ACIDIFICATION AND POLYMERIZATION OF ACTIN

by

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When salt is added to a solution of the globular protein, actin, it polymerizes to yield a solution of increased viscosity. Also, as has been found by DUBUISSON[1] and has been observed in this Laboratory, the pH of the solution decreases. The question arises whether these phenomena are immediately interdependent. DUBUISSON implies that they are[1]. The polymerization of actin by salt, however, involves the other effects of salt upon protein solutions[2]. Since one of these effects is a decrease in pH of proteins more alkaline than the isoelectric point, the possibility exists that the decrease in pH of actin following the addition of salt is a salt-effect unrelated to polymerization. This possibility was investigated to further understand the polymerization of actin solution and its role in muscular contraction.

MATERIALS AND METHODS

Actin was prepared according to FEUER *et al.*[3] The details of this process need not be repeated, but it should be mentioned that the recommended extractions of the muscle residue[3] in 0.4% $NaHCO_3$ and in 0.01 M Na_2CO_3 — 0.01 M $NaHCO_3$ were both done.

The serum and egg albumins used were both crystalline products of the Armour Laboratories. The myosin was prepared according to SPICER's[4] modification of SZENT-GYÖRGYI's[5] procedure. It was made salt-free by dialysis.

To study the effects of salt upon these proteins salt was added to solutions of each while they were being stirred by a magnetic stirrer and while the pH was being followed by means of a Macbeth pH meter. Eight ml of each of the protein solutions was made 0.1 M KCl by adding 0.2 ml 4 M KCl; and 8 ml was made 0.02 M $CaCl_2$, 0.02 M $MgCl_2$, or 0.016 M phosphate buffer (pH 7.1), respectively by adding appropriate amounts of concentrated solutions of each salt. Then records of the pH in the solutions were made at five and ten second and minute intervals after the salt was added until no further change occurred. The process of polymerization was followed at 25° by measuring the time of efflux in a Cannon-Fenske viscometer until no further changes occurred. The times of efflux were converted to apparent specific viscosities (η sp) by substituting in the following equation:

$$\eta_{sp} = \frac{t}{t_0} - 1$$

where t = seconds of efflux of a solution containing actin and salt and t_0 = seconds of efflux of a solution containing only salt.

RESULTS

When a solution of G-actin is made 0.1 M KCl the pH decreases immediately upon the mixing of the salt (Figs. 1, 2 and 3). This has occurred in a large number of experiments, some of which are recorded in Table I. Also, the pH of serum albumin solution (5 mg/ml), of egg albumin solution (5 mg/ml), and of myosin solution*, under the conditions in which actin was studied, decreases when salt is added (Table II and Fig. 1).

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* When myosin is suspended in salt-free water the amount dissolved is less than 9 mg/ml; however, myosin is soluble to a limited extent in water (5) and the salt-effect on pH is real.

TABLE I

CHANGES OF pH AND OF APPARENT SPECIFIC VISCOSITIES (η_{sp}) OF ACTIN UPON ADDITION OF 0.1 *M* KCl

*pH**		*η_{sp} maximum*	
Before KCl	*After KCl*	*Before KCl*	*After KCl*
8.8	7.2	0.13	0.84
9.2	7.8	0.25	1.45
8.9	7.8	0.04	0.94
7.8	7.5	0.06	0.47
8.9	7.3	0.10	0.66
8.9	7.3	0.34	9.0**
7.9	7.5	0.00	0.67
7.3	6.8	0.2	1.4

* pH values obtained before and after polymerization with a Beckman pH meter.
** Estimation of extremely viscous actin.

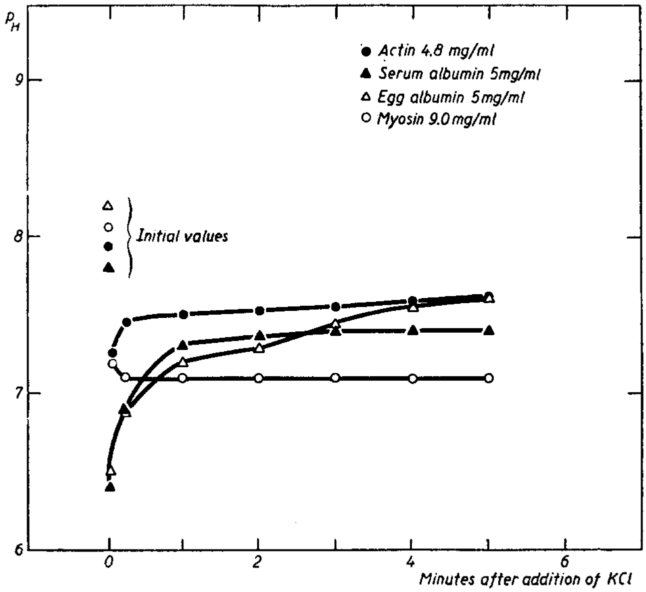

Fig. 1. The effect of KCl upon the pH of the solutions of four proteins. The initial pH's of the albumins and of myosin were adjusted so as to be similar to that of actin. Final concentration of KCl, 0.1 *M*.

