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Adsorption of Water Vapor on Myosin A and Myosin B

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The moisture sorption isotherms of Myosin A and Myosin B were measured at 7°. The Bradley isotherm equation could be applied to the experimental water sorption behavior of both proteins up to a water activity of 0.8. Residual potassium chloride bound to the proteins affects the isotherm in all regions.

Water and its interactions with proteins have received a great amount of attention over the past 20 years. Proteins sorb water vapor by binding water molecules to specific hydrophilic sites at low water activities, followed by condensation or multimolecular adsorption as the humidity increases (Cassie, 1945; Pauling, 1945). By chemically modifying the adsorption groups of various proteins, it was found that at low water activities the water first combines with amino groups and then with carboxyl and hydroxyl groups as the water content of the system increases (Kanagy and Cassel, 1957; Leeder and Watt, 1965; Mellon, 1947). Using an isopiestic method, Bull and Breese (1970) found that protein hydration correlates strongly with the sum of the polar residues minus the amide residues.

Mathematical expressions which describe the adsorption of gases on solid adsorbents have met with limited success when applied to protein-water vapor systems. Bull (1944) applied the BET equation (Brunauer *et al.*, 1938) to water vapor adsorption data of various globular proteins and reported agreement up to a water activity of 0.5; Ling (1965) and Mellon and Hoover (1950) applied the Bradley multilayer adsorption equation (Bradley, 1936) to fibrous proteins and synthetic polypeptides and found agreement with experimental data up to water activities as high as 0.95. Ling and Nagendank (1970) showed that 95% of water in frog muscle follows behavior predictable on the basis of the Bradley adsorption equation. This result supports Ling's postulate (1965) that water in living cells might exist in the form of polarized layers.

During the past several years, our laboratory has reported on the use of moisture sorption isotherms as a basis for studying chemical (Kapsalis, 1969; Kapsalis *et al.*, 1964; Salwin, 1959, 1963) and textural (Kapsalis *et al.*, 1970) changes in freeze-dried meat and meat products. It was the objective of this study to investigate the water sorption properties of two major proteins in muscle, Myosin A and Myosin B (actomyosin), to determine the degree to which both proteins contribute to the water sorption of the whole muscle, and since the Bradley equation has been shown to describe water sorption behavior in the multilayer sorption region where most sorption equations fail, we applied it to the sorption data of Myosins A and B. As this work was in progress, Palnitkar and Heldman (1971) reported the isotherm of Myosin B.

EXPERIMENTAL SECTION

Both proteins were extracted from bovine arm bone muscle immediately after slaughter, and all subsequent operations were carried out at 3° to minimize thermal denaturation. Myosin A was isolated by the method of Perry (1953) and Myosin B (actomyosin) by the method of Morita and Tonomura (1960). Criteria of purity for both proteins were based on their behavior in the ultracentrifuge. After the final isoelectric precipitation, both proteins were exhaustively dialyzed against distilled water at 0°. Dialyzed proteins were lyophilized and stored at −20°.

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Adsorption and desorption isotherms of the solid proteins were measured at 7° on a modified McBain-Bakr spring balance. Details of this balance have been previously described (Wolf *et al.*, 1972). The water vapor pressure was regulated by a condenser thermally controlled to ±0.1°. Each point on the isotherm represents an average of two measurements made during the experimental run. The length of time for an adsorption-desorption cycle was 2 weeks.

RESULTS

The adsorption-desorption cycles for Myosin A and Myosin B are shown in Figures 1 and 2. A comparison of the adsorption isotherms of these proteins with that of freeze-dried muscle is given in Figure 3.

The adsorption data were treated according to the Bradley equation as used by Ling (1965)

$$\log_{10} P_0/P - K_4 = K_2 K_1^{a}$$

where a = amount of water vapor adsorbed at pressure P, P_0 = saturation water vapor pressure, and K_1 and K_2 = constants which are functions of the field of the sorptive polar groups, the dipole moment of the sorbed gas, the polarizability of the gas, and of the temperature. The term K_4 is included to account for the difference between the heat of evaporation from the polarized surface and from the bulk liquid. The value for K_4 used in our work is the value given by Ling (1965) for the sorption of water vapor on collagen. A constant of +2 is added to the log ($\log P_0/P - K_4$) term in the graphical representation of the Bradley equation so that all plotted points are of positive sign. Figures 4 and 5 depict the agreement of the adsorption data for Myosins A and B to the Bradley equation.

