Development of a method for producing Fab/F(ab')2 fragments from a full-length monoclonal antibody for bioanalytical assays

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PURPOSE

Monoclonal antibodies (mAb) comprise of two Fab fragments and one Fc fragment or one F(ab)'2 fragment and one Fc fragment. While a full-length mAb is frequently used as an assay reagent for bioanalysis, mAb fragments are required in certain cases. For example, to build a sandwich assay for detection of anti-drug antibodies (ADA) for therapeutic antibodies, Fab or F(ab)'2 fragment is used instead of a full-length mAb as capture reagent. This is because therapeutic antibodies, either humanized or fully human, are in many ways indistinguishable from the ADA generated in patients, especially in Fc fragment. When ADA detection methods utilizes anti-human Fc antibodies as the detection reagent, the full-length mAb drug will be directly bound by the detection reagent, causing interference. Preparation of a Fab or F(ab)'2 fragment is therefore needed.

METHOD(S)

In this study, a method is being developed for enzymatic digestion of therapeutic antibodies to generate monovalent Fab or bivalent F(ab')2 fragments (Figure 1). With such reagents becoming available, a sandwich ADA assay formats can be expanded to allow anti-human Fc antibodies as detection reagents. To standardize the method, we explored various digestion conditions, including type of proteases (i.e., pepsin, papain, and IdeS), digestion-time (1, 2, 3, 4, and 6 h), protease to antibody ratio (1:10, 1:20, 1:40 and 1:80 w/w), IgG isotypes (human IgG1-κ, IgG1-λ, and IgG4-κ). The digestion products were quantified by NanoDrop and purified by dialysis (10K MWCO) and Protein A/G/L magnetic bead methods.

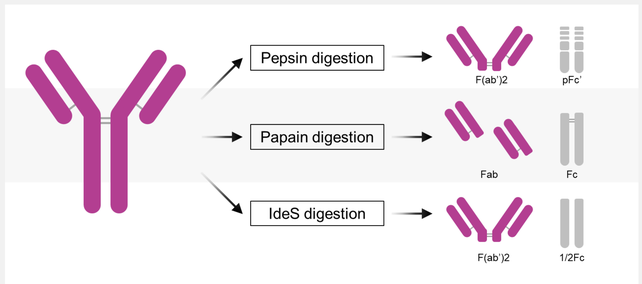

Figure 1 Assay Design Antibody digestion by protease. Digestion by the enzyme pepsin normally produces one F(ab')2 fragment and numerous small peptides of the Fc portion. Papain is primarily used to generate two Fab fragments and one Fc fragment. IdeS Protease cleaves IgG at a single site below the hinge region, yielding F(ab')2 and 1/2Fc fragments.

RESULT(S)

Preparation of Fab fragments

We have digested *Homo* IgG with the three proteases (pepsin, papain, and IdeS), using the same procedure, and have compared the recovery and properties of resulted Fab fragment under several conditions:

1. For treatment with pepsin dissolved in 0.2 M acetate buffer (pH = 4.0) and digested for 1 - 6 h at 37℃ by pepsin. The pepsin-IgG ratio was 1:10 to 1:80 (w/w).

2. For treatment with papain dissolved in digestion buffer (contain 0.02 M EDTA and 0.02 M cysteine) and digested by papain at 37℃.

3. IdeS digestion of IgG at a ratio of 1 µg:1 U.

The concentration of the products after the digestion was determined by NanoDrop. IgG standards were used as calibration of SDS-PAGE. The intensities of product bands were measured by the Image Pro Plus software. The recoveries of Fab/F(ab')2 fragments were >90% based on SDS-PAGE calculations. In addition, optimal conditions for the digestive reaction were assessed by ELISA method. The digestion products were coated on 96-well plates and detected with goat anti-human Fab-HRP (Goat anti-Fab mAb-HRP) versus mouse anti-human Fc-HRP (Mus anti-Fc mAb-HRP). Assay results of an IgG1 showed that pepsin efficiently cleaved IgG1 to produce F(ab')2 fragments and that Fc appeared to be gone (Figure 2). Results of IgG4 digestion showed a similar pattern (data not shown).

