Hepatic glycogen levels vary depending on the metabolic state of the individual, but could easily reach 2 mg/ml. In glycogen-storage diseases, the amount of glycogen present in the liver can be much higher, and in such conditions glycogen inhibition of the glucose-6-phosphatase enzyme via the transport protein T1 could contribute to the patient's hypoglycaemia. This is the first report of the physiological concentration of any metabolite significantly inhibiting the activity of glucose-6-phosphatase via T1 in intact liver microsomes. However, around the time this work was undertaken, Nordlie & Robbins [6] reported intermediate metabolite inhibition in rabbit liver microsomes by fructose 6-phosphate, ribose 6-phosphate, phosphoenolpyruvate, glyceraldehyde 3-phosphate, dihydroxyacetone and fructose 1-phosphate, although not at physiological concentrations.

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The influence of anions, ionic strength and organic solvents on the interaction between actin and myosin subfragment 1

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In 1984 it was proposed that the binding of actin (A) to myosin (M) subfragment 1 (S1) occurs in a two-step reaction

$$A + M \overset{K_1}{\rightleftharpoons} A-M \overset{K_2}{\rightleftharpoons} AM$$

In the first step myosin binds relatively weakly to actin to form an 'attached state' and then isomerizes to the 'rigor-like' complex [1]. This work examines the influence of anions, ionic strength and organic solvents on the two-step reaction.

F-actin was prepared as described in [2] and covalently labelled with *N*-(1-pyrenyl)iodoacetamide as described in [3]. The fluorescence of pyrene-labelled actin is quenched by 75% upon binding to S1 and has been shown to monitor the isomerization specifically [4]. S1 was prepared by chymotryptic digestion of rabbit muscle myosin [5].

The overall affinity of actin for S1 (K_{ass}) was measured by fluroescence titration [6]. The binding of actin to S1 is sensitive to changes in hydrostatic pressure and rapid pressure perturbation induces two relaxations in the system. Analysis of the concentration dependence of the relaxations allows the rate and equilibrium constants to be defined [4]. The rate of association of actin with S1 was also measured by stopped flow [3].

Abbreviation used: S1, myosin subfragment 1.

Fluorescence titration in the presence of different anions indicates that, at 0.1 M concentrations, replacing chloride with other monovalent anions (acetate, propionate, bicarbonate and bromide) has only a small effect on the affinity of actin for S1, a small decrease in (K_{ass}) being observed in each case. An increase in KCl concentration reduces the association constant significantly [7–9]. At 0.5 M-KCl the association constant was 0.3×10^6 M^{-1}. However, organic ions (acetate and propionate) at 0.5 M caused only a small reduction in the affinity from that observed at 0.1 M (Table 1).

Pressure relaxation experiments give a more detailed understanding of the effects of ionic strength and specific anions than the titration experiment. At 0.1 M the difference in K_{ass} observed between acetate and chloride is reflected in a change in K_2. Increasing either acetate or chloride to 0.5 M reduces K_2 by a factor between 4 and 5; chloride in addition reduces K_1 by a factor of 10. These results are consistent with an effect of ionic strength on K_2 and a specific effect of chloride on K_1. Addition of ethyleneglycol or dimethylsulphoxide to 0.1 M-chloride affected only K_2 significantly, a 2- and 8-fold reduction being observed, respectively. Increasing the chloride concentration in the presence of ethyleneglycol had little effect on either K_1 or K_2.

Table 1. *Equilibrium and rate constants for the interaction of pyrene–actin with S1*

k_{+2} was > 1000 (s^{-1}) under all conditions. The buffer used was 20 mM-imidazole (pH 7 at 20°C). In addition, chloride buffers contained 5 mM-$MgCl_2$; all others contained 5 mM-acetate. K_{ass} was determined by titration, K_1 and K_2 by pressure jump amplitudes, k_{+1} by stopped flow and pressure jump, and k_1 as k_{+1}/K_1.

Potassium salt	K_{ass} (M^{-1})	K_1 (M^{-1})	K_2	k_1 ($M^{-1}.s^{-1}$)	K_{-1} (s^{-1})
0.1 M-Chloride	11×10^6	7×10^4	160	12.8×10^5	18
0.5 M-Chloride	0.3×10^6	0.7×10^4	42	2.3×10^5	33
0.1 M-Acetate	9.1×10^6	7×10^4	130	9.6×10^5	14
0.5 M-Acetate	1.1×10^6	4.6×10^4	25	3.2×10^5	7
20% (w/v) Dimethylsulphoxide/ 0.1 M-chloride	5.0×10^6	8×10^4	62	3.2×10^5	4
40% (w/v) Ethyleneglycol/ 0.1 M-chloride	2.0×10^6	10×10^4	20	2.4×10^5	2

The rate constant for the association of actin and S1 (k_{+1}) was the same at all ionic strength on k_{+1}. Increasing chloride from 0.1 M to 0.5 M reduced k_{+1} by a factor of 4–5, but as K_1 is reduced by a factor of 10 then chloride, in addition to reducing the 'on-rate' in the same way as acetate, destabilizes the attached state by increasing k_{-1}. Increasing acetate from 0.1 M to 0.5 M or by adding the organic solvent decreased the association rate constant by a factor of 3–4 and as K_1 did not change k_{-1} must also change by a factor of 3–4.

