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Monolithic chromatographic supports employed in virus separation and purification

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The particular structure of monolithic chromatographic supports has been proved highly advantageous in analytic separation and purification of large biomolecules. Characterized by very high porosity, high binding capacity, and high flow rate based on convective mass transport, monoliths are particularly applicable in virus research. Monolith chromatography has hardly any limitation to be applied in processing of virus particles, huge macromolecular complexes, and viral genomes, especially when being distinct from nucleic acid forms present in the host cell. The majority of trials published so far, have exploited ion exchange carriers, although other chemistries are also applicable, like hydrophobic interaction when concentrating viruses from marine environment. According to papers published over the past decade, viruses of different sizes, structures and morphologies, even virus-like particles, have successfully been purified from tissue homogenates or cell lysates. Moreover, following virtual separation by overlapping chromatograms obtained from separate experiments, a real separation of three virus species, and a distant strain of one of them (four viral fractions in total) was accomplished from laboratory prepared mixture. One of the most recent achievements is a proof of principle that virus chromatographic feature could easily be modified without abolishing its structural stability, or its biological activity. As adenoviruses have been used in almost a quarter of human gene therapy trials, the experiment was conducted with an adenoviral vector. The deletion of just two negatively charged amino acids from the main structural protein was efficient in shortening chromatographic retention of the recombinant adenovirus. In this way, it would be possible to shift virus particles away from particular interfering substances present in the crude lysate. The concept might be followed to facilitate chromatography-approach purification by engineering modifications contributing to virus separability, besides those contributing to its therapeutic functionality.

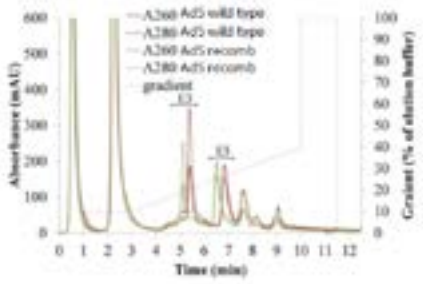

Figure: Shortened retention time of both the unassembled hexon protein (E3) and the virion fraction (E5) obtained for hexon-modified adenoviral vector. The shifted Ad5 recombinant missed just two negatively charged amino acids in the hexon protein compared to original virus particle displaying wild-type capsid. Following short low-speed centrifugation and filtration, the cell lysate was loaded to QA monolithic support with 20 mM Tris, 5% glycerol, pH 7.5, and eluted with a gradient of loading/eluting buffer, where the eluting buffer additionally contains 2 M NaCl. The flow rate was 2 mL/min.

Recent Publications

1. Krajacic M, Ravnikar M, Strancar A and Gutiérrrez-Aguirre I (2017) Application of monolithic chromatographic support in virus research. Electrophoresis 38:2827-2836.
2. Ruscic J, Ambriovic-Ristov A, Majhen D, Kolundzija S, Barut M, Benihoud K and Krajacic M (2016) Manipulating adenoviral vector ion-exchange chromatography: Hexon versus fiber. Journal of Separation Science 39:4299-4304.
3. Ruscic J, Gutiérrrez-Aguirre I, Tusek Znidaric M, Kolundzija S, Slana A, Barut M, Ravnikar M and Krajacic M (2015) A new application of monolithic supports: The separation of viruses from one another. Journal of Chromatography A 1388:69-78.
4. Ruscic J, Gutiérrrez-Aguirre I, Urbas L, Kramberger P, Mehle N, Skoric D, Barut M, Ravnikar M and Krajacic M (2013) A novel application of methacrylate based short monolithic columns: Concentrating Potato spindle tuber viroid from water samples. Journal of Chromatography A 1274:129-136.

5. Krajacic M, Ivancic-Jelecki J, Forcic D, Vrdoljak A and Skoric D (2007) Purification of plant viral and satellite double-stranded RNAs on DEAE monoliths. Journal of Chromatography A 1144:111-119.

Biography

Mladen Krajacic is a Full Professor at University of Zagreb where he completed his PhD in Virology. As a Head of the Laboratory and Principal Investigator in several research projects, he has been leading the molecular biology investigations on viruses, sub-viral agents (viroids, satellite RNAs) and non-cultivable bacteria. During the last decade, he has dedicated to chromatography on monolithic supports and its appliance in separation of viral and sub-viral ribonucleic acids, as well as virus particles. His results have been published in prominent scientific journals in which he has regularly been employed as a Reviewer. He was supervising a number of young research fellows who have received positions from well recognized institutions across the world.

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