ELECTROPHORETIC INVESTIGATIONS OF CRYSTALLISED MYOSIN

by

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Various authors have studied the electrophoretic behaviour of myosin solutions: BAILEY[1], ZIFF AND MOORE[2], DUBUISSON[3], JACOB[4]. In these experiments the chief stress has been laid upon an analysis of the components that can be extracted direct from the muscle. The present work has been undertaken with a view to studying the influence of different ions, and p_H values, on the crystallised myosin of SZENT-GYÖRGYI. A short note about the work is given in *Nature*[5].

Experimental procedure

Crystallised myosin was prepared from rabbit muscle according to SZENT-GYÖRGYI[6]. It was crystallised only once. The crystals were centrifuged down, dissolved in the desired buffer, and then dialysed overnight against two litres of buffer solution, with continuous stirring, at 4° C. The solutions were then centrifuged in a laboratory centrifuge at 10000 r.p.m. for one hour. Any sediment of insoluble material which happened to occur was discarded. The concentration of the myosin solution was determined with a refractometer, and the myosin diluted to the required concentration, 0.1–0.4% protein, with buffer solution. As buffer solution was used veronal acetate buffer with K, Ca, or Mg (MICHAELIS). To this was added KCl, $CaCl_2$, or $MgCl_2$, to attain the desired ionic strength. Since the protein concentration was kept very low, these buffers had sufficiently great buffer capacity in the p_H range 2–9 which was investigated.

The investigations were carried out in a TISELIUS electrophoresis apparatus[7] with the optical arrangement of SVENSSON[8] and for a thermostat temperature of 0° C. It was possible here to measure mobilities with no apparent disturbances even for powers up to 8 watts.

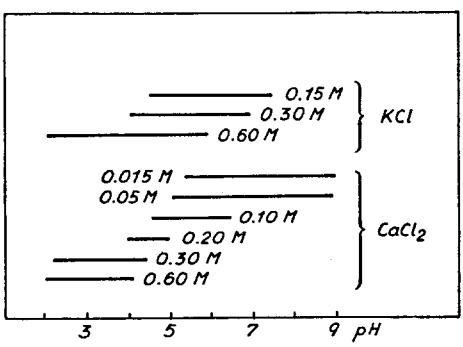

Fig. 1. The dependence of precipitations zones on p_H for myosin at different salt concentrations.

Since it was of great interest in these investigations to know the precipitation zones of the crystallised myosin, some qualitative experiments were made with all the salt concentrations, and p_H values, which were used in the later experiments. The results of these experiments are given in Fig. 1. Only the results for KCl, and $CaCl_2$ solutions are given in the figure since no noteworthy differences exist between the precipitation zones in $CaCl_2$ and $MgCl_2$. The actual end points of the precitipation zones are not sharp, and one can very often cross over them in the experi-

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ments since all the myosin does not precipitate. It follows from the experiments that a great difference exists between the precipitation zones for KCl and $CaCl_2$ solutions. Thus, myosin is insoluble for an ionic strength of 0.60 in KCl at p_H 6, while in $CaCl_2$ at the same ionic strength only between p_H 4 and 5.

The electrophoretic homogeneity of myosin

In order to examine the electrophoretic homogeneity of the crystallised myosin, some experiments were made with a 0.4% myosin solution in the p_H range 7.15–7.80 (ionic strengths 0.30–0.60). Both ascending and descending boundaries were nearly symmetrical and moved with the same uniform velocity. The diagrams showed only one component after a period of 8 hours. For longer periods the component divided into

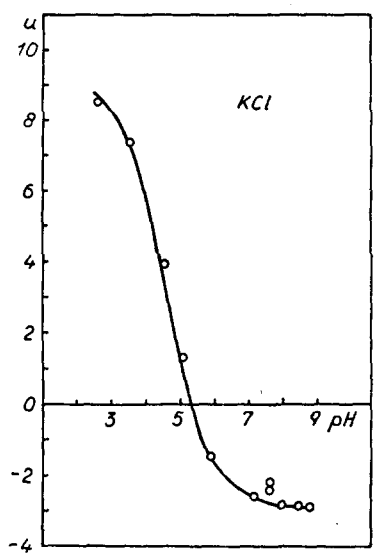

Fig. 2. The electric mobility — p_H curve of myosin in different potassium chloride solutions (0.1–0.5 M KCl).

