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Abstract

Taking Advantage of Nature's Benefits: Soluble and Stable Antigen Straight Out of the Pathogen †

Didier Clénet [1], Léna Clavier [1], Benoit Strobbe [1], Christel Le Bon [2], Manuela Zoonens [2] and Aure Saulnier [3,*]

[1] Bioprocess R&D Department, Sanofi Pasteur, 69280 Marcy l'Etoile, France; Didier.Clenet@sanofi.com (D.C.); lena.clavier@hotmail.fr (L.C.); benoit.strobbe@laposte.net (B.S.)
[2] CNRS/Université Paris 7, UMR 7099, Institut de Biologie Physico-Chimique, F-75005 Paris, France; lebon@ibpc.fr (C.L.B.); zoonens@ibpc.fr (M.Z.)
[3] Analytical Sciences R&D Department, Sanofi Pasteur, 69280 Marcy l'Etoile, France
* Correspondence: Aure.Saulnier@sanofipasteur.com
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Abstract: Integral membrane proteins (MP) exhibit specific tridimensional conformation and topology that define their various functions. Pathogen surface antigens, encompassing many MP, are at the forefront of the viral strategy which is broadly targeted by the host immune response. These antigens are present in equilibrium under different oligomeric forms with distinctive epitopes, and to obtain them in a soluble form and/or stable constitutes a real risk. The solubilization of a full-length MP directly from a pathogen to rapidly obtain a native antigen mimicking the original conformation of the MP at the pathogen surface is the process development reported in this work. Rabies virus (RABV) was used as a model for this demonstration and its full-length glycoprotein (G) was stabilized in amphiphatic polymers (A8-35 amphipols). The stability of the soluble RABV-G was evaluated under various stress conditions (temperatures, agitation and light exposures) and a long-term stable RABV-G formulation, suitable for the freeze-drying process, was defined using a design of experiment approach. RABV-G/A8-35 in liquid form was shown to be antigenically stable at 5 °C and 25 °C for one month, and a dedicated kinetic model predicted its stability up to 1 year at 5 °C. To mitigate the RABV-G/A8-35 sensitivity to mechanical stress, a solid form of RABV-G/A8-35 and a freeze-drying process were considered, resulting in a 2-year thermally stable product at 5 °C, 25 °C and 37 °C. To the best of our knowledge, this is the first time that a natural full-length MP, extracted from a virus and trapped in amphipols, was kept antigenically stable in the long term, in a defined freeze-dried form out of any refrigerated storage conditions. These results described an easy process to obtain a pure, well conformed native-like antigen of interest, from a circulating pathogen which is of concern for diagnostic (quantification/characterization assays), therapeutic and vaccine strategies. After the physical characterization of the protein, the identification of RABV G/A8-35 neutralizing epitopes has been underway before in vivo testing.

Keywords: G glycoprotein from rabies virus; amphipol-trapped integral membrane protein; A8-35 formulation study; stable freeze-dried A8-35 formulation