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Modulation of the properties of elastin-like polypeptides by structure variations

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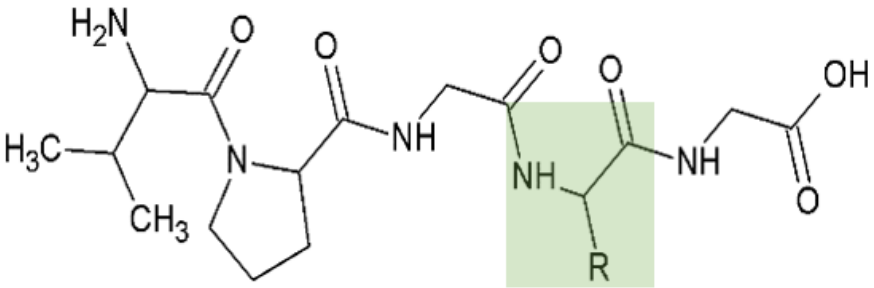
Introduction

Elastin-like polypeptides (ELPs) with VPGXG repeating sequence are artificial biomacromolecules derived from hydrophobic domain of tropoelastin.[1] Their characteristic feature is coacervation (self-association) characterized by transition temperature T_t. ELPs are soluble in water or buffer below its T_t and they form β -structures and aggregates above its T_t . Up to date, the reversibility of the coacervation at specific temperature T_t has been studied in many research areas, e.g. in drug delivery, protein purification and tissue engineering.[2] Moreover, the transition temperature can be modulated by both extrinsic (pH, ionic strength) and intrinsic factors (primary sequence and chain length).[3]

The aim of our study was to characterize secondary structure behavior and to define T_t with respect to primary sequence of ELPs with general formula $H(VPGLG)_2(VPGXG)_2(VPGLG)_6$-OH, where we used the hydrophobic (Gly, Ala, Leu), hydrophilic (Gln, Asn, Tyr), acidic (Glu) and basic amino acids (His, Lys) as the variable element X.



Scheme 1: Chemical structure of elastin-like polypeptide subunit VPGXG

Table 1: Properties of prepared ELPs

peptide	sequence	MW (Da)	pI*	pI (X)
ELP 1	H-$(VPGLG)_2(VPG\mathbf{G}G)_2(VPGLG)_6$-OH	4301.47	6.09	5.97
ELP 2	H-$(VPGLG)_2(VPG\mathbf{A}G)_2(VPGLG)_6$-OH	4169.33	6.09	6.0
ELP 3	H-$(VPGLG)_2(VPG\mathbf{L}G)_2(VPGLG)_6$-OH	4253.51	6.09	5.98
ELP 4	H-$(VPGLG)_2(VPG\mathbf{K}G)_2(VPGLG)_6$-OH	4283.53	10.72	9.74
ELP 5	H-$(VPGLG)_2(VPG\mathbf{H}G)_2(VPGLG)_6$-OH	4301.47	8.13	7.59
ELP 6	H-$(VPGLG)_2(VPG\mathbf{Y}G)_2(VPGLG)_6$-OH	4353.53	5.96	5.66
ELP 7	H-$(VPGLG)_2(VPG\mathbf{E}G)_2(VPGLG)_6$-OH	4285.41	3.03	3.22
ELP 8	H-$(VPGLG)_2(VPG\mathbf{Q}G)_2(VPGLG)_6$-OH	4283.45	6.09	5.65
ELP 9	H-$(VPGLG)_2(VPG\mathbf{N}G)_2(VPGLG)_6$-OH	4255.39	6.09	5.41

defined by Peptide Property Calculator.[4]

Solid Phase Peptide Synthesis and HPLC-MS purification

Peptides were synthesized using SPPS protocol by Fmoc/tBu strategy on Wang resin (L= 1.12 mmol/g, mesh 100 200). The coupling steps were performed using DIC/oxyma/AA in molar ratio 3/3/3. Subsequent Fmoc deprotection was provided by 20 % piperidine in DMF for 5 and 15 min. After synthesis, all peptides were cleaved by cleavage cocktail consisting of DMC/anisole/thioanisole/TIS/TFA (5/2/1/2/90).

Crude peptides were purified by RP-HPLC on Shimadzu L CMS-2020 system equipped with a splitter and ESI-MS and PDA detection at 210 nm. The mobile phases were 0.1 % formic acid in distilled water and acetonitrile. For separation, C18 column (Jupiter 4 μm Proteo 90 Å AXIA, 250 × 10 mm) was used. The flow rate was 2.5 ml/min in the suitable slow gradient profiles.

Temperature measurements

ELPs were dissolved in phosphate buffer (pH 7.5) to concentration 0.1 mg/ml. Peptides structural changes were studied by circular dichroism (CD) spectroscopy (Figure 1). Temperature dependence of CD spectra were measured in 1 mm quartz cell in spectral range from 190 nm to 280 nm with temperature step 10 °C, scanning speed 10 nm/min and time response 8 sec. To compare molar ellipticity changes at two different wavelengths (Figure 2A), peptide sample was heated with gradient 1 °C/min and for each measurement the temperature equilibration was 30 sec. Temperature interval was 5 °C to 90 °C.

