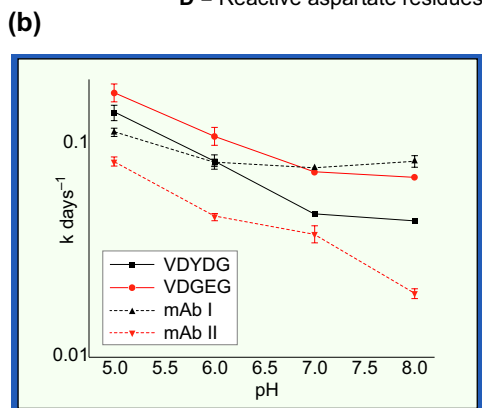


**Figure 3.5** (a) The differences between the primary sequences of two closely related mAbs (mAb I and mAb II), (b) pH rate of Asp isomerization in two closely related mAbs and respective model peptides.

Adapted from [Wakankar et al. \(2007\)](#), kindly provided by A. Wakankar.



An X-ray crystal structure for mAb I Fab showed there was a Tyr residue located within H-bonding distance of the labile Asp residue due to the structural folding of the polypeptide chain. An H-bond-donating residue such as Tyr could potentially catalyze Asp reactivity, especially under pH conditions where the Asp is ionized, that is, at  $\text{pH} > \text{pK}_a$  of the Asp ( $\text{pH} > 4$ ). The lack of a similar catalytic effect in the peptide model VDYDG could be attributed to the flexibility of the peptide which does not favor a stable H-bond formation between the Asp and the Tyr.

### Deamidation and Asp isomerization in mAbs

As in most proteins deamidation of mAbs is prevalent, as has been reported in the literature ([Chelius, Rehder, & Bondarenko, 2005](#); [Daugherty & Mrsny, 2006](#); [Gaza-Bulsecu, Li, Bulsecu, & Liu, 2008](#); [Harris et al., 2001](#); [Kroon, Baldwinferro, & Lalan, 1992](#); [Liu, Gaza-Bulsecu, Faldu, Chumsae, & Sun, 2008](#); [Paborji, Pochopin, Coppola, & Bogardus, 1994](#); [Timm, Gruber, Wasiliu, Lindhofer, & Chelius, 2010](#); [Vlasak & Ionescu, 2008](#); [Zheng & Janis, 2006](#)). Isomerization also can occur, proceeding through the cyclic succinimide intermediate. The isomerization can occur at Asn sites where deamidation occurs or at Asp residues. Again there have been many literature reports on Asp isomerization in mAbs ([Cacia et al., 1996](#); [Harris et al., 2001](#); [Huang, Li, Wroblewski, Beals, & Riggins, 2005](#); [Liu et al., 2008](#); [Pace, Wong, Zhang, Kao, & Wang, 2013](#); [Sreedhara, Cordoba, Zhu, Kwong, & Liu, 2012](#); [Vlasak & Ionescu, 2008](#); [Wakankar et al., 2007](#); [Zheng & Janis, 2006](#); [Zhang, Yip, & Katta, 2011](#)). Asp isomerization may have an impact on the activity of the mAb, especially if the degradation occurs in one of the