3957 Corte Cancion, Thousand Oaks, CA 91360; telephone 805-388-1074; fax 805-388-7252; www.ap-lab.com

Size Exclusion Analysis of T-Sirt3

Tsutomu Arakawa
Alliance Protein Laboratories
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Purpose

The study is to analyze the size distribution of T-Sirt3 by size exclusion chromatography (SEC).

Experimental procedure

Superdex-200 column (1 X 30) was equilibrated with 0.1 M Na phosphate, 0.2 M arginine, pH 6.8 at a flow rate of 0.5 ml/min or with 0.1 M phosphate, 0.5 M NaCl, pH 7.2 at a flow rate of 0.2 ml/min. A 0.1 ml aliquot of 4 mg/ml T-Sirt3 sample was injected into the column. The elution was monitored with the absorbance at 280 nm.

Results

Fig.1 shows the SEC profile of T-Sirt3 in 0.1 M phosphate, 0.2 M arginine, pH 6.8. Two main elution peaks were observed. The arrows indicate elution positions of lysozyme (14,300) and BSA monomer (68,000) and trimer (200,000). Both elution peaks of T-Sirt3 were incisitent with the molecular weight of the sample of 32,000.

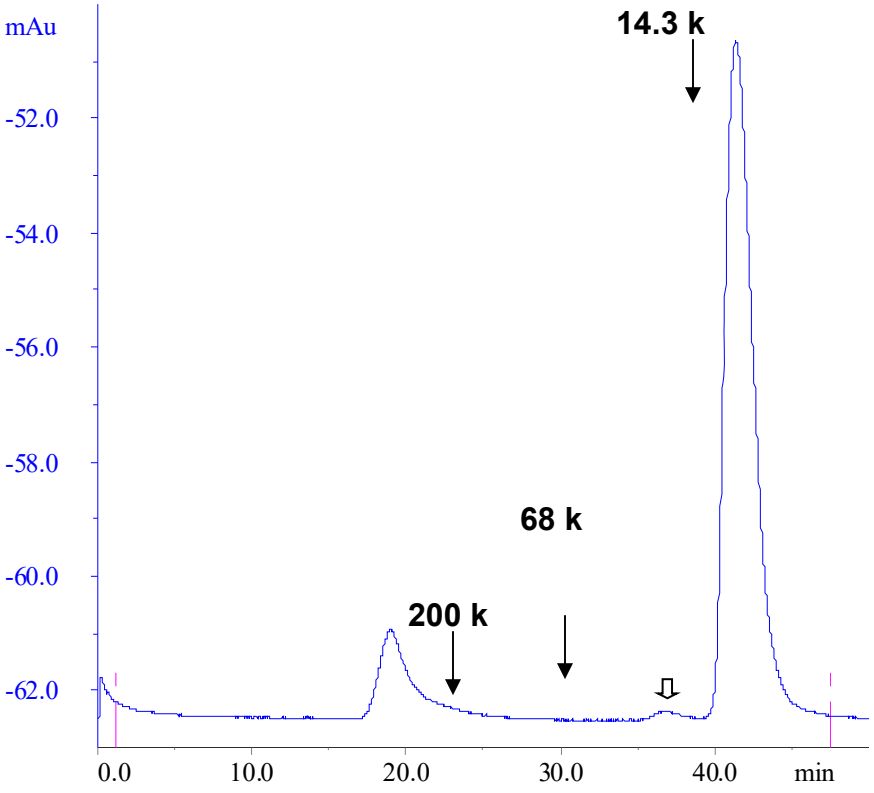

Fig.1 SEC profile of T-Sirt3 in 0.1 m Na phosphate, pH 6.8

⇩This peak may be your protein.

Fig.2 shows the SEC profile in 0.1 M Na phosphate, 0.5 M NaCl, pH 7.2: note that the flow rate was reduced to 0.2 ml/min. The results are essentially identical. These 2 main elution peaks were collected and their UV absorbance spectra determined, showing a maximum at 260 nm. The loaded sample also shows a UV absorbance peak at 260 nm (see attached file).

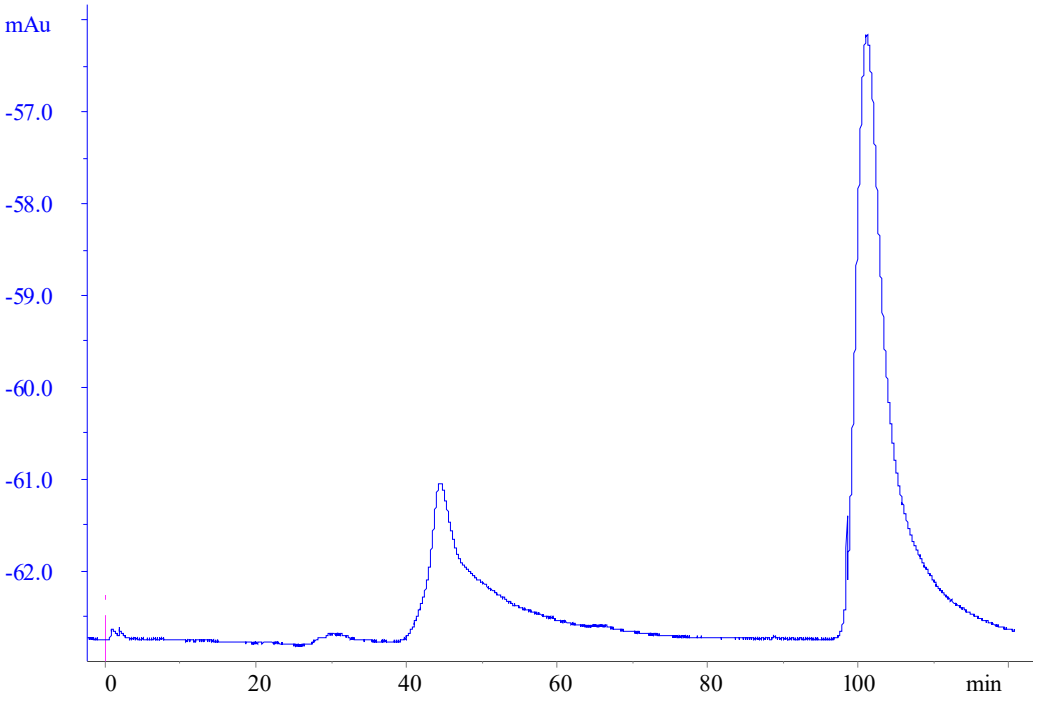

Fig.2 SEC profile in 0.1 M Na phosphate, 0.5 M NaCl, pH 7.2

Conclusion

SEC analysis of T-Sirt3 showed two main elution peaks due to nucleic acids. The load also showed a UV absorbance peak at 260 nm.