-term stability and PEI molar mass dependency were very similarly observed in Au-PEI[*Red*]s (data omitted).

For biological application, stability in physiological media is critical. Based on a previous study,[14] citrate-stabilized AuNPs showed immediate instability in phosphate buffered saline (PBS), otherwise Au-PEIs exhibited improved stability in PBS over 48 h period relevant to cell exposure assays (Figure 3). In the long-term stability study, molar mass of PEI impacts the colloidal stability of Au-PEIs[15] as shown in figure 2. Figure 3

demonstrates how significant it is against physiological environment. Furthermore, the effect of synthetic pathways to the physico-chemical properties of resulted Au-PEIs was clearly observed by this study. The overall stability of Au-PEI[Red]s exhibited significant improvements compared to those of Au-PEI[*LE*]s. Based on these results, we select Au-PEI25kB[*Red*]s, as the most stable Au-PEI, and conducted

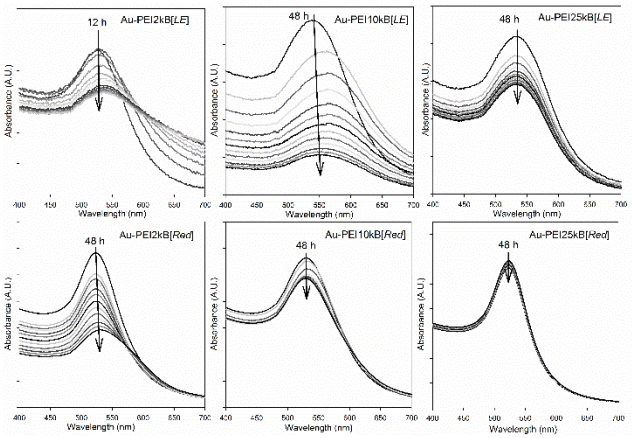


Figure 3. Stability of Au-PEIs in PBS over time, as monitored by UV-Vis: (Top) Au-PEI[*LE*]s, and (Bottom) Au-PEI[*Red*]s; PEI2kB, -10kB, and -25kB, from left to right in both panels.

further colloidal stability tests. Over a wide range of pH values (pH 1.5 ~ 12), Au-PEI25kB[*Red*]s showed remarkable stability over 12 h (data omitted). Overall, the resistance against acid destabilization is greatly improved relative to citrate AuNPs and all of the remaining Au-PEI synthetic conditions. Thermal stability of Au-PEI25kB[*Red*]s was evaluated by UV-Vis from (20 to 60) °C, a relevant temperature range for most biological assays. The constancy of the SPR band (from UV-Vis spectra, data omitted) confirm that the Au-PEI25kB[*Red*]s are stable with respect to temperature variations over the tested range.

4 CONCLUSION

The positively charged gold nanoparticles, Au-PEIs were prepared via two different pathways; 1) ligand modification, and 2) reduction process. In summary, we demonstrated that each synthetic process resulted in differentiated product formation and their physico-chemical properties, by systematic examination considering reaction condition and the dependency of relative molar mass/backbone structure of PEIs. The physico-chemical properties and the conjugation mechanisms of Au-PEI[*LE*] and Au-PEI[*Red*] were characterized by an orthogonal analyses system including DLS, UV-Vis, TEM, and ATR-FTIR measurements. Furthermore, the colloidal stabilities of all produced Au-PEIs were investigated under various physiologically relevant conditions. We found that the consequential fates of Au-PEIs were significantly dependent on born pathways. Notably, Au-PEI25kB[*Red*]s showed the most remarkable stability in such circumstances, which is very critical to be applied in nanomedicine and nanobiotechnology. We are currently working on this material for advanced studies including hybridization with biological entities to be gene transfection tools.

REFERENCES

[1] Y. Ding, Z. W. Jiang, K. Saha, C. S. Kim, S. T. Kim, R. F. Landis and V. M. Rotello, *Mol. Therapy*, 22, 1075, 2014.

[2] A. Elbakry, E. C. Wurster, A. Zaky, R. Liebl, E. Schindler, P. Bauer-Kreisel, T. Blunk, R. Rachel, A. Goepferich and M. Breunig, *Small*, 8, 3847, 2012.

[3] Z. Z. Chen, L. F. Zhang, Y. L. He and Y. F. Li, *Acs Appl. Mat. Interfaces*, 6, 14196, 2014.

[4] M. Y. Lee, S. J. Park, K. Park, K. S. Kim, H. Lee and S. K. Hahn, *Acs Nano*, 5, 6138, 2011.

[5] W. J. Song, J. Z. Du, T. M. Sun, P. Z. Zhang and J. Wang, *Small*, 6, 239, 2010.

[6] M. Thomas and A. M. Klibanov, *Proc. Nat. Acad. Sci., U.S.A.*, 100, 9138, 2003.

[7] G. Kramer, H. M. Buchhammer and K. Lunkwitz, *Coll. Surf. A-Physicochem. Engineer. Aspects*, 137, 45, 1998.

[8] O. Boussif, F. Lezoualch, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J. P. Behr, *Proc. Nat. Acad. Sci., U.S.A.*, 92, 7297, 1995.

[9] E. C. Cho, J. W. Xie, P. A. Wurm and Y. N. Xia, *Nano Letters*, 9, 1080, 2009.

[10] U. Taylor, S. Klein, S. Petersen, W. Kues, S. Barcikowski and D. Rath, *Cytometry Part A*, 77A, 439, 2010.

[11] R. R. Arvizo, O. R. Miranda, M. A. Thompson, C. M. Pabelick, R. Bhattacharya, J. D. Robertson, V. M. Rotello, Y. S. Prakash and P. Mukherjee, *Nano Letters*, 10, 2543, 2010.

[12] P. Zhang, B. B. Li, J. W. Du and Y. X. Wang, *Coll. Surf. B-Biointerfaces*, 157, 18, 2017.

[13] R. I. MacCuspie, *J. Nanopart. Res.*, 13, 2893, 2011.

[14] T. J. Cho, R. I. MacCuspie, J. Gigault, J. M. Gorham, J. T. Elliott and V. A. Hackley, *Langmuir*, 30, 3883, 2014.

[15] T. J. Cho, J. M. Pettibone, J. M. Gorham, T. M. Nguyen, R. I. MacCuspie, J. Gigault and V. A. Hackley, *Langmuir*, 31, 7673, 2015.

[16] T. J. Cho and V. A. Hackley, National Institue of Standards and Technology https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.1200-26.pdf, 2018.