A nanoplatform based on self-assembled plant-made nanoparticles with multiple applications

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Abstract
Protein nanoparticles with self-assembly capabilities are of utmost relevance in 'bottom-up' nanobiotechnological approaches. Several examples are available which exemplify this concept for instances derived from the plant world. Most of them derive from plant viruses, natural nanoparticles self-assembling with a high efficiency [1]. These viral nanoparticles (VNPs) can take several forms, which mainly fall into two biological categories. Either the particles enclose and pack a nucleic acid (virions) or they do not (virus-like particles, VLPs). For some 'in vitro' applications, the presence of the nucleic acid does not involve any added inconvenience. However, when the application involves exposure of living entities to the VNP, or the modification imposed is incompatible with a normal viral life cycle, it is normally preferable to deal with VLPs.

We have been developing a VNP platform based on *Turnip mosaic virus* (TuMV), a plant virus belonging to the genus *Potyvirus*. VNPs derived from viruses in this genus are elongated flexuous rods, approximately 750-850 nm long and approx. 15 nm wide [2]. Initial VNPs were generated in plants exposed to an infectious TuMV clone, developed by us years ago [3]. To deal with virions, these were purified from plants infected with natural or genetically modified TuMV. Extracts of the infected plants were subjected to standard protocols of virion purification. For VLPs, plants were infiltrated with genetic constructs coding exclusively for one single protein, the viral protein CP which is the only building block to make VLPs. These were also purified following similar procedures.

Both types of VNPs can be genetically or chemically modified for nanobiotechnological exploitation with several purposes. In the genetic manipulation, viral genes (normally the gene encoding the CP) are modified to confer the sought properties to their derived proteins. For instance we have made this protein longer so that the derived VNP can expose hundreds or thousands of copies of peptides or proteins on its external surface. The corresponding derived VNPs have been used to increase peptide antigenicity by orders of magnitude. They have also been deployed to increase substantially our ability to detect the presence of antibodies in biological fluids, the basis for new simple and ultrasensitive approaches for the diagnosis of diseased conditions. The CP gene can also be made shorter, still preserving the self-assembling capability of the CP. By doing so, we have made substantial progress in defining the size limit of the protein which retains self-assembly. This basic study of the properties of this system will now allow us to try to express much larger peptides and proteins in our VNPs.

The chemical modification approach involves fusing peptides or proteins 'in vitro' to the previously purified VNP. Other macromolecules can be fused, too. Usual procedures for this involve the use of bi-headed chemical reagents mediating the fusion. We have used this experimental approach for enzyme immobilization onto TuMV VNPs. The characterization of the resulting structures has revealed that the initial VNPs participate in the formation of higher ordered macromolecular assemblies involving both the exogenously added enzyme and the VNPs. The enzymatic activity associated to the new structures was several times higher than that of the non-immobilized enzyme indicating the applicability of this approach in nanobiocatalysis.

Current achievements in all these directions will be presented and discussed in the general context of the progress in developing this nanoplatform further. New possibilities in other areas, such as nanomaterials or nanodevices with new properties will also be explored.

References

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