On the electronegativity of atoms and their influence on the isoelectric point of casein.

by

K. Laki.

The electric charge of a protein particle in absence of salts is determined by the pH of the solution. At pH below the isoelectric point the protein particle takes up hydrogen ions in excess and aquires hereby a positive charge. At the opposite side of the isoelectric point the protein particle aquires a negative charge the hydroxyl ions being in excess. The quantity of the charge taken up by the protein particle increases as we depart from the isoelectric point. The charge has its maximum at a pH where the protein particle reaches the maximum of its acid or base combining capacity.

Curve A in figure 1. shows schematically how the acid combining capacity of casein (Hammarsten) changes with the pH, the shape of the curve being determined from the data of titrations combined with pH measurements. The curve reaches the abscissa at pH 4,8 (the isoelectric point of the casein) and becomes assymptotic at pH 2 showing that the protein reached here the maximum of its acid combining capacity and also the maximum of the positive charge that can be obtained.

If, at a pH where the casein particle has a positive charge, we add a salt which neutralises this charge,[1] then the casein particle will have no charge in excess and will have its isoelectric point at this pH. If this neutralisation happened at a pH at which the charge of the casein is not yet maximal, on further acidification the protein particle becomes positively charged again. Curve B in figure 1. gives an example of this case. The charge of the casein is neutralised at pH 4 by the addition of a salt. On further acidification the casein particle takes up a positive charge again. It is clear that if we neutra-

lise the charge of the casein at a pH where the charge maximum has already been reached (pH 2), on further acidification it aquires no charge again. It follows that in presence of salt the isoelectric point depends on the extent to which this salt can neutralise the charge of the casein particle. On the pH scale the isoelectric point becomes thus displaced from its original position. This displacement is a measure of the electric charge by which the salt neutralises the casein particle.

At the isoelectric point the casein precipitates from the

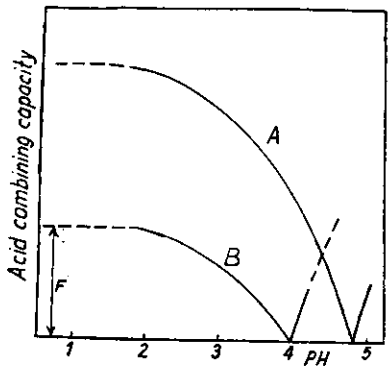

Fig. 1.

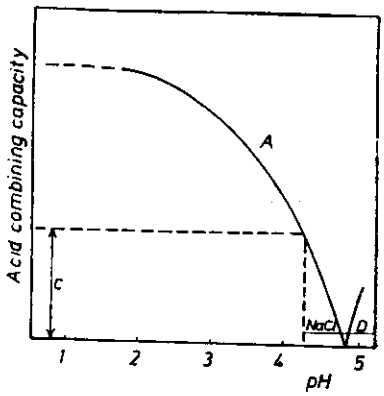

Fig. 2.

solution, and also precipitates in the neighbourhood of it indicating that the casein particle needs a certain quantity of charge to remain in solution. Correspondingly the casein has, instead of a point, a zone of precipitation on the pH scale.

Some precipitation zones, obtained in the presence of halogen salts of alkali metals, can be seen on fig. 3 taken from the preceding paper (Erdős). Strict comparison of the single zones is impossible because the left end of some of these zones is not defined, and so the exact position of the isoelectric point can not be given (calling the middle of the zones the isoelectric point).

The fact that in case of certain salts only the right end of these zones is defined can be explained in the following way. In figure 2 the line D represents the precipitation zone of the casein in the presence of NaCl. It can be seen from the figure that the casein remaines precipitated in a fairly large

pH zone until its charge exceeds a certain quantity (C) which is necessery to bring the casein into solution. Now let us suppose that a certain salt displaces the zone and the isoelectric point. In this case curve B in fig. 1. shows how the casein can be charged again by altering the pH. The comparison of the charge quantities (C and F in fig. 2. and 1.) shows that the amount of charge in this case is insufficient even at pH 2 to charge the casein properly and to bring it into solution.

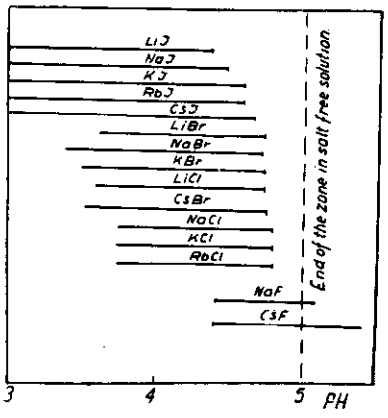

Fig. 3.

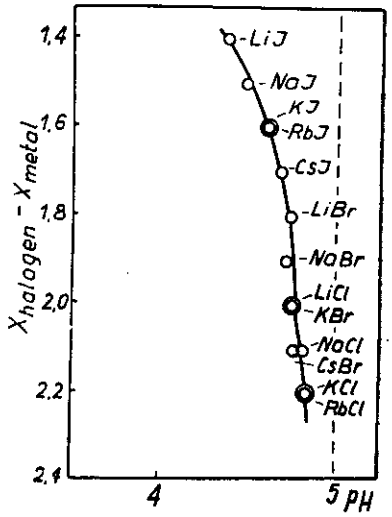

Fig. 4.

The casein thus would remain undissolved throughout the whole acid pH range. It follows from these that in cases where the precipitation zones are displaced to some extent, the zones will extend to cover the whole acide pH range having no defined end to the left. This is the case e. g. with NaJ and LiJ in fig. 3.

