Depolymerization of F-Actin by Concentrated Solutions of Salts and Denaturing Agents[1]

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The effectiveness of a series of concentrated solutions of salts and organic compounds in causing the depolymerization of F-actin has been determined by viscosity measurements. In the presence of intermediate concentrations of effective compounds a quasi-equilibrium state of partial depolymerization is reached in less than 5 min., and shows little further change for several hours. With most compounds, depolymerization is reversible upon dialysis. The extent of partial depolymerization in the presence of urea and potassium iodide is independent of pH between pH 6.8 and 8.2. Depolymerization by potassium iodide and by urea is partially reversed by sodium sulfate. The results cannot be explained by effects of the solvent on any one of the following: covalent bonds, hydrophobic bonds, binding of anions to cationic sites, or effects on the structure of water. Compounds which are effective in decreasing the activity coefficient of the model peptide, acetyltetraglycine ethyl ester, are also effective in depolymerizing F-actin, and compounds which increase the activity coefficient of this peptide are generally ineffective in depolymerizing F-actin. Although other effects may also be important, the results suggest that the depolymerization of F-actin by the solvents examined may be explained, in large part, by the effect of the solvent on the activity coefficients of peptide and amide groups which become exposed to the solvent upon depolymerization.

It is well known that the polymer F-actin undergoes depolymerization in the presence of concentrated solutions of certain salts or urea. We have examined this reaction in detail in an attempt to gain some insight into the nature of the forces which are involved in the depolymerization of this protein. The forces which are involved in the depolymerization of a protein are not necessarily the same forces which hold the polymer together. Nevertheless, elucidation of the nature of the solvent–protein interactions which cause depolymerization is of interest in its own right and provides an indication, if not a direct demonstration, of the types of forces which may be involved in maintaining the structure of the polymer.

It was shown by Straub that, although both 0.1 *M* NaCl and NaI cause the polymerization of G-actin to F-actin, 0.5 *M* NaI depolymerizes F-actin while 0.5 *M* NaCl has no depolymerizing effect.[2] Guba showed that KI causes a complete depolymerization of F-actin almost instantaneously, while KBr and KCl cause a time-dependent and incomplete decrease in the viscosity of F-actin at 0°.[3] Depolymerization by SCN^- or alkali was studied by Bárány, *et al.*,[4] and by Kasai, *et al.*[5] Szent-Györgyi and Joseph showed that depolymerization brought about by KI and by urea is reversible under the proper experimental conditions and that a given state of depolymerization by these agents could be attained from both directions, which suggests that the polymerization–depolymerization process has some of the characteristics of an equilibrium reaction.[6] Holtzer, *et al.*, showed by ultracentrifugation that actomyosin is dissociated to myosin and G-actin by the following sodium or potassium salts at the indicated concentrations, in solutions which were maintained at an ionic strength of 0.6 with KCl or NaCl: *p*-toluenesulfonate, 0.2; SCN^-, 0.2; I^-, 0.3; ClO_4^-, 0.3; Br^-, 0.6; NO_3^-, 0.65; and Cl^-, >2.0 *M*.[7]

The depolymerization and dissociation of a number of other proteins is brought about by salts and by denaturing agents of the urea–guanidinium class.[8] The order of effectiveness of anions in depolymerizing glutamic dehydrogenase, for example, is $SCN^- > I^- > ClO_4^- > Cl^-$[9] and the order of effectiveness in the dissociation of an inhibitor of a bacterial diphosphopyridine nucleotidase is LiBr > guanidinium chloride > NaI > $NaClO_4 \sim LiNO_3$ > NaBr > LiCl > $(CH_3)_3HNCl$ > CsCl > $NaNO_3$ > NaCl > KBr > NH_4Cl > KCl.[10] These orders are similar to those for the depolymerization of F-actin, and it appears probable that the mechanisms of protein–solvent interaction which cause actin depolymerization also contribute to the dissociation of these and other proteins.