In Figure 6 we compare the isotherms of a Myosin B preparation with the same protein for which no attempt was made to exclude potassium chloride after the final isoelectric precipitation.

DISCUSSION

Both Myosins A and B have similar sigmoidal-shaped isotherms which are characteristic of the sorption of water on proteins. The desorption branches of both isotherms show a moderate degree of hysteresis. A certain amount of water remains irreversibly bound to proteins under the conditions of the experiment, even after prolonged pump-down in the sorption tube (2 and 1.5% bound water for Myosin A and Myosin B, respectively).

A comparison of the isotherms of the proteins with that of the muscle (Figure 3) shows that Myosin A has a higher capacity for water uptake at water activities (A_w) above 0.7. The fact that Myosin B absorbs less than either Myosin A or muscle above 0.7 may be due to irreversible linkages formed between the Myosin and Actin during lyophilization. As a result, Myosin B is restricted in its capacity to swell and take up water at these higher water activities.

The Bradley isotherm equation was found to fit the adsorption data for both proteins up to a water activity of 0.80 (Figures 4 and 5). The deviation from linearity between A_w 0.80–1.0 is probably due to the inherent limita-

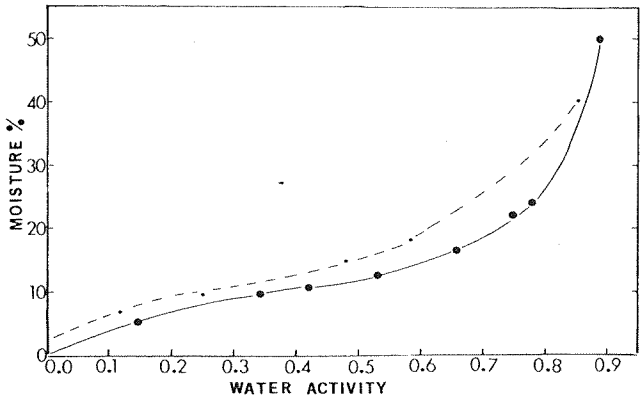

Figure 1. Moisture sorption–desorption isotherm for Myosin A at 7°. ———, adsorption; -------, desorption.

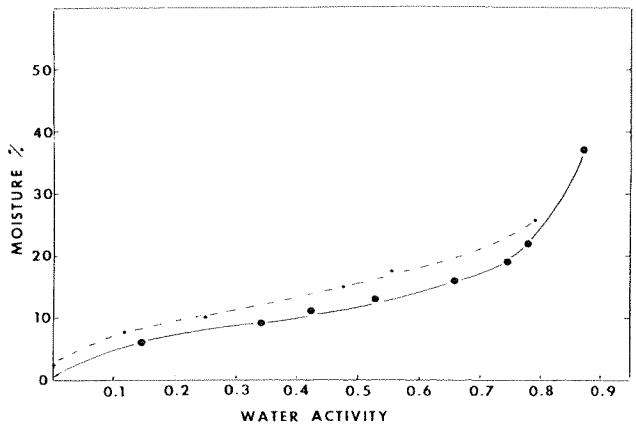

Figure 2. Moisture sorption–desorption isotherm for Myosin B at 7°. ———, adsorption; -------, desorption.

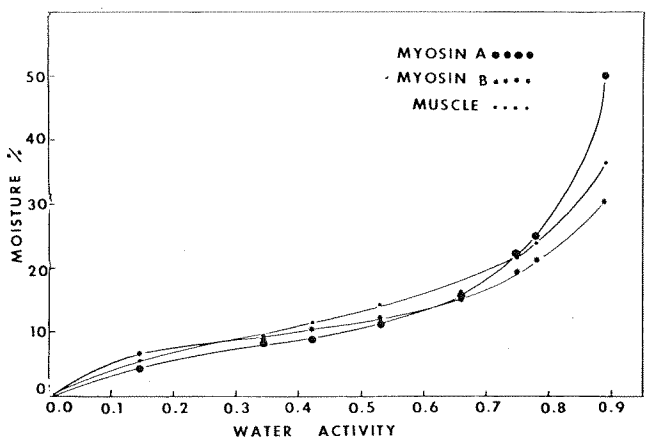

Figure 3. Moisture sorption isotherms for Myosin A, Myosin B, and muscle at 7°.

