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MEASUREMENTS OF THE ELECTRIC CHARGE AND ION-BINDING OF THE PROTEIN FILAMENTS IN INTACT MUSCLE AND CORNEA, WITH IMPLICATIONS FOR FILAMENT ASSEMBLY

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Microelectrode techniques can be used to record a Donnan potential from muscle and corneal tissue, where the bounding membranes have been removed or inactivated. From this potential, due to the different concentrations of freely-diffusible ions inside and outside the tissue, the net internal fixed-charge concentration of the nondiffusible proteins can be calculated (e.g., Collins and Edwards, 1971). We have combined such observations with x-ray diffraction measurements of the distances between the protein filaments, and can deduce the charge per unit length of the filaments *in situ*. (Elliott et al., 1978, footnote 1).

For muscle (glycerinated rabbit muscle and mechanically and chemically skinned rat and frog fibers), the fact that the Donnan potential does not change as the sarcomere length is changed gives further information, and we can calculate separately the charges on the thick myosin-containing filaments and the thin actin-containing filaments. As the ionic strength of the bathing solution increases, the thin filament charge does not change, but the thick filament charge increases swiftly, becoming more negative (at physiological pH). The thin filament charge is ~ 1–2×10^4 e μm^{-1}, the thick filament charge is 4×10^4 e μm^{-1} in a 5 mM KCl solution and 12×10^4 e μm^{-1} in a 100 mM solution.[1]

This concentration dependence of the thick-filament charge probably means that the myosin-containing filaments bind negative ions, and we have therefore further investigated the differential binding of chloride and phosphate ions. Both of these have been shown to bind, and the maximum charge is achieved in a solution containing ~5 : 1 chloride : phosphate. Magnesium decreases the thick-filament charge (from $\sim 12 \times 10^4$ e μm^{-1} to $\sim 8 \times 10^4$ e μm^{-1} when the external magnesium is increased roughly three-fold from 1 mM) but will only do so in the presence of phosphate ions.[2] These ion-binding effects in muscle have a concentration

[1]G. R. S. Naylor and Elliott, G. F. Manuscript in preparation.

[2]Bridgman, T. D., E. M. Bartels, and G. F. Elliott. Manuscript in preparation.

dependence seemingly impossible to explain on the law of mass action, so we must suppose that the sites act in a cooperative manner.

We have used the term "ion-binding" to refer to ions that are associated with the fixed-charge matrix of the total system and are thus not freely diffusible in and out of the system. We do not mean to imply that these are necessarily convalently bound and prefer to believe that they are associated electrostatically with the matrix. Possibly it would be better to use the older term "absorbed ions."

In muscles with longer sarcomeres (barnacle muscle) we have recorded different Donnan potentials from microelectrodes placed in the A-bands and I-bands, and whose position is observed by light microscopy. These potentials are in accord with the known numbers of thick and thin filaments, and with the expected charge ratios from our measurements in other muscles.[3]

In cornea, the measured fixed charge is similar to that in muscle, and shows a similar dependence on the concentration of the bathing solution (in this case buffered NaCl). Unlike the muscle case, it is not obvious that all the fixed charge is associated with the protein (collagen) filaments, because this tissue contains glycosaminoglycans which contribute to the charge and whose position is still uncertain. If we assume that all the charge is on the filaments and on glycosaminoglycans bound to the filament surfaces, the filament negative charge is $\sim 7.5 \times 10^4$ e μm^{-1} in phosphate buffer ($\mu = 0.02$, pH 7) and $\sim 22 \times 10^4$ e μm^{-1} in the same buffer with NaCl added to bring the ionic strength up to 0.15 (Goodfellow, 1975). Here again we have clear evidence of chloride binding.

Both in muscle and in cornea the fixed charge decreases as the pH is reduced from the physiological value, and passes through zero at the isoelectric point (pH ~4.5 in muscle, depending on the ionic strength, and pH ~4 in cornea). At lower pH's the Donnan potential, and the fixed charge, is positive, as we would expect. (Collins and Edwards, 1971; Elliott et al., 1978; Goodfellow, 1975).

For the muscle filaments, the measured thin filament charge and the thick filament charge at minimum ion-binding agree reasonably well with those calculated by Elliott (1973) from the known protein content (and amino-acid composition) of the filaments. A comparison of our ion-binding measurements in both tissues with reports in the literature of ion-binding to myosin (e.g., Sarkar, 1950) and collagen molecules in solution shows that there is no case where the ions which we find to bind *in situ* have not been reported to bind in solution. At the same time a quantitative consideration suggests that the amount of binding is much enchanced when the molecules are assembled into filaments, and that the pH conditions for binding are different. Our working hypothesis is that much of the ion-binding in these fibrous proteins takes place on networks (clusters) of charged amino-acid side chains set up between the molecules as they assemble together. These ion-binding clusters (Loeb and Saroff, 1954) might be an important feature of fibrous protein assembly; we suggest that they be called intermolecular Saroff sites.

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DETECTION OF INTRAMOLECULAR INTERACTIONS OF LYSYL AND N-TERMINAL AMINO GROUPS OF REDUCTIVELY METHYLATED PROTEINS BY ^{13}C NUCLEAR MAGNETIC RESONANCE

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Detailed information on the intramolecular interactions of protein amino groups has been largely unobtainable except to a limited extent from crystallographic studies. Reductive methylation with $NaCNBH_3$ and (^{13}C)-formaldehyde introduces a specific probe for ^{13}C nuclear magnetic resonance (NMR) investigation of protein amino groups (1, 2). We have shown that the chemical shifts of ^{13}C-enriched dimethylamino groups are sensitive to the

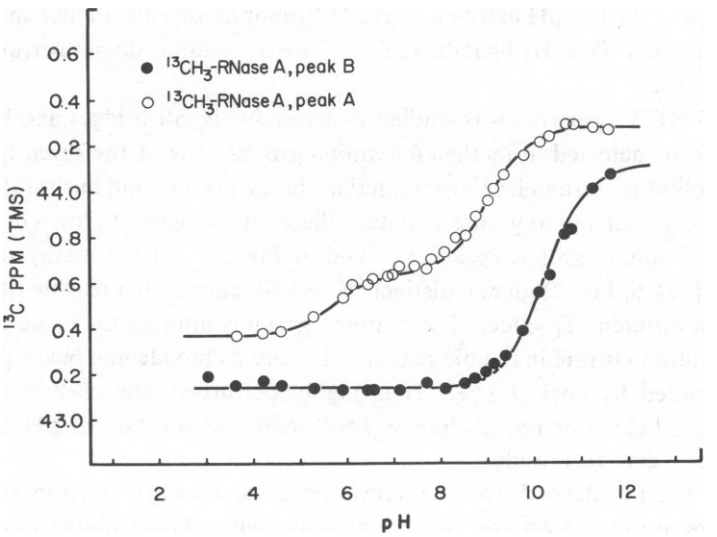

Figure 1 The ^{13}C NMR titration behavior of the [^{13}C]-ε-N,N-dimethyl lysyl residues of reductively methylated ribonuclease-A. Peak *A* is assigned to dimethyl lysine 41 and peak *B* to the remaining dimethyl bulk lysine residues. (^{13}C NMR spectra were obtained at 45.3 MHz from 7-ml samples of ~25 mg/ml methylated RNase A.)