yielded fucose, galactose, and glucose on hydrolysis and which appeared to be identical with that of the digestive gland. Glycogen was not demonstrable in the cuticle; if it was present at all, the amount was negligible as compared with the acid mucopolysaccharide.

Quantitative distribution of the acid mucopolysaccharide in the cuticle and digestive gland is summarized in Table 1.

The cuticle contains an average of 937 mg of acid mucopolysaccharide per 100 gm (dry weight). In the digestive gland, about 10 to 25 percent of the total polysaccharide is in the form of acid mucopolysaccharide.

Recently many interesting observations on the correlation between calcification and distribution of acid mucopolysaccharide have been reported. Rubin and Howard (*11*) observed acid mucopolysaccharide in association with calcification in bones and calcified cartilages and suggested that calcium-binding capacity was due to the presence of the acid mucopolysaccharide. Studies on various types of human tissues have led to the conclusion that a mucopolysaccharide with specific properties is involved in almost all biological calcification. In the study of the organic matrix of the shell of the hen's egg, Simkin and Tyler (*12*) suggest that both acid mucopolysaccharide and protein components of the egg shell are vitally important to the calcification mechanism. Travis (*2*) has observed that the mucopolysaccharide shows some cyclic changes in the digestive gland and the integument in relation to the intermoult stages. On the basis of these observations it is envisaged that the presence of acid mucopolysaccharide in the cuticle of the crab may have an important role in the calcification of the cuticle (*13*).

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## Titration of the Protein Paramyosin

*Abstract.* The titration curve of an aqueous solution of crystalline paramyosin shows a unique buffering zone which can be attributed to a shift in the *pK* of its glutamic acid residues. A possible explanation of this phenomenon on the basis of the presence of polyanionic segments in the molecule is discussed.

In the course of studies on the solubility characteristics of the protein paramyosin and the physiological behavior of muscles containing this protein (*1*), it became apparent that its sharp crystallization point around *p*H 6.4 may be of prime importance to its physiological role. The amino acid composition of paramyosin, as determined by Kominz *et al.* (*2*), shows no amino acid residues which should have a *pK* in this range. The protein contains less than 1 percent histidine but about 158 residues of glutamic acid, 120 of aspartic acid, 73 each of lysine and arginine, and 13 of tyrosine, out of 800 amino acid residues (100,000 molecular weight); of the carboxylic groups, 112 are present as amides. No significant amount of phosphate could be detected.

We have titrated solutions of paramyosin over the *p*H range from 2 to 12, in the hope of determining whether or not crystallization is associated with pecularities in the titration curve.

The procedure was as follows. Paramyosin, isolated as previously described (*1*), was dissolved in 0.3*M* KCl and dialyzed against water. The precipitated paramyosin was isolated by centrifugation and dissolved in 0.3*M* KCl to give a final concentration of 1 percent protein. Thirty milliliters of this solution was placed in a beaker above a magnetic stirrer, under a nitrogen atmosphere, and adjusted to *p*H 12 with KOH. The solution was then titrated with 0.1*N* HCl, with a microburette, and the *p*H was read continuously with a Beckman model G *p*H meter with external electrodes. A blank, consisting of 30 ml of 0.3*M* KCl, was titrated over the same *p*H range, and its curve was subtracted from that of the protein sample. The results, designated as the net titration curve, are shown in Fig. 1. The protein precipitated at a *p*H value near 6.5, as had previously been found to be the case (*1*). The precipitation did not in itself alter the behavior of the protein toward added acid, since it was possible to start on the acid side of the curve with precipitated protein and titrate in the opposite direction with no change in the shape of the curve.

The titration curve shows a buffering zone around *p*H 11.5, corresponding to the known arginine content of the protein. The large buffering zone around *p*H 10 corresponds to the lysine, tyrosine, and terminal amino groups. A large buffering zone is found near the center of the curve, with a *pK* of about 6.3, corresponding to the titration of 130 groups per 800 amino acid residues. No buffering zone for carboxyl groups is found around *p*H 4.3, although such a zone would have been expected on the basis of the known content of glutamic acid. The buffering zone at the low *p*H end of the curve could correspond to free aspartic acid residues as well as terminal carboxyl groups.