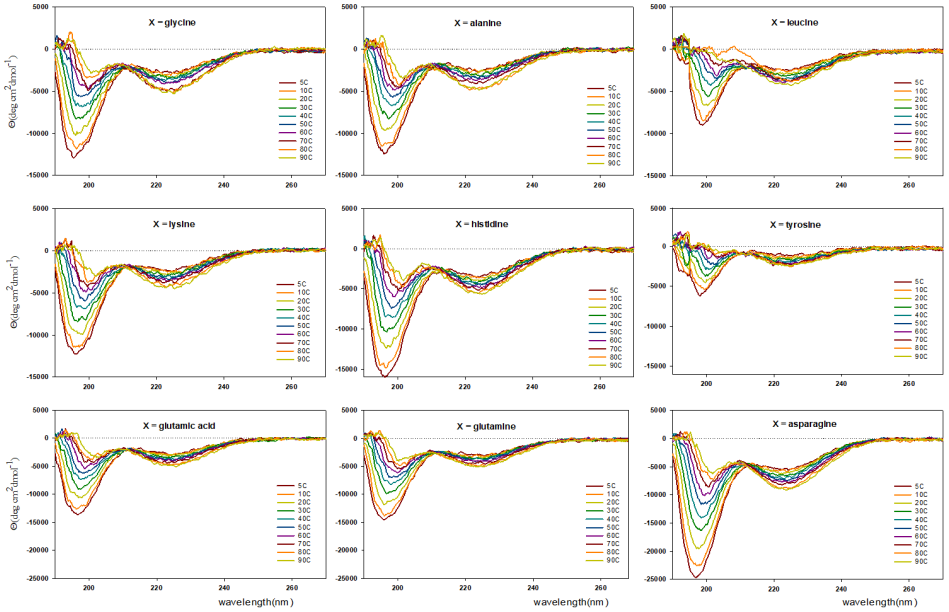


Figure 1: CD spectra of elastin like polypeptide ELP1-9. The CD spectra at low temperature are characterized by high proportion of random coil (distinct minimum at 197 nm) and low proportion type II β -turns (less-pronounced minimum at 224 nm). In general, the secondary structure distribution is gradually inverted with increasing temperature. The differences in intensity of CD spectra for discrete peptide is caused by presence of β -turn structure already presented at low temperature.

Turbidity profiles and transition temperatures (Figure 2B) were estimated using UV-Vis spectroscopy (OD at 400nm). The sample (concentration 1 mg/ml in phosphate buffer pH 7.5) was placed in 1 cm quartz cell and continually stirred and heated with temperature gradient 1 °C/min in the temperature range from 10 °C to 70 °C.

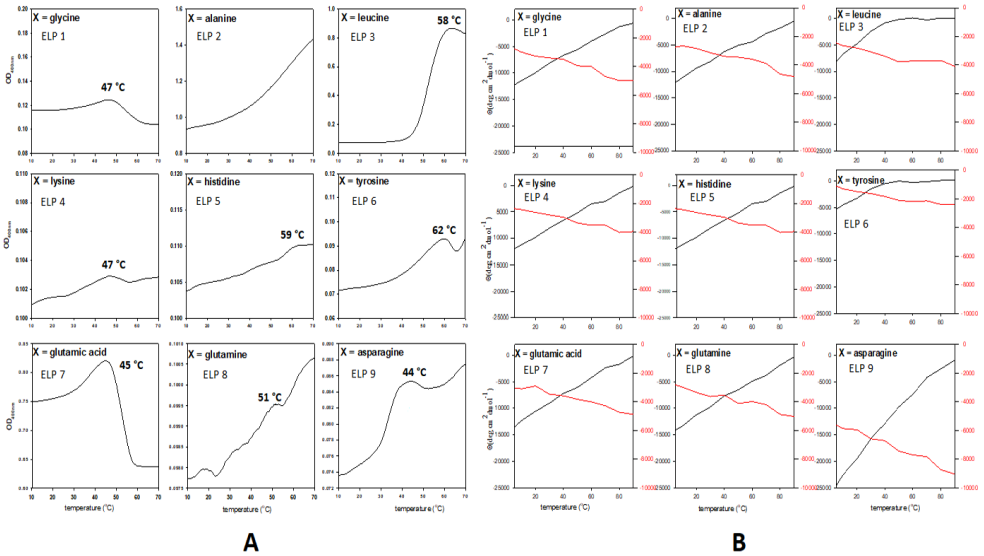


Figure 2: Temperature measurements: A) ELPs coacervation profiles and B) ELPs molar ellipticity (Θ) changes at 197 nm (black) and 224 nm (red)

Conclusion

Turbidity profile and transition temperature for each prepared ELP were estimated with exception of the peptide containing alanine, where aggregation was observed immediately after dissolving in PBS buffer. Pronounced coarcervation was observed for ELPs with hydrophobic (L) or polar (Q and N) and negative (E and T) side chain.

To obtain the information of secondary structure dependency on temperature changes, CD spectroscopy was employed. For all studied ELPs, we observed higher portion of unordered conformation in combination with type

II β-turns at low temperature (5 °C). The distribution of type II β-turns increased with rising temperature and simultaneously the distribution of unordered structures decreased. However, the presence of β-turn structures was observed for ELPs containing bulky side chains (T, N and L) or negative charge (E) at low temperature. ELPs with polar (Q and N) and negative (E) side chain showed pronounced structural transition from unordered structure to type II β -turns. On the other hand, saturation of β-turns if any was observed for peptides containing X with bulky side chain (L, K, H and T). For ELPs, the reversibility of their secondary structure changes with increasing and afterwards decreasing temperature was confirmed, but on the different time scale for discrete ELPs.

References

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