Table 3.1 Electrospray fragments of mAb B (Figure 3.8) collected from size exclusion chromatography

Residues[a]	Expected mass	Observed mass	Cleavage site
HC: 1–222	23,619.5	23,617.3	Ser–Cys
HC: 1–223	23,722.6	23,721.0	Cys–Asp
HC: 1–224 (−18 Da)	23,837.7	23,818.1	Asp–Lys
HC: 1–224	23,837.7	23,836.5	Asp–Lys
HC: 1–225	23,965.9	23,964.6	Lys–Thr
HC: 1–227	24,204.1	24,203.1	His–Thr
HC:228–449 w/G0	26,415.7	26,414.6	His–Thr
HC:227–449 w/G0	26,552.9	26,556.5	Thr–His
HC:225–449 w/G0	26,782.2	26,782.0	Asp–Lys
HC:225–449 w/G1	26,944.2	26,942.1	Asp–Lys
HC:225–449 w/G2	27,106.2	27,105.9	Asp–Lys
HC:224–449 w/G0	26,897.2	26,897.0	Cys–Asp
HC:224–449 w/G1	27,059.3	27,059.2	Cys–Asp

[a]G0, G1, and G2 refer to Fc oligosaccharides with 0, 1, or 2 galactose residues, respectively.
From Cordoba et al. (2005)

Nonreducible cross-linking in mAbs

The most common types of cross-linking in mAbs are disulfide bonds and dityrosine formation, both as a result of oxidation. The disulfide bonds can usually be broken by use of reductants such as β-mercaptoethanol, whereas the dityrosine cross-links tend to be nonreducible. Other nonreducible cross-links have been observed. Usami et al. observed nonreducible SDS PAGE bands above the light chains in a human mAb that formed after incubation at pH 4 or 10 for 14 days at 37 °C (Usami et al., 1996). Jiskoot et al. detected a nonreducible SDS PAGE band at 88 kDa in two murine mAbs after incubation at pH 10 for 32 days at 37 °C (Jiskoot et al., 1990). A major nonreducible SDS PAGE band at 88 kDa was also observed after incubation at ~37 °C of the mAb OKT3 in phosphate-buffered saline with polysorbate 80 at pH 7. This band was also detected following incubation for 3 years at 5 °C. Peptide mapping showed that the cross-links were between heavy and light chains specifically between L46-52 and H99-121 (Kroon et al., 1992). No further characterization was done so the exact chemical mechanism for formation on the nonreducible cross-links was not determined. However, it appeared likely due to an oxidative degradation pathway since samples incubated with an argon overlay or that contained thiosulfate resulted in a delay of formation of the 88 kDa band (Rao & Kroon, 1993). A nonreducible species was detected by reducing SDS PAGE and reducing capillary electrophoresis in several humanized mAbs and found to be the result of a thioether bond between Cys 223 of the heavy chain and the C-terminal Cys residue of the light chain (Tous et al., 2005; Figure 3.16). This bond was found in samples before incubation at high temperature, and increased with duration of incubation at 40 °C, approximately 14% for 40 °C storage versus 0.4% at 5 °C