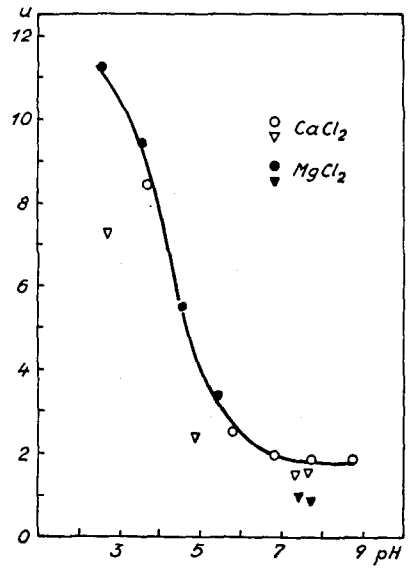

Fig. 3. The electric mobility—p_H curve of myosin in different calcium and magnesium chloride solutions (0.03 M–0.24 M). o < 0.1 M salt; ▽ > 0.1 M salt.

two. Even after 30 hours these were so slightly separated that nothing definite can be said about whether this phenomenon corresponds to two real components or whether it is an anomaly. For the studies which we have made one can therefore, from an electrophoretic point of view, regard the crystallised myosin as a homogeneous material. The time for one experiment never exceeded 8 hours.

Electrophoresis of myosin in the presence of various salts

Our experimental results for the mobility of myosin in solutions of various salts are given in Tables I, II, and III, and Figs 2 and 3.

It follows from these values that myosin in potassium chloride behaves essentially as most proteins. Crystallised myosin has here an isoelectric point at about p_H 5.4. Owing to the insolubility of myosin near this point, it has not been determined more accurately. On the acid side of the isoelectric point the myosin moved towards the

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TABLE I

THE MOBILITY OF MYOSIN IN POTASSIUM CHLORIDE SOLUTIONS FOR DIFFERENT pH VALUES. PROTEIN CONCENTRATION 0.1–0.2 %

Salt concentration	pH	u·10⁵ cm²/volt·sec
0.10 M KCl 0.05 M K-veronal-acetate buffer	2.60	+8.5
	3.53	+7.4
	4.50	+3.85
	7.50	—2.22
	8.30	—2.90
0.25 M KCl 0.05 M K-buffer	7.14	—2.60
	7.80	—2.90
0.5 M KCl 0.05 M K-buffer	4.98	+1.21
	5.75	—1.51
	7.50	—2.32
	8.65	—2.82

TABLE II

THE MOBILITY OF MYOSIN IN CALCIUM CHLORIDE SOLUTIONS FOR DIFFERENT pH VALUES. PROTEIN CONCENTRATION 0.1–0.4 %

Salt concentration	pH	u·10⁵ cm²/volt·sec
0.02 M $CaCl_2$ 0.018 M Ca-veronal-acetate buffer	3.68	+8.1
0.06 M $CaCl_2$ 0.018 M Ca buffer	5.76	+2.23
	6.80	+1.82
	7.75	+1.58
	8.70	+1.55
0.10 M $CaCl_2$ 0.018 M Ca buffer	2.69	+7.15
	7.40	+1.12
0.22 M $CaCl_2$ 0.018 M Ca buffer	4.89	+2.1
	7.60	+1.27
	7.75	+1.22

TABLE III

THE MOBILITY OF MYOSIN IN MAGNESIUM CHLORIDE SOLUTIONS FOR DIFFERENT pH VALUES. PROTEIN CONCENTRATION 0.1–0.4 %

Salt concentration	pH	u·10⁵ cm²/volt·sec
0.025 M $MgCl_2$ 0.010 M Mg veronal acetate buffer	2.54	+11.1
	3.57	+9.2
	4.50	+5.4
	5.13	+3.3
0.10 M $MgCl_2$ 0.02 M Mg buffer	7.40	+1.03
0.15 M $MgCl_2$ 0.01 M Mg buffer	7.66	+0.68

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cathode, and on the base side towards the anode. The mobility was, within the limits of experimental error, essentially independent of the salt concentration.

No isoelectric point could be detected in experiments in $CaCl_2$, and $MgCl_2$, solutions within the p_H range investigated, neither was there any indication that myosin has an isoelectric point. In these experiments the values of the mobility decreased to a constant value at higher p_H, thus even at p_H 8 the myosin moved towards the negative electrode.