The information presented here only allow a few general statements about the nature of the reactions in the two-step binding. The equilibrium constant for the formation of the attached state appears to be unaffected by ionic strength, in the range studied here, or by the presence of the two organic solvents. The presence of chloride ions at concentrations well above 0.1 M does destabilize the attached state by increasing the rate at which the two proteins dissociate. There is a general effect of both ionic strength and organic solvent on the rate of formation of the attached state. The equilibrium of step 2 is affected by ionic strength and the effect appears to be relatively independent of the nature of the anion used. A similar effect is produced by the organic solvents.

The overall conclusion is that increases in ionic strength or the addition of organic solvent has very little effect upon K_1 but will produce a marked reduction in K_2. These treatments may be useful for trapping actomyosin in the attached (A–M) state for structural studies.

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Effect of phosphate and sulphate on the interaction of actin and myosin subfragment 1

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The binding of actin to myosin subfragment 1 (S1) has been shown to take place in two steps, as follows [1, 2]:

$$A+M \overset{K_1}{\rightleftharpoons} A-M \overset{K_2}{\rightleftharpoons} A.M$$

Scheme I

where A–M is a relatively weakly attached state and A.M is a rigor-like attached state analogous to the rigor state in muscle. Two-step binding is also observed when nucleotide is bound to S1 [3, 4]. Phosphate binds to both S1 [5] and to acto.S1 [6] and competes with ATP binding. Sulphate (SO_4^{2-}) also competes with ATP binding to S1 and acto.S1 [6]. We have studied the effects of phosphate and sulphate anions on the two-step interaction of actin and S1.

F-actin was prepared as described by Lehrer & Kerwar [7]. Actin was fluorescence-labelled with pyrene by the method of Kouyama & Mihashi [8] with the modifications of Criddle *et al.* [9]. The fluorescence of pyrene-labelled actin is quenched by 80% when S1 binds and this quenching monitors the isomerization step [2]. S1 was prepared by chymotryptic digestion of rabbit myosin as described by Weeds & Taylor [10]. All experiments were performed at pH 7.0 and at a constant ionic strength of 0.175 M.

The effects of phosphate and sulphate on the kinetic parameters in Scheme I were measured using a variety of techniques. Rates of ATP-induced dissociation of acto.S1 were measured by stopped flow fluorimetry. The observed rates were proportional to the concentration of ATP and the reaction was competitively inhibited by phosphate and sulphate. Analysis of this inhibition yielded association constants for phosphate (27 M^{-1}) and sulphate (190 M^{-1}) with acto.S1. The affinity of S1 for actin was measured by fluorescence titration [3]. The association constant for actin and myosin in the presence of increasing concentrations of phosphate and sulphate were measured. K_{ass} values at saturating concentrations were calculated by extrapolation and were reduced 30-fold and 10-fold by phosphate and sulphate, respectively (Table 1). The variation of K_{ass} with ligand also gave a measure of the association constants for the ligand with S1. These were 340 M^{-1} and 1400 M^{-1} for phosphate and sulphate, respectively. K_1 was measured in the presence of phosphate and sulphate using pressure relaxation as previously described [2] and was reduced 5-fold by both 70 mM-phosphate and 35 mM-sulphate (Table 1). The rate constant of acto.S1 association (k_{+1}) was measured by both stopped flow and pressure relaxation and was reduced 4- to 6-fold by 90 mM-phosphate and 3- to 4-fold by 20 mM-sulphate. k_{-1} was calculated from k_{+1}/K_1 and showed no change in the presence of phosphate or sulphate. The rate of displacement of labelled actin from acto.S1 on mixing of excess unlabelled actin was measured by stopped flow. Phosphate (60 mM) and sulphate (30 mM) both increased the rate by about 3-fold. This rate gives an accurate estimate of $k_{-1}/(1+K_2)$. K_2 was

Abbreviation used: S1, myosin subfragment 1.

Table 1. *Kinetic parameters for the interaction of actin and S1 and the effect of phosphate and sulphate on these parameters*

Ionic strength was kept constant at 0.175 mM by mixing buffers (a) 0.1 M-cacodylate/60 mM-KCl/5 mM-$MgCl_2$, (b) 90 mM-phosphate/5 mM-$MgCl_2$, and (c) 20 mM-cacodylate/47 mM-K_2SO_4/5 mM-$MgCl_2$. Values for K_{ass} are extrapolated to infinite concentrations of anions; other values are at concentrations of anions limited by ionic strength. K_2 and k_{-1} were calculated as described in the text.

	Control	Phosphate	Sulphate
K_{ass} (M^{-1})	3×10^7	1×10^6	3×10^6
k_{+1} ($M^{-1}\,s^{-1}$)	1×10^6	2×10^5	2.8×10^5
$k_{-1}/1+K_2$ (s^{-1})	0.14	0.38	0.4
K_1 (M^{-1})	5.94×10^4	1.14×10^4	1.15×10^4
k_{-1} (s^{-1})	17×10^4	18	24
K_2	120	46	59