

Measuring protein masses by electrospray mass spectrometry

Reference: Nelson, and Cox, *Lehninger Principles of Biochemistry*, 4th ed., pp. 102-105

In the electrospray mass spectrometry technique, proteins are vaporized from an aerosol of charged droplets formed from an acidic solution. The positive ions are analyzed. The maximum positive charge on a protein cation is determined primarily by the *number of basic residues* in the protein: in the fully protonated state, each basic residue (plus the terminal amino group) contributes +1; acidic groups contribute 0.

However, in practice, many of the ions are less than fully-protonated, so a series of peaks is obtained, corresponding to ions of the form $(M + nH)^{n+}$, with n running from 1 to some maximum value; M is the mass of the (neutral) protein. The mass spectrometer does not determine mass directly, of course, but rather determines the *ratio of mass to charge*. Let us represent this mass-to-charge ratio, for any ion, by m . Then, for each ion:

$$m = \frac{(M + nH)}{n}$$

where H is the atomic mass of hydrogen.

(Obviously, ions with greater n will appear at lower values of m .) What we want to determine is not m but M , so we need to figure out n ; *i.e.*, we need to assign n values to each peak in the series of peaks we obtain from a given protein.

Consider any two successive peaks (m_1 and m_2) in the spectrum.

For the higher mass peak: $m_2 = \{M + nH\} / n$ (eqn. 1)

Writing down the corresponding equation for m_1 :

$$m_1 = \{M + (n+1)H\} / (n+1). \quad (\text{eqn. 2}) \quad (m_2 > m_1)$$

Treating eqn. 1 and 2 as simultaneous equations, we can solve for n .

$$1 \Rightarrow M = n(m_2 - H) \quad (\text{eqn. 3})$$

$$2 \Rightarrow M = (n+1)(m_1 - H) \quad (\text{eqn. 4})$$

$$3 \text{ and } 4 \Rightarrow \therefore n(m_2 - H) = (n+1)(m_1 - H)$$

$$\therefore nm_2 - nH = nm_1 + m_1 - nH - H$$

$$\therefore n(m_2 - m_1) = m_1 - H$$

$$\therefore n = (m_1 - H) / (m_2 - m_1)$$

This formula allows assignment of n (an integer) to each peak in the series.

But: $m = \{M + nH\} / n$ so: $M = n(m - H)$

And so, we now know the values of M and n

Example: The observed spectrum of horse heart myoglobin consists of a series of peaks; these include: $m = 1211.80$; 1131.12 ; 1060.46 ; 998.11 ; etc. *Calculate the mass of myoglobin.*

Consider a sequential pair of peaks, e.g.: $m_2 = 1211.80$; $m_1 = 1131.12$

$$n = (m_2 - H) / (m_2 - m_1) = 1130.11 / 80.68 = 14.007, \text{ i.e., } n = 14 \text{ for peak } 1211.80;$$

$$\therefore M = n(m - H) = 14 (1211.80 - 1.00794) = 16,951.09$$

(A more accurate value is obtained by performing the same calculation for each peak and averaging the results.)