The complete absence of a buffering zone around *p*H 4.3 and the presence of a large zone around *p*H 6.3 suggest that the *pK′* of the glutamic acid residues present in the protein has been shifted by approximately 2 *p*H units. Such large shifts in *pK′* are not common in proteins but are common in synthetic polymers. In the case of synthetic polyanions, the shift has been accounted for on the basis of the strong electric field which appears when charges are concentrated along the polymer chain (*3*). Others have sought to explain this effect in proteins on the basis of hydrogen bonding (*4*) or masking of groups by bound water (*5*). However, in view of the solubility behavior of paramyosin, the possibility that polyanionic segments are present in this protein sounds attractive to us.

This possibility has prompted us to calculate the number of charges per unit length of protein molecule necessary to generate an electric field large enough to shift the *pK* of the glutamic acid residues by approximately 2 *p*H units. The following formula, derived by Hartley and Roe (*6*) and modified by Katchalsky (*7*), for polyanions in solution, was used:

$$pH = pK' - 1n\frac{1-\alpha}{\alpha} + \frac{0.4343\,\varepsilon\,\psi}{kT} \quad (1)$$

where *pK′* is the *pK′* of the monomeric group (in this case 4.3), *T* is the abso-

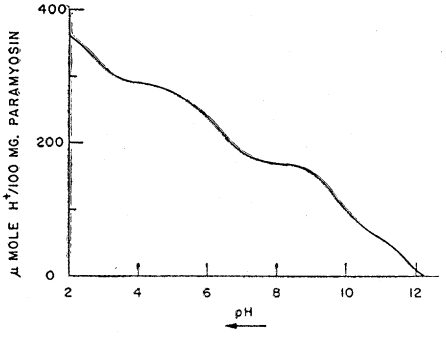


Fig. 1. Net titration curve of paramyosin dissolved in 0.3*M* KCl. The solution was adjusted to *p*H 12 and titrated to *p*H 2 with 0.1*N* HCl.

lute temperature, a is the degree of dissociation of the monomeric group, $\psi$ is the electric field, $k$ is Boltzmann's constant, and $\varepsilon$ is the elementary electronic charge. On the assumption that half-dissociation of the monomeric group in Fig. 1 occurs at *p*H 6.3, the electric field ($\psi$) was calculated to be approximately 100 mv. The surface-charge density necessary to generate a field of this strength was calculated from the following expression derived by Overbeek (*8*) (the Gouy-Chapman model of an electric double layer was used, and it was assumed that the molecular surface roughly approximates a plane surface):

$$\sigma_0 = \sqrt{\frac{DcRT}{2\pi}}\, 2 \sinh\left(\frac{F\psi_0}{2RT}\right) \qquad (2)$$

where $\sigma_0$ is the surface-charge density, $c$ is the concentration of the counterions in the medium at infinite distance from the face of the molecule, $D$ is the dielectric constant of the medium, $R$ is the gas constant, and $F$ is Faraday's constant; the other terms are the same as those appearing in Eq. 1. The value for $\sigma_0$ was found to be $1.5 \times 10^{14}$ charges/cm$^2$. This corresponds to 2.4 charges per 5.1-A length of a cylinder having a diameter of 9.8 A. On the assumption that the polypeptide chains within the paramyosin have the form of an a-helix (*9*), a polyglutamic acid segment could have a maximum of 3.8 charges per 5.1-A length.

The possibility that the high charge density is due to the presence of polyglutamic acid segments along the molecule can be tested by partial digestion of the paramyosin and examination of the fragments formed for the presence of highly acidic polypeptides. Preliminary studies with paper electrophoresis, in which tryptic digests of paramyosin were used, have indicated the presence of a fast-moving negative component which could correspond to a polyanion (*10*).

