学位（博士）論文要旨

(Doctoral thesis abstract)

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論 文 題 目 (Title)	In vivo immunogenicity of amorphous aggregates made of misfolded anti-EGFR single domain antibody (VHH)

論文要旨（2000 字程度）
(Abstract(400 words))
※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。
(in English or in Japanese)

Generation of an unwanted immune response, as a form of anti-drug antibodies (ADAs), is a major concern when developing biotherapeutic drugs. Protein aggregation has long been suspected to generate ADAs without any concrete conclusion regarding their biophysical and biochemical nature that make some aggregates immunogenic while others are not. Firstly, here I show the immunogenicity of an anti-EGFR V_{HH} (V_{HH}-7D12) antibody, a potential anti-cancer drug, relating to the aggregates nature. To this end, I produced four types of amorphous aggregates: two heat-aggregated V_{HH}s incubated at 65 °C (V_{HH}-65) and 95 °C (V_{HH}-95), a misfolded V_{HH} isolated from the insoluble fraction of the *E. coli* lysate (V_{HH}-Ins), and a low solubility misfolded V_{HH} produced by miss-shuffling the SS bonds of the native V_{HH}-7D12 (V_{HH}-Mis). Biophysical and biochemical measurements indicated that V_{HH}-7D12 was indeed natively folded, monomeric, and β-sheeted; that V_{HH}-65 was partially unfolded and formed aggregates with a Z-average (Zave) of 771 nm, whereas V_{HH}-95 was unfolded and formed aggregates of 1722 nm; and that both V_{HH}-Ins and V_{HH}-Mis were misfolded, having non-native intermolecular SS bonds and aggregates size of 1846 nm and 1951 nm, respectively. Immunization experiments showed that the native V_{HH} was barely immunogenic, V_{HH}-95 was not immunogenic, while V_{HH}-65 was mildly immunogenic, as assessed by IgG using ELISA. By contrast, both V_{HH}-Ins and V_{HH}-Mis, having an aggregation propensity similar to that of

V_{HH}-95, were highly immunogenic. These findings indicate the critical role of the biochemical and biophysical attributes of the amorphous aggregates in generating an immune response against a protein, rather than just their sizes. Next, I investigated the specificity of the immune response induced by V_{HH}-Mis by comparing the IgG specificity using an analogous V_{HH} (V_{HH}-9G8), having 77% identical sequence to V_{HH}-7D12 and possessing a common framework but distinct complementarity determining regions (CDRs). In 60% of mice immunized with V_{HH}-Mis, the anti-V_{HH}-7D12 IgG titer was stronger than the anti-V_{HH}-9G8 titer (**Group-1**). In the remaining mice (40%; **Group-2**), the anti-V_{HH}-7D12 and anti-V_{HH}-9G8 titer were almost identical. We rationalized these results by hypothesizing that mice in **Group-1** produced IgG mostly against the V_{HH}-7D12's CDRs, whereas in **Group-2** mice, they targeted the V_{HH}'s framework. The IgG specificity against V_{HH}-7D12 and V_{HH}-9G8 was essentially unchanged over 17 weeks in both groups. Further, in all mice (**Group-1 & 2**) re-immunized with native V_{HH}-7D12, the IgG titer against V_{HH}-7D12 increased sharply but not against V_{HH}-9G8. On the other hand, none of the three **Group-1** mice re-immunized with native V_{HH}-9G8 showed an immune response against V_{HH}-7D12 or V_{HH}-9G8. Whereas, in **Group-2** mice (three/three) re-immunized with V_{HH}-9G8, the IgG titers against both V_{HH}s increased but slowly. Flow-cytometric studies showed that V_{HH}-Mis immunized mice generated a higher number of effector and central memory T-cells. Overall, these observations indicate that amorphous aggregates made of a misfolded V_{HH} can induce serum IgG with memory against its natively folded self but with a nil-to-moderate immune response against natively folded V_{HH} analogs.