Evaluation of Fab/F(ab')2 binding performance

To further evaluate the Fab/F(ab')2 fragment performance, we analyzed the samples from pepsin digestion of *Homo* IgG1-λ (an antibody against IFN-γ), a mixture of F(ab')2 and pFc' fragments. The ELISA-based analysis was conducted with coated target (IFN-γ, 1 µg/mL) and detection reagents (anti-human Fab and Fc antibodies-HRP, 1:20,000). Sample analysis results are shown in Table 1. The effective recoveries of F(ab')2 produced after 1 - 3 h digestion were higher than 90.0%.

Table 1 Sample analysis results

Sample	Mus anti-Fc mAb (Mean OD450)	Goat anti-Fab mAb (Mean OD450)	Fab Conc. (ng/mL)	%CV (%)	Effective recovery (%)	Sample	Mus anti-Fc mAb (Mean OD450)	Goat anti-Fab mAb (Mean OD450)	Fab Conc. (ng/mL)	%CV (%)	Effective recovery (%)
Blank	0.054	0.059	-	10.35	-	Blank	0.062	0.060	-	12.72	-
Undigested	1.124	0.542	1,000.0	0.92	-	Undigested	1.203	0.540	1,000.0	3.84	-
1h 1: 20	0.104	0.622	1,160.9	4.55	116.09	3h 1: 20	0.057	0.502	912.3	0.82	91.23
1h 1: 40	0.131	0.619	1,154.5	1.17	115.45	3h 1: 40	0.057	0.512	933.3	5.38	93.33
1h 1: 80	0.152	0.661	1,248.6	0.14	124.86	3h 1: 80	0.060	0.531	975.7	0.62	97.57
2h 1: 20	0.066	0.612	1,138.9	1.23	113.89	4h 1: 20	0.056	0.416	728.2	3.10	72.82
2h 1: 40	0.068	0.586	1,079.5	1.38	107.95	4h 1: 40	0.057	0.425	747.4	5.64	74.74
2h 1: 80	0.073	0.611	1,136.3	2.30	113.63	4h 1: 80	0.072	0.456	811.9	1.50	81.19

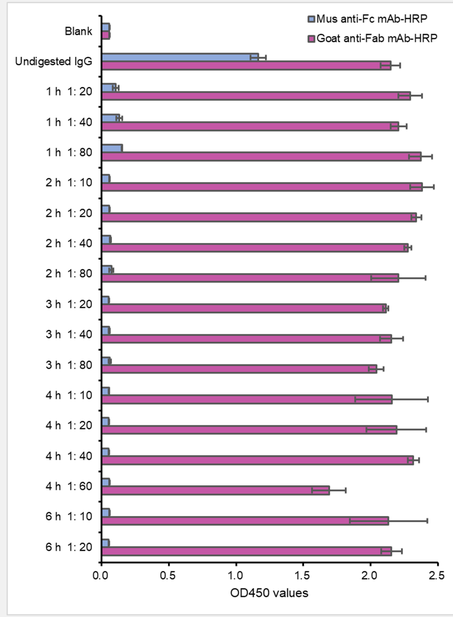

Figure 2 Preparation of F(ab')2 fragments from an IgG1. Pepsin dissolved in 0.2 M acetate buffer (pH=4.0) was mixed with IgG1 samples and then digested by pepsin for 1-6 hours at 37°C. The pepsin-IgG1 ratio was 1:10 to 1:80 (w/w). The products of all digestion conditions were quantified and they were diluted to 1,000 ng/mL. Undigested IgG1 standards (1,000 ng/mL) and digestion products (1,000 ng/mL) were coated on plates and then F(ab')2 and Fc fragment levels were detected directly using Goat anti-Fab mAb-HRP and Mus anti-Fc mAb-HRP, respectively.

CONCLUSION(S)

- Digestion of human IgG1 and IgG4 with pepsin resulted into a complete cleavage into F(ab')2 fragments and degradation of Fc fragments. While IdeS Protease produced an equivalent quantity of F(ab')2 and Fc fragments with a similar efficiency, removal of the intact Fc fragment was required as an additional step. If the Fab fragments were desired, papain could be used with yield of Fab/F(ab')2 fragments being over 90%. We have subsequently utilized either Fab or F(ab')2 as a capture reagent for ADA detection.
- Our results provide a practical method for the preparation of Fab/F(ab')2 fragments from IgG1 or IgG4 antibody therapeutics. The optimized conditions described here are broadly applicable to different IgG isotypes across many therapeutic antibodies.