Since, for all the salts investigated, the crystallised myosin shows a relatively low mobility above p_H 7, and therefore for the long times and high salt concentrations which were used there was a danger that the real values could be obscured by a superimposed flow in the liquid, the results were checked by the addition of 1% sugar to the myosin solutions before the start of the experiment*. Sugar has no charge, and the movement which this shows can therefore be considered mainly to have its cause in anomalies caused by disturbance. The measurement of the true mobility of myosin can then be made relative to the sugar component. Photographs taken from such experiments are reproduced in Figs 4 and 5. The results of such measurements are given in Table IV. It follows from these experiments that sugar shows a mobility of $\mu = -0.08$ in the presence of KCl, and about $\mu = -0.25$ in the presence of $CaCl_2$, in the p_H range 7–9. The relative measurements show that the differences between the mobilities of myosin in potassium, and calcium chloride, solutions are larger than before, when this correction is applied. In the curves given previously (Figs 2 and 3) this correction is already applied.

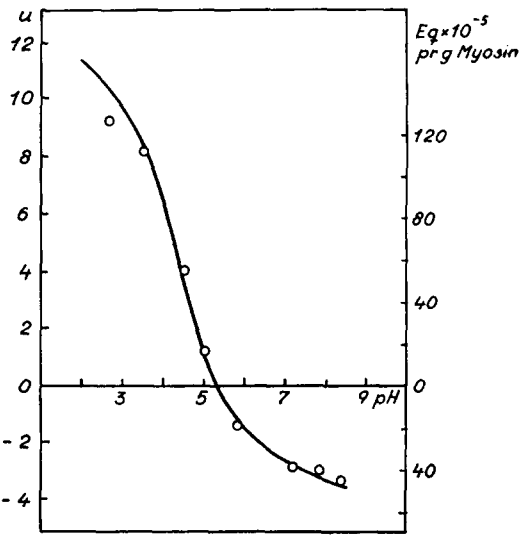

Fig. 4. Comparison of the titration curve of DUBUISSON (smooth curve) and our electric mobility values for myosin in potassium chloride solutions.

TABLE IV

THE MOBILITY OF SUGAR IN DIFFERENT SALT CONCENTRATIONS. CONCENTRATION OF SUGAR 1%

Salt concentration	pH	mA	u·10⁵ cm²/volt·sec
0.50 M KCl 0.10 M K buffer	7.5	70	—0.08
0.02 M $CaCl_2$ 0.02 M Ca buffer	3.60 3.60	10 20	—0.30 —0.20
0.10 M CaC_2 0.02 M Ca buffer	2.60 7.40	28 35	—0.23 —0.47
0.10 M $MgCl_2$ 0.02 M Mg buffer	1.4 7.4	45 35	—0.14 —0.22

* For this helpful suggestion our thanks are due to Mr B. ENOKSSON.

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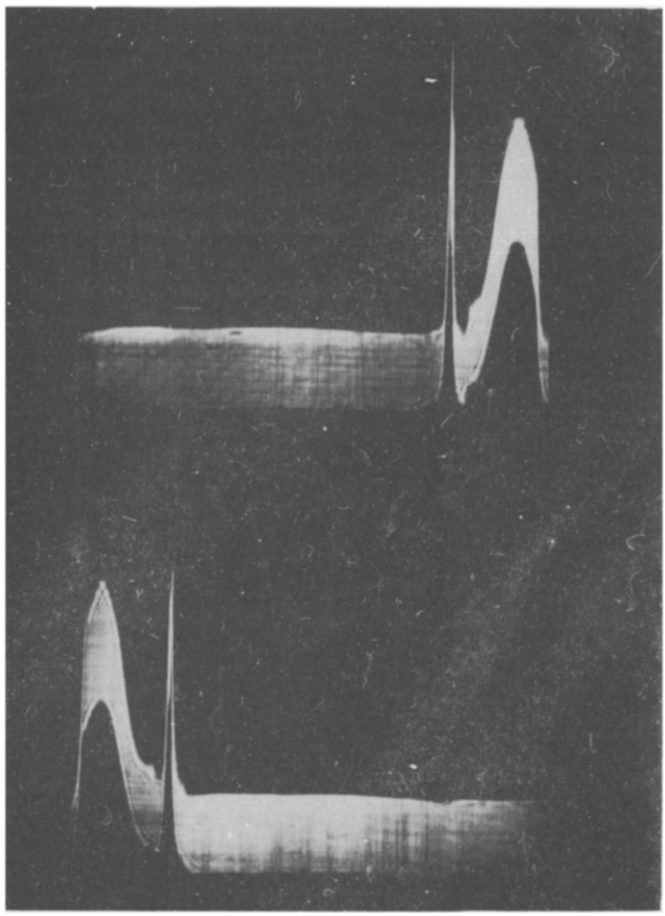

Fig. 5. Electrophoretic patterns of the boundaries of myosin and sugar in potassium chloride (0, 0.5 M KCl, 0.1 M K-veronal, p_H 7.5, Current 70.0 mA, 100 volts, 10 h). The sharp peak is myosin.

