Dendritic poly(L-lysine) for drug delivery

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Introduction

This application note presents the characterization of a potential non-viral gene carrier that has shown transfection ability without significant cytotoxicity *in vitro*. L-Lysine ($C_6H_{14}N_2O_2$, abbreviated: Lys or K, 146 g/mol) is an amino acid present in all proteins in the human body.
Here we investigate a polymer of this amino acid at different pH buffers. Dendritic poly(L-lysine) of the 6th generation (KG6) is a starburst polypeptide with an expected molar mass of $(2^1+2^2+2^3+2^4+2^5+2^6)\cdot 146 \sim$ 18400 g/mol.
Under acidic conditions, the molecule is predicted to be positively charged (the isoelectric point of the single amino acid is ~ 8.9).
Figure 1 shows the predicted structure for this molecule at low pH, as modeled by ChemDraw.

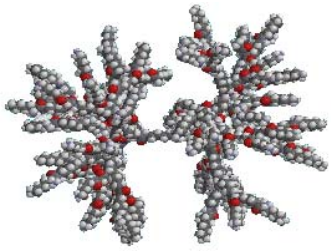

Figure 1: Molecular structure of KG6 at low pH; dendritic form due to cationic charged branches.

When the solvent environment is changed such that the cationic charge neutralizes, the molecule will 'collapse' as the flexible arms no longer need to stretch out. A visual representation of this situation is shown in figure 2, again modeled with ChemDraw.

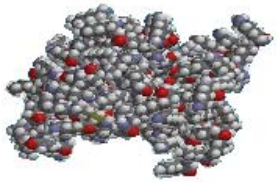

Figure 2: Molecular structure of KG6 at pH near the isoelectric point, the molecule is compact.

Experimental

KG6 was synthesized in the standard copolymer fashion, and subsequently purified.[i] Samples were dialyzed in different pH buffers (pH 5, 6, 7, and 8) and then measured in the Zetasizer Nano after filtration through a 200nm pore size Anodisk membrane (Whatman).

Results

All samples appear clear. Dynamic light scattering (DLS) measurements were performed in automatic mode Samples were prepared at a concentration of 0.5 mg/mL and generated a strong light scattering signal in order to measure their correlation function.

Hydrodynamic Size

In dynamic light scattering (DLS) the fluctuations of the scattered light are the result of thermal motion of the molecules and these are analyzed by measuring the correlation function of the signal. This leads to the diffusion coefficient when applying knowledge of the viscosity of the diffusing medium (aqueous buffer in this case).

We can obtain a size distribution for the hydrodynamic size of the scattering object. This size is the size of an equivalent sphere that has the same diffusion coefficient as the polypeptide molecules. We look at the volume size distribution analysis, shown in figure 3.

At high pH the size is smallest, at low pH we observe the largest mean value for the distribution. The intermediate pH distributions are in between the two peaks. DLS results are summarized in Table 1.

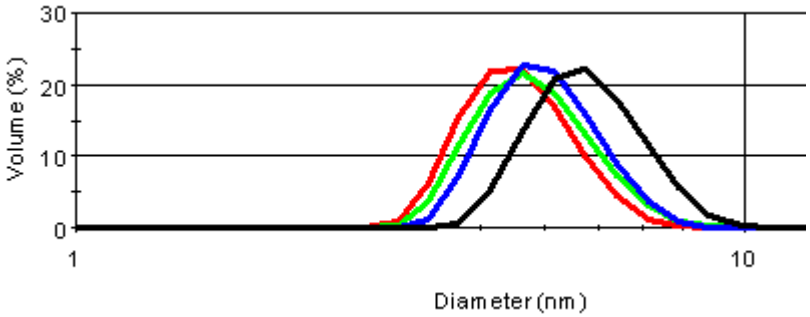

Figure 3: Size Distribution (%volume versus diameter[nm]) of KG6 at pH 5.0 (**black**), pH 6.0 (**blue**), pH 7.0 (**green**) and pH 8.0 (**red**)

Buffer condition	Mean size of Peak (d.nm)	Polydispersity Index (PDI)	MW estimate, starburst polymer	MW estimate, globular protein
pH 5.0	5.7 nm	0.13	21.8 kDa	38.4 kDa
pH 6.0	4.9 nm	0.14	13.8 kDa	27.9 kDa
pH 7.0	4.7 nm	0.14	11.7 kDa	24.9 kDa
pH 8.0	4.5 nm	0.15	9.9 kDa	22.1 kDa

Table 1: Peak information summary for KG6 under different pH conditions. Data from volume distribution, estimates are based on empirical calibration curves.

All peaks appear relatively polydisperse with a polydispersity index (PDI) between 0.13 and 0.15. We can estimate the molecular weight from the measured hydrodynamic size. The Zetasizer Nano software has several MW estimation models, here we look at the starburst polymer model and compare it to the globular protein. At pH 5.0 where KG6 is a stiff 'star-polymer' we find that the estimate of 22 kDa is in reasonable agreement with the true 19 kDa. For pH 8.0 on the other hand, KG6 is forming a compact sphere, and the globular protein model provides the better prediction.

The intermediate samples are less well modeled since they neither behave like a dendrimer, nor like a globular compact sphere.

The molecular structure of lysine may be used to predict its charge[ii] in solution, shown in fig. 4. The charge on the single amino acid is expected to be close to zero at pH8 – leading to a higher influence of van-der-Waals attraction, stronger hydrogen bonds and overall 'shrinkage' of the dendrimer.

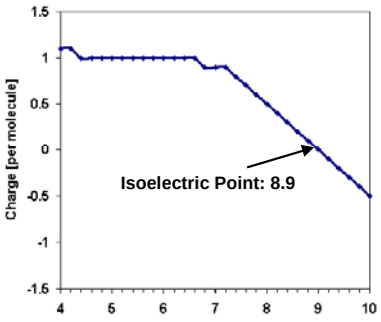

Figure 4: Charge vs. pH for lysine

Summary

A dendritic polypeptide (KG6) was characterized by dynamic light scattering. Results support the existence of a shape change as a function of pH of the buffer. The protein is compact and globular at pH8, but 'fluffy' and starburst-like at pH5. This result is expected from the behavior of the individual amino acid L-lysine.

Zetasizer Nano System

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined dynamic, static, and electrophoretic light scattering measurements, providing the researcher with a wide range of sample properties, including the size, molecular weight, and zeta potential. The system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications.

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[i] T. Okuda, S. Kawakami, T. Maeie, T. Niidome, F. Yamashita, M. Hashida. *J. Controlled Release* **114**(1):69-77 (2006)

[ii] http://www.scripps.edu/~cdputnam/protcalc.html

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