8.81b Let the fractions of the acidic NH_3^+ group, the α-COOH (pK=1.83) group, and the COOH side chain (pK=3.96) be given by f_1, f_2, and f_3, respectively. The net charge on the dipeptide results from a combination of the fraction of the NH_3^+ group (f_1), the fraction of the α-COO^- group ($1 - f_2$), and the fraction of the side chain COO^- group ($1 - f_3$). Remembering that f is the fraction of undissociated acid, and because the last two groups contribute negative charges, the net charge on the dipeptide, Q, can be expressed as

$$Q = f_1 - (1 - f_2) - (1 - f_3)$$

For pH = 3.00,

$$f_1 = \frac{1}{1 + 10^{3.00-9.82}} = 1.00$$

$$f_2 = \frac{1}{1 + 10^{3.00-1.83}} = 0.063$$

$$f_3 = \frac{1}{1 + 10^{3.00-3.96}} = 0.90$$

The NH_3^+ group is fully protonated, the α-COOH group is nearly fully dissociated, and the side chain carboxyl group is only slightly dissociated. The net charge on the dipeptide is

$$Q = 1.00 - (1 - 0.063) - (1 - 0.90) = -0.037$$

For pH = 7.00,

$$f_1 = \frac{1}{1 + 10^{7.00-9.82}} = 1.00$$

$$f_2 = \frac{1}{1 + 10^{7.00-1.83}} = 6.8 \times 10^{-6} \approx 0.00$$

$$f_3 = \frac{1}{1 + 10^{7.00-3.96}} = 9.1 \times 10^{-4} \approx 0.00$$

The NH_3^+ group is fully protonated whereas the side chain COOH group and the α-carboxyl group are completely dissociated. The net charge on the dipeptide is

$$Q = 1.00 - (1 - 0.00) - (1 - 0.00) = -1.00$$

At the isoelectric point, the dipeptide has no charge; that is,

$$Q = f_1 - (1 - f_2) - (1 - f_3) = 0$$
$$f_1 + f_2 + f_3 = 2$$

As the above calculations above show, even at pH as high as 7.00, the fraction of NH_3^+ is 1.00. (f_1 remains 1.00 for lower pH.) On the other hand, at this pH, both carboxyl groups are completely dissociated. The isoelectric point has to be much less than 7.00 for appreciable fractions of COOH groups to remain associated, but not so acidic to inhibit dissociation completely. Nevertheless, at the isoelectric point, $f_1 = 1.00$, and

$$f_1 + f_2 + f_3 = 1.00 + f_2 + f_3 = 2$$
$$f_2 + f_3 = 1$$
$$\frac{1}{1 + 10^{\mathrm{pI}-1.83}} + \frac{1}{1 + 10^{\mathrm{pI}-3.96}} = 1$$
$$\frac{1 + 10^{\mathrm{pI}-3.96} + 1 + 10^{\mathrm{pI}-1.83}}{\left(1 + 10^{\mathrm{pI}-1.83}\right)\left(1 + 10^{\mathrm{pI}-3.96}\right)} = 1$$
$$2 + 10^{\mathrm{pI}-3.96} + 10^{\mathrm{pI}-1.83} = \left(1 + 10^{\mathrm{pI}-1.83}\right)\left(1 + 10^{\mathrm{pI}-3.96}\right)$$
$$2 + 10^{\mathrm{pI}-3.96} + 10^{\mathrm{pI}-1.83} = 1 + 10^{\mathrm{pI}-3.96} + 10^{\mathrm{pI}-1.83} + 10^{2\mathrm{pI}-1.83-3.96}$$
$$10^{2\mathrm{pI}-1.83-3.96} = 12\mathrm{pI} - 1.83 - 3.96 = \log 1 = 0$$
$$\mathrm{pI} = \frac{1.83 + 3.96}{2} = 2.90$$

At pH = pI, the situation is not much different from pH = 3.00 as described above.