Mobility ($u \cdot 10^5$ $cm^2/volt \cdot sec$) = −2.32 (myosin)
−0.08 (sugar)
−2.24 (corrected)

The conclusion we come to is that while the mobility of myosin in the p_H range measured for KCl solutions shows an isoelectric point on changing the p_H, this is not the case for $CaCl_2$, and $MgCl_2$, solutions. If such a point exists it must lie above p_H 9.

In order to see whether these differences appeared even for other types of experiments, an investigation was made of the state of crystallised myosin in 0.1 M $CaCl_2$ in Ca-veronal-acetate buffer at p_H 7. No differences appeared here between this myosin and myosin dissolved in KCl. The two have the same sedimentation constant and concentration dependence in the ultracentrifuge. The relative viscosity is the same in the two solvents.

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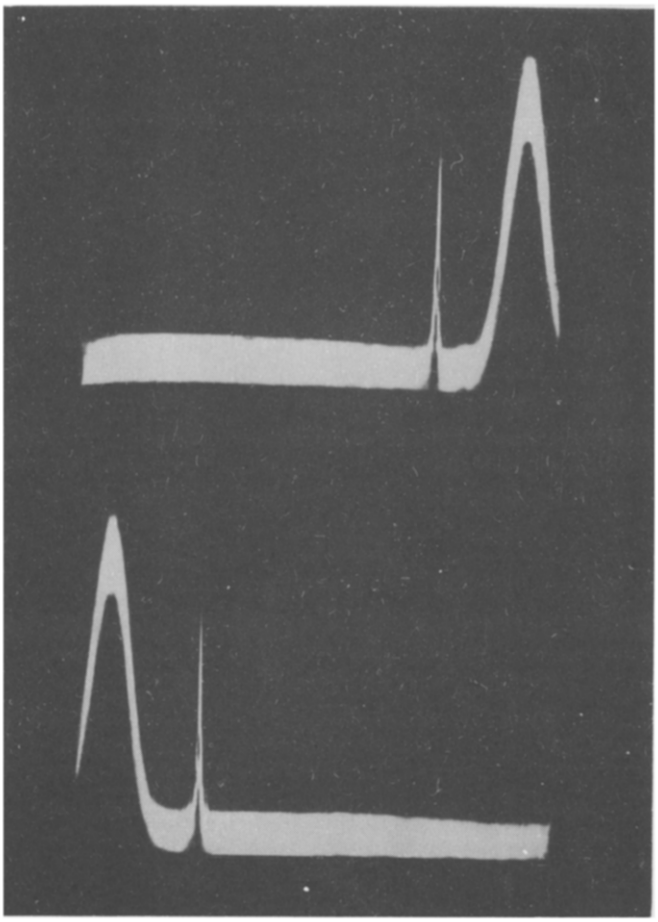

Fig. 6. Electrophoretic patterns of the boundaries of a solution containing myosin and sugar in calcium chloride (o, 0.1 M $CaCl_2$, 0.02 Ca-veronal, p_H 7.4, Current 35.0 mA, 196 volts, 5 h). The sharp peak is myosin.

Mobility ($u \cdot 10^5$ cm^2/volt·sec) — 1.25 (myosin)
—0.47 (sugar)
—1.72 (corrected)

DISCUSSION

The experiment shows that myosin has a specificity for doubly charged ions. It is much larger than has been observed for any other protein. What makes the conditions so easy to observe in this case may be the great solubility of myosin in salt solutions of divalent ions. For other proteins where one can possibly expect the same result, the observations are prevented by the proteins being insoluble. Whereas in potassium chloride solutions the zones of insolubility are directly connected with the isoelectric point, in Ca-, and Mg-solutions the connection with the electrophoretic mobility does not seem to be pronounced.

The curve for the electrophoretic mobility of myosin in KCl can be displaced so

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that it coincides with the titration curve which DUBUISSON[9] found for myosin in KCl. This shows that in this case the mobility is directly determined by the hydrogen (or hydroxyl) ions which are dissociated from the myosin. On the other hand, the curves for Ca (or Mg) and K-myosin can only be made to coincide below p_H 4.5 if the Ca-curve is moved downwards by the value given by the limiting value. This indicates that the residual mobility shown by myosin in $CaCl_2$ is due to non-specific salt effects and that Ca (or Mg) ions are bound in a specific way.

From our experiments it does not seem possible to assume, with SZENT-GYÖRGYI[10], that the insolubility in small concentrations of KCl should depend on a displacement of the isoelectric point solely by the change of the binding condition of the potassium ions. One can imagine, rather, that in small concentrations of potassium ions the calcium ions, possibly still bound, play a role. Experiments with myosin in mixtures of potassium and calcium ions may therefore be of interest.

Some preliminary experiments with such mixtures of salts have been made. Myosin at p_H 7.4 is completely and also reversibly soluble at the ionic strength of $\mu = 0.3$ in KCl and $CaCl_2$, very slightly soluble at the ionic strength of $\mu = 0.15$ in both salts, and practically insoluble for lower concentrations. The dissolution effects of KCl and $CaCl_2$ are not additional. Thus, for instance, myosin is only slightly soluble if we mix $\mu = 0.15$ KCl and $\mu = 0.15$ $CaCl_2$, and practically insoluble if the solution consists of $\mu = 0.075$ KCl and $\mu = 0.075$ $CaCl_2$. But neither do the effects of KCl and $CaCl_2$ seem to be contradictory. If myosin is dissolved in $\mu = 0.3$ KCl, the addition of $\mu = 0.0003$ to 1.0 $CaCl_2$ does not cause any appreciable change in the solubility of myosin. The same is true if myosin is dissolved in $\mu = 0.3$ $CaCl_2$ and different amounts of KCl are added.

Experiments with such mixtures have shown that the mobility of myosin is markedly influenced by the presence of both salts. It was observed, for instance, using a buffer with constant ($\mu = 0.3$) KCl concentration and increasing amounts of $CaCl_2$, that the mobilities were decreasing and at $\mu = 0.3$ KCl and $\mu = 0.3$ $CaCl_2$ the myosin did not move at all. Adding more $CaCl_2$ the mobility changed sign and our myosin, which had previously migrated to the anode, now moved towards the cathode. From a physiological point of view, such a possibility to change the sign of the charge of myosin must be of great importance. More experiments on mixed salt solutions are being made, and before their completion no further conclusions can be drawn.

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SUMMARY

The electrophoretic mobility of crystallised myosin in potassium chloride solutions shows no special characteristics compared with the conditions with other proteins. In solutions containing calcium or magnesium chloride the crystallised myosin migrates towards the negative electrode throughout the investigated p_H range (p_H 2–9). Whereas in KCl solutions an isoelectric point is found at p_H 5.4 there is no indications of such a point for myosin dissolved in $CaCl_2$. In salt mixtures,

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which so far are little studied, one can at one and the same p_H (p_H 7.4) obtain myosin which migrates towards either the negative or the positive electrode or which does not migrate at all, depending on the variation of the ratio K/Ca.

RÉSUMÉ

Comparé à celui des autres protéines, le comportement électrophorétique de la myosine cristallisée dans des solutions de chlorure de potassium ne présente aucun caractère particulier. Dans des solutions de chlorure de calcium ou de magnésium, la myosine cristallisée se dirige vers l'électrode négative, quel que soit le p_H, entre p_H 2 et p_H 9. Dans des solutions de chlorure de potassium, on trouve un point isoélectrique à p_H 5.4; un tel point n'existe pas pour la myosine dissoute dans $CaCl_2$. Dans des mélanges de sels, encore peu étudiés, on peut obtenir à un même p_H (p_H 7.4) de la myosine qui se dirige soit vers l'électrode positive, soit vers l'électrode négative, ou qui reste immobile, selon la valeur du rapport K/Ca.

ZUSAMMENFASSUNG

Die elektrophoretische Beweglichkeit kristallisierten Myosins in Kaliumchloridlösungen zeigt bei Vergleich mit den Bedingungen bei anderen Eiweisskörpern keine besonderen Eigenschaften. In Lösungen, die Kalzium- oder Magnesiumchlorid enthalten, wandert das kristallisierte Myosin im ganzen untersuchten p_H-Gebiet (p_H 2–9) zur negativen Elektrode. Während in KCl-Lösungen bei p_H 5.4 ein isoelektrischer Punkt gefunden wird, besteht kein Anzeichen eines solchen Punktes für Myosin in $CaCl_2$-Lösung. In Salzlösungen, die bisher wenig untersucht wurden, kann man bei demselben p_H (p_H 7.4) Myosin erhalten, dass entweder zur negativen oder positiven Elektrode wandert, oder überhaupt nicht wandert, je nach dem Verhältnis K/Ca.

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