Experimental

Crude actin was prepared according to Bárány and Bárány,[11] with the modification that the muscle pulp was extracted with three volumes of *n*-butyl alcohol before acetone extraction. The actin was extracted from the acetone powder and further purified by the procedure for "cold-extracted actin" described previously.[12] The actin was polymerized and stored in a solution containing 0.1 *M* KCl, 10^{-3} *M* MgCl, 10^{-3} *M* tris(hydroxymethyl)aminomethane buffer (Tris), pH 7.6, and 2×10^{-4} *M* sodium ATP. Actin was

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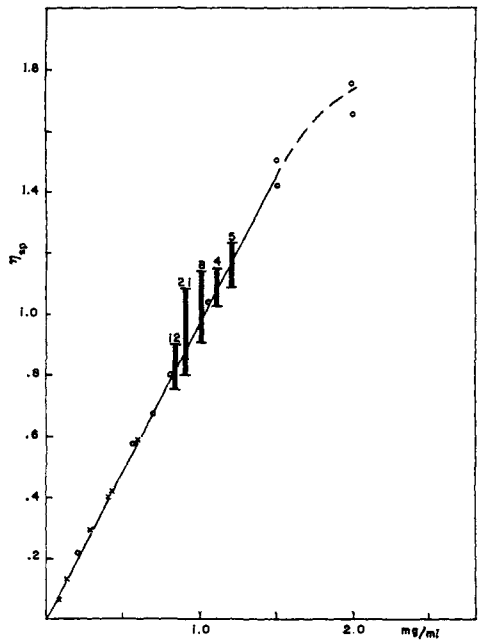

Figure 1. Dependence of the specific viscosity of F-actin solutions on protein concentration: O, this work; ×, data of Maruyama and Gergely.[16] The vertical bars show the range of viscosities of the F-actin solutions which were used for the depolymerization studies. The numbers above the bars refer to numbers of different preparations at that concentration.

polymerized and stored under nitrogen after deaeration under vacuum. Stock solutions of F-actin had a protein concentration of approximately 4 mg./ml. For viscosity measurements the protein concentration was adjusted to approximately 1 mg./ml.

Chemicals were of the highest grade commercially available. Compounds were recrystallized or redistilled, if necessary, until their physical properties agreed with those in the literature. ATP was obtained from the Sigma Chemical Co.

Protein was determined by the biuret method,[11,13] which was calibrated by Kjeldahl nitrogen determination, based upon 16.1% nitrogen content.[14] Inorganic phosphate was determined by the method of Rockstein and Herron.[15] Optical rotation was measured in a Rudolph Model 80 photoelectric spectropolarimeter with an oscillating polarizer prism. The light source was a General Electric A-H6 water-cooled high-pressure arc. All measurements were made in the wave length range of 310–600 mμ in a 10-cm. polarimeter tube with quartz end plates.

Viscosity measurements were carried out with Ostwald viscosimeters with an outflow time for water of about 30 sec. and a total volume of 2 ml. in a water bath at 25 ± 0.02°. Under the experimental conditions employed, the observed specific viscosity was found to increase linearly with increasing F-actin concentration in the range 0–1.5 mg./ml. (Figure 1), in agreement with the results of Maruyama and Gergely.[16] The viscosity measurements shown in Figure 1

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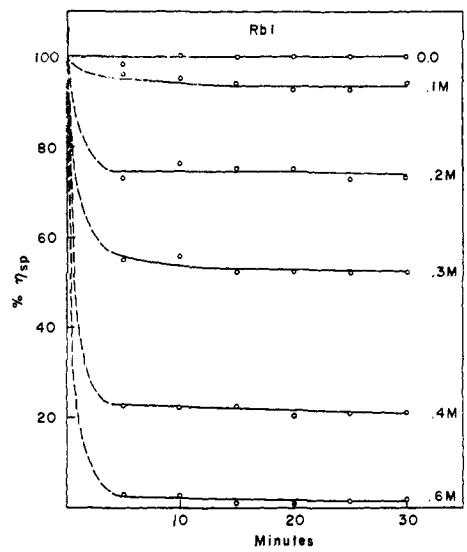

Figure 2. Effect of RbI on the viscosity of F-actin solutions, 1.1 mg./ml., in 0.1 *M* KCl, 10^{-3} *M* $MgCl_2$, 10^{-3} *M* Tris buffer, pH 7.6, and 2×10^{-4} *M* ATP, at 25°. The concentrations of RbI are given to the right of the curves.

were made after 1 hr. and were found to remain constant for 20 hr. Depolymerization measurements were made with actin concentrations between 0.8 and 1.2 mg./ml. in the linear portion of this curve. The vertical bars and the numbers in Figure 1 show the observed range of specific viscosity determinations for the F-actin solutions which were used in the depolymerization experiments. Although the specific viscosity increases