(1) Polymeric actin can go through a relatively large range of structural transitions from a rather 'closed' structure to a rather 'opened' one. (2) A particular state of actin is not solely determined by phalloidin but results from the interaction of all factors which can influence the structure of the actin polymer. Factors acting in the opposite destabilizing direction (as compared to phalloidin) are evidently hydrogen ions and sonication. (3) ATPase activity is possible only in a limited range of actin filament structure, which must be neither too 'closed' nor too 'opened'. Fig.2 presents diagrammatically in which way the different combinations of presence or absence of phalloidin and presence or absence of sonication may influence actin structure at different values of pH. According to this scheme there is inhibition of ultrasonic ATPase activity by phalloidin at neutral pH (cf. [7]) because actin structure remains too 'closed'

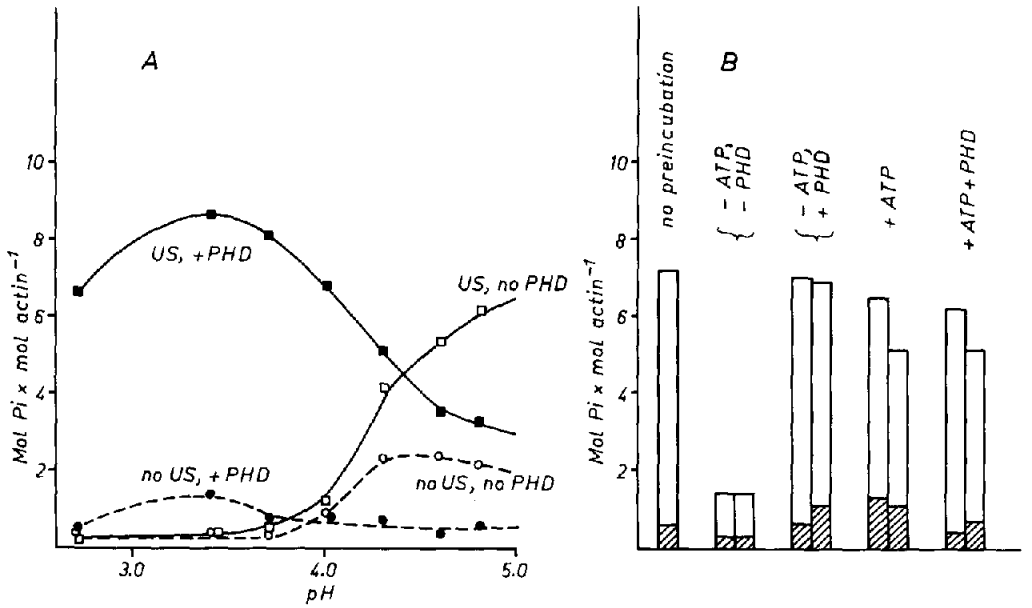

Fig.1. (A) Ultrasonic and steady state ATPase activity of polymeric actin at low pH in the presence or absence of phalloidin. 1.45 mg of actin were polymerized overnight at + 4°C in 60 mM KCl, 1 mM Tris-HCl, pH 7.4, 0.1 mM ADP. To each sample ATP (4 ml) was added to a final concentration of 1 mM and the pH was adjusted with acetate buffer to the desired value. When phalloidin was present, it was added prior to ATP. The final concentrations in the assays used for measurement of ATPase activity were 1.2 mg/ml actin (26 μM), 0.9 mM Tris-HCl, pH 7.4 and 0.08 mM ADP (both from the polymerizing medium), 10 mM acetate buffer, 1 mM ATP and no or 32 μg/ml (40 μM) phalloidin. At each pH the content of inorganic phosphate (P_i) was measured from 3 samples: to one sample trichloroacetic acid (TCA) was added immediately after adding ATP, to a second sample TCA was added after 30 min storage at 20°C (without sonication), to a third sample TCA was added after 30 min sonication at 20°C. Ultrasonic ATPase activity was calculated from the difference in P_i content between the third and the first sample, steady state ATPase activity from the difference between the second and the first sample. (●, ○) Steady state ATPase activity; (■, □) ultrasonic ATPase activity (US); (○, □) no phalloidin (PHD) present; (●, ■) PHD present. (B) Effect of pre-incubation at pH 4.0 on the ATPase activity of actin at the same pH in the presence of phalloidin polymerization and concentration of actin as in fig.1A. Ultrasonic ATPase activity was measured either immediately after transference of actin to pH 4.0 (no pre-incubation) or after 10 min (left bar of each group) and after 30 min (right bar of each group) pre-incubation at 35°C. During pre-incubation the samples contained ADP (0.08 mM) and Tris-HCl (0.09 mM) from the polymerizing medium as well as 10 mM acetate buffer, pH 4.0. If the samples contained during pre-incubation ATP and/or PHD, the concentrations were 1 mM ATP and 40 μM PHD. After pre-incubation the samples were divided into one part which was sonicated for 30 min at 20°C and another part which stood without sonication for 30 min at 20°C. To a third part TCA was added immediately after pre-incubation. The hatched sections of the bars represent the content in P_i of those parts which were not sonicated. The amount of P_i which was evolved in the sonicated samples is indicated by the total height of the bars. The calculations were performed as in fig.1 (A). Note that after the time of pre-incubation all samples contained 40 μM PHD and 1 mM ATP.

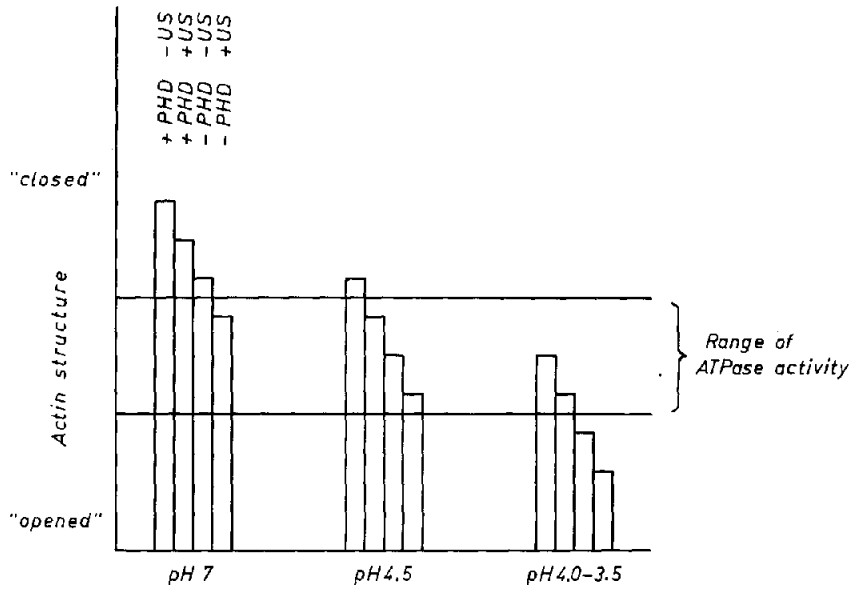

Fig.2. Schematic presentation of the influences which are presumably exerted (in the presence of ATP) by pH, sonication and PHD on the polymer structure of actin. It is supposed that ATPase activity of actin is possible only when the polymer structure is within the range indicated. The diagram only indicates which combination of influences shifts actin into the range favorable for ATPase activity, it does not indicate the magnitude of the ATPase activity. PHD: presence of phalloidin, US: treatment with ultrasound.

for ATPase activity but there is activation by phalloidin (both of the spontaneous as well as of the ultrasonic ATPase) at lower pH values because without phalloidin actin structure would be too 'opened'. In the absence of both ATP and phalloidin acidic pH 'opens' actin structure to such an extent, that irreversible denaturation occurs.

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