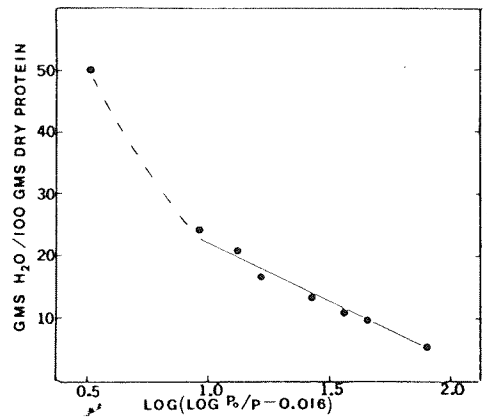

Figure 4. Adsorption data for Myosin A in terms of the Bradley isotherm equation.

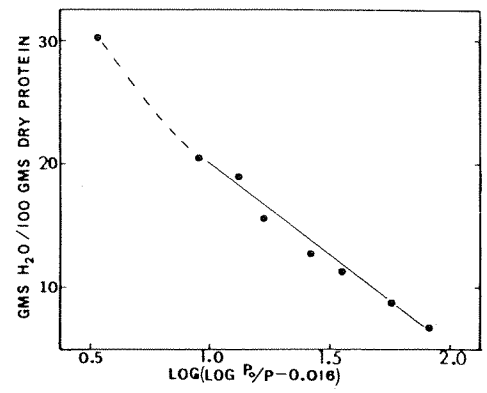

Figure 5. Adsorption data for Myosin B in terms of the Bradley isotherm equation.

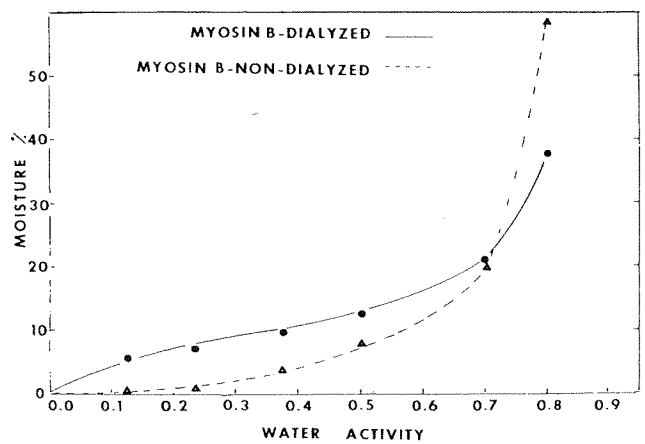

Figure 6. Moisture sorption isotherms of Myosin B dialyzed and Myosin B nondialyzed at 7°.

tions of the Bradley equation. This is known as the region of capillary condensation, where maximum swelling and solution effects may occur in the adsorbent (protein). The Bradley equation contains no terms which are suitable for coping with structural or solution changes that result from these processes.

Bull and Breese (1970) and Gal and Bankay (1971) report that sodium chloride bound to protein produces a characteristic change in the water vapor sorption process at medium and high water activities. Since the isolation of both Myosin A and Myosin B involves an isoelectric precipitation from a 0.6 *M* KCl solution, we wished to see how the isotherm would be affected if no effort was made to remove the traces of KCl that are bound to the precipitated protein. Figure 6 shows clearly that not only is the isotherm of Myosin B affected at medium and high A_w values if the KCl is not removed, but at low A_w the isotherm is depressed. The isotherm for Myosin B in which the salt is removed by exhaustive dialysis exhibits the normal sigmoid shape. Palnitkar and Heldman (1971) reported the isotherm for Myosin B in which no attempt was made to ensure the removal of salts. Their isotherm compares almost exactly with our salt-contaminated Myosin B isotherm.

CONCLUSIONS

Myosin A and Myosin B give the typical sigmoid-shaped isotherm for the water adsorption process of proteins. A model isotherm equation (Bradley equation) provides a good fit of the experimental adsorption data up to water activities of 0.80. Residual potassium chloride

bound to Myosin B profoundly affects the isotherm in all regions.

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