*Note added in proof.* Kensal Van Holde of the chemistry department, University of Illinois, has calculated by an independent method the charge density that could give rise to the suggested shift in $pK'$ of the glutamic acid residues in paramyosin. He has used the model proposed by T. Hill [*Arch. Biochem. Biophys.* **57**, 229 (1955)] which consists of a charged cylinder of infinite length, radius $b$, and closest center-to-center approach for small external ions of distance $a$. The titration curve should, in the region of the $pK'$ of the above groups, be expressed by

$$pH = pK' + 1n\,[\alpha/(1-\alpha)] - \frac{}{0.868w(c - \alpha n)}$$

where $\alpha$ is the fraction of groups ionized, $n$ is the total number of acid groups, $c$ is the number of positive groups in the neighborhood, and $w = F_e/Z^2KT$ [$Z$ is the actual charge and $F_e$ is the electrostatic free energy for charging the model in question, the expression for which is given by Hill (see above)]. For a shift of $pK'$ of 2.0 *p*H units, assuming that cationic groups are sufficiently remote to be neglected in this calculation and that the cylinder has the radius of an $\alpha$-helix ($a = 7$ A; $b = 5$ A), the number of charges in a length of 5.1 A is 3.7.

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## On the Mechanism of Action of Chlorpromazine

*Abstract.* Chlorpromazine is shown to be a powerful electron donor. Observations are described supporting the assumption that the therapeutic action of this drug is connected with this property.

Redox reactions between organic substances as a rule involve a change between two forms differing by two H atoms (two electrons plus two protons). Michaelis (*1*) pointed out that in this process the electrons may transfer one by one, and he demonstrated the intermediate state. Accordingly, substances regarded as oxidants or reductants are expected to have two stable configurations differing from each other by two hydrogen atoms.

J. Weiss (*2*) discovered that complexes may have properties which indicate an electron transfer from one molecule to the other within the complex. Mulliken (*3*) and his associates further developed this theory of "charge transfer." In such charge-transfer complexes an electron is supposed to go from the highest filled orbital of the donor to the lowest empty orbital of the acceptor; this transfer involves no change in configuration in classical chemical terms. In most cases studied by Mulliken the electron donated spent only a small fraction of its time with the acceptor and needed additional energy, supplied by the absorption of light, to be almost completely transferred to the acceptor.

The findings obtained in one of our laboratories indicated that complexes may be formed by biologically important substances in which an electron is practically wholly transferred from the donor to the acceptor, even in the ground state. There was reason to believe that such charge-transfer reactions are a frequent occurrence and so may play a role in reactions connected with a transfer of energy. The question arose whether charge transfer might play a role in the mechanism of drug action. Preliminary experiments indicated that this may be the case. For instance, various alkaloids were found to act readily as donors in complexes with iodine dissolved in chloroform.

Chlorpromazine has striking biological activity. It is, therefore, of great interest that, as indicated in this report, chlorpromazine also has striking properties as an electron donor, and the question arises whether the tranquilizing action of this drug may not be due to its charge-transfer properties. It is not possible to predict from its biological activity whether a pharmacologically active substance is an electron acceptor or an electron donor. If the drug acted, for example, on the electric double layer on the cell surface, where there is a positive charge on the outside and a negative charge on the inside, then an electron transfer on the outside would lead to a depolarization and thus to excitation, while a donation on the inside could be expected to lead to a hyperpolarization and, with it, to inhibitions. So, the final action depends on the affinities and location. What can be said, in a general way, is that one would expect pharmacological activity from a good donor rather than from a good acceptor, since the charge transfer depends to a great extent on the overlap of wave functions and a strong donor property involves a rather extended wave function. Whatever the case may be, one would expect pharmacological agents to show exceptionally strong donor or acceptor properties if their biological activity is actually due to a charge transfer.