Since the left end of the zones in many cases is not defined the middle of the zones can not be ascertained and given as the isoelectric point. We are thus confined to the defined right end of the zones if we want to use them as a basis for comparison.

In figure 4. the circles represent the right ends of the precipitation zones of fig. 3. The abscissa gives the pH scale, the ordinata the values of electronegativity differences of the atoms composing the salt. It can be seen that the displacement

of the precipitation zones is correlated to the values of electronegativity differences.

The electronegativity represents the power of an atom in a molecule to attract electrons,[2] and MULLICAN[3] pointed out that the average of the first ionisation energy and the electronaffinity of an atom can be the measure of its electronegativity.

This correlation of electronegativity values with the power of salts to displace the isoelectric point suggests that the ionisation energy and electronaffinity of atoms play a role in the chargeing process in which the charge of the casein particle is neutralised.

In the following discussion an attempt will be made to explain — at least qualitatively — how the electronegativity differences can be brought into connection with the charge given to the protein particle.

In the past few years a new general theory of the solid matter has been developing.[4] According to this theory the solid matter can be regarded as a large molecule containing a great number of atoms which are arranged in a fashion which gives a regular lattice system. Because of this regular arrangement the valence electrons of these atoms belong to the whole system of atoms having common energy bands. This theory has been successful in interpreting many of the various properties of solids.

There is an increasing number of observations about the behaviour of the protein molecules which can be explained by picturing the protein praticle as a piece of solid matter[5] built up by C, N, O, H atoms. These atoms or a part of them are arranged in such a manner as to give a regular lattice at least in one dimension. The H atom in the hydrogen bond plays an important part in building up the lattice.

In the following treatment this new theory of solid matter will be accepted for the casein particle and discussed what sort of changes occur when a new atom is brought to the surface of the particle.

Suppose, that an atom such as Na having a low ionisation energy is brought on the surface of the casein particle the lattice atoms of which have a higher ionisation energy. Then it might happen that the electron of the Na atom leaves its core and enters into the continuum built up by the atoms

of the casein particle. The result of this is that the Na will be ionised and positively charged and the lattice atom nearest to the surface negatively charged.

In a case where the difference between the ionisation energies is small, the chargeing of the casein also may occur if the lattice atom has a greater electron affinity compared with that of the adsorbed atom. Then the lattice atom attracts an electron from the adsorbed atom, thus the surface atom of the casein will have a negative charge, the adsorbed atom a positive charge.

It follows therefore that two factors determine the magnitude of the charge given to the surface of the casein particle by the adsorption of a foreign atom: 1) the difference between the ionisation energies and 2) the difference between the electron affinities of the adsorbed atom and the lattice atoms.

These two factors can be symbolised in the following way: 1.) $J_l - J_a$ symbolises the magnitude of the charge caused by the difference in the ionisation energies of the adsorbed atom (J_a) and the lattice atom (J_l). 2.) $E_l - E_a$ represents the magnitude of the charge caused by the difference of the electron affinities of the lattice (E_l) and adsorbed (E_a) atom.

Adding up these two symbols we get the following expression:

$$J_l - J_a + E_l - E_a = J_l + E_l - (J_a + E_a)$$

$J_l + E_l - (J_a + E_a)$ represents the total charge given to the casein molecule.

The sum of $J + E$ (ionisation enregy and electron affinity) devided by 130 is identical with the electronegativity values (X) of the atoms.[2] Taking this into consideration the charge given to the casein particle can be represented by the differences of the electronegativities of the adsorbed (X_a) and the lattice composing (X_l) atoms: $X_l - X_a$.

Now if a Cl atom is brought to the surface of the casein particle and this atom behaves in the opposite way as the Na atom (it has a higher ionisation energy and greater electron-affinity than the lattice atom,) the magnitude of the charge given to the casein particle can be represented by the following symbole: $X_b - X_l$. Where X_l means the electronegativity of the lattice atom and X_b represents the electronegativity of the adsorbed Cl atom.

If both Na and Cl atoms are simultaneously on the surface, the resulting charge can be given by adding up these two expressions:

$$X_i - X_a + X_b - X_i = X_b - X_a$$

The symboles representing the electronegativities of the lattice atoms fall out and there remains the difference of the electronegativities of the Cl and the Na atoms: $X_b - X_a$.

The Na and Cl atoms according to this picture are ionised on the surface as Na and Cl ions.

The symbole $X_b - X_a$ says that the magnitude of the charge given to the casein particle is related to the values of the electronegativity differences of the salt composing atoms, and so gives the same result which is born out by the experiment.

Literature.

1. *Takeo Ito,* Biochem. Z., *233,* 444, 1931.

2. *L. Pauling,* The nature of the Chemical bond. (Cornell Univ. Press, 1939.)

3. *R. S. Mulliken,* J. Chem. Phys., *2,* 782, 1934., *3,* 573, 1935.

4. *F. Seitz, R. P. Johnson,* J. Applied Phys., *8,* 84, 1937., *8,* 186, 1937., *8,* 246, 1937.

F. Seitz, The modern theory of solids. (McGraw-Hill Book Comp. 1940.)

5. *F. Möglich* a. *M. Schön,* Naturwiss., *26,* 199, 1938.

P. Jordan, Naturwiss., *26,* 693, 1938.

N. Riehl, Naturwiss., *28,* 601, 1940.

N. Riehl, N. W. Timoféeff-Ressovsky u. *K. G. Zimmer.* Naturwiss., *29,* 625, 1941.