The condition under which actin was studied and which was produced in albumin and myosin solutions was that of pH. The pH of actin is increased by the residual Na_2CO_3 and $NaHCO_3$ carried over in its preparation, so a mixture of 0.01 *M* Na_2CO_3 and 0.01 *M* $NaHCO_3$ (final concentration of each about $2 \cdot 10^{-4}$ *M*) was added to the solutions of albumin and myosin. When albumin and myosin were not made alkaline, or when actin was dialyzed to reduce the pH, the addition of salt was also followed by a decrease of pH (Tables II and III).

The decrease in pH of actin produced by KCl is also produced by $CaCl_2$, $MgCl_2$, and phosphate buffer (Fig. 2). It is likewise produced by the addition of $CaCl_2$ to serum albumin and to myosin, and by the addition of $MgCl_2$ and of phosphate buffer to serum albumin.

The decrease in the pH of actin when KCl is added is followed by an increase in pH (Figs. 1 and 3). This has been observed in more than ten experiments. The rise in pH is

TABLE II

EFFECT OF 0.1 M KCl UPON THE pH OF ALBUMIN AND MYOSIN

	Egg	*Serum*	*Myosin*
pH before salt	5.70	5.70	6.70
Lowest value obtained after salt	5.10	5.55	6.25
pH 5 min after salt	5.05	5.55	6.25

TABLE III

EFFECT OF DIALYSIS OF G-ACTIN UPON THE CHANGE OF pH WHEN MADE 0.1 M KCl

	Dialyzed	*Control*
pH before salt	6.00	8.10
Minimum pH after salt	5.45	7.50
pH 5 min after salt	5.45	7.80

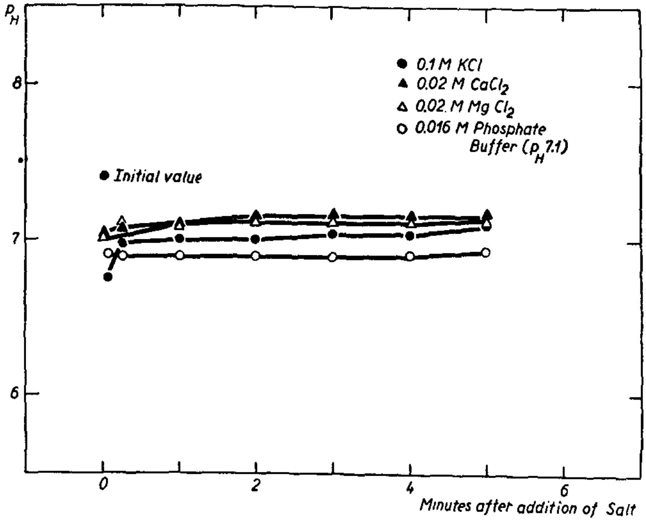

Fig. 2. The effect of the addition to four salts upon the pH of actin.

exagagerted when the concentration of actin is reduced (Fig. 3); in fact, it was first observed in low concentrations of actin. It occurs only to a limited extent when $CaCl_2$ or $MgCl_2$ is added (Fig. 2). The increase is more gradual than the decrease and results in a final value which, although more acid than the initial value, is not as low as the minimum immediately following the mixing of the salt. This increase in pH occurs in all the proteins, the pH of which has been adjusted with carbonate, except myosin (Fig. 1). It is obliterated in actin by dialysis (Table III) and by adding 0.016 M phosphate buffer instead of KCl (Fig. 2). It fails to occur in albumin and in myosin when the pH is not adjusted before adding salt (Table II).

DISCUSSION

A solution of KCl which has been exposed to air is usually slightly acid (about pH 5.5) and when such a solution is added to a less acid solution of protein the possibility exists that the carbonic acid introduced accounts for the decrease in pH. This was

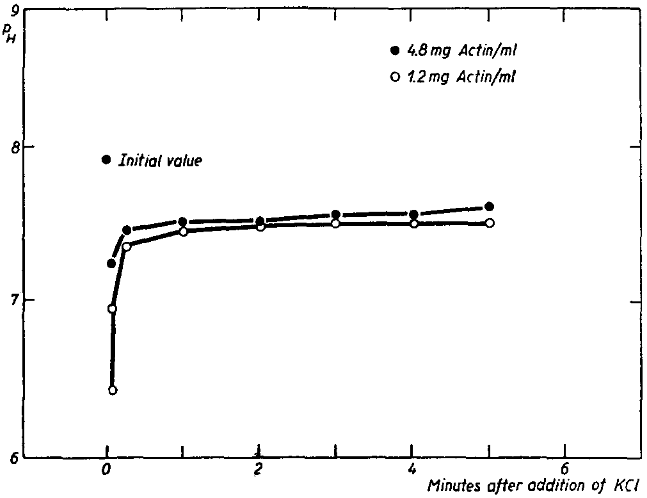

Fig. 3. The effect of concentration of actin upon the magnitude of the change of pH produced by the addition of KCl. Final concentration of KCl, 0.1 *M*.

tested by adjusting the 4 *M* KCl to a pH greater than that of the protein. When this was added to the protein, the decrease of pH still occurred.

The decrease in pH which follows the addition of salt to actin and the other proteins studied above is in accordance with SÖRENSEN's[6] finding that when salt is added to a negatively charged protein solution the pH decreases, *i.e.*, the net charge on the protein molecule increases. This is true of all proteins[2] and the above data show that actin is no exception.

The decrease in acidity which follows the increase upon the addition of salt varies from one preparation of actin to another. In general, the decrease varies directly with the difference between the isoelectric point of actin (about 4.6) and the pH of the actin solution, *i.e.*, it is greater when the initial pH is 8.0 than when it is 7.0, and it fails to occur when the initial pH is reduced to 6.0 by dialysis. Also, it is greater in dilute actin than in concentrated (Fig. 3). The coincidence between the presence or absence of carbonate buffer and the increase in pH, as with the albumins to which carbonate was added and actin from which it was removed by dialysis, suggests that the increase may be due to the removal of carbon dioxide, therefore decreasing the carbonic acid and relatively increasing the sodium ion concentration. This explanation is supported by the failure of the pH to increase when a stronger buffer than the carbonate-bicarbonate (0.016 *M* phosphate buffer) is added to actin (Fig. 2).

Whether the production of H-ions or of OH-ions is essential for polymerization is difficult to establish and only can be considered indirectly. The pH of unpolymerized actin solution is usually between pH 7.5 and 9.0 and of polymerized actin between 7.0 and 8.0 (Table I). There is little, if any, correlation between the amount of change of viscosity and the change in pH upon the addition of salt; however, actins which undergo large changes of viscosity tend to also undergo large changes of pH (Table I). Actin which will not polymerize (treated with apyrase or aged at 25° for 16 hours) still undergoes a reduction of pH when made 0.1 *M* KCl. The converse experiment, to wit, actin which will not change in acidity but will polymerize upon the addition of salt, is impossible. Such an experiment would have to be done at the isoelectric point and at that pH actin precipitates.

The changes of viscosity and of pH which follow the addition of KCl, contrary to DUBUISSON'S[1] finding, are not concomitant. The decrease in pH follows immediately after salt is added. Typical actin requires one to three minutes after KCl is added for the first changes in viscosity to appear. About ten minutes are required for maximum viscosity to be reached[7]. Actually it is the increase in pH which accompanies polymerization of ordinary actin; however, the dialyzed actin, which only changed pH in one direction (Table III), polymerized as usual. The possibility that the increase in pH of actin is due to deamination of adenylic acid which might be involved in polymerization was explored. Polymerized actin was analyzed for NH_3 by two methods, but neither indicated NH_3 that was produced during polymerization.

SUMMARY

The production of H-ion when salt is added to actin solution appears to be the usual effect of salt upon a protein solution and not to be prerequisite for polymerization. The pH decreases immediately after the salt is added but polymerization, as judged by changes in viscosity, does not begin for one to three minutes. The pH of actin also decreases when no polymerization occurs; the H-ion is not the result of polymerization.

RÉSUMÉ

La production d'ions H qui a lieu lorsque l'on ajoute du sel à une solution d'actine semble être l'effet habituel de l'addition de sel à une solution de protéine et ne pas être nécessaire à la polymérisation. Le pH décroît immédiatement après l'addition de sel mais la polymérisation, à en juger d'après les variations de la viscosité, ne commence pas avant une ou trois minutes. Le pH de l'actine décroît aussi s'il n'y pas de polymérisation; les ions H ne résultent pas de la polymérisation.

ZUSAMMENFASSUNG

Das Auftreten von H-Ionen nach Salzzugabe zu einer Lösung von Aktin scheint der gewöhnliche Effekt von Salz auf eine Proteinlösung zu sein und nicht eine der Polymerisation notwendigerweise vorausgehende Erscheinung. Das pH nimmt unmittelbar nach der Salzzugabe ab, aber die Polymerisation beginnt, nach den Viskositätsänderungen zu schliessen, erst nach einer oder drei Minuten. Das pH des Aktins nimmt auch ab, wenn keine Polymerisation stattfindet; die H-Ionen sind nicht das Ergebnis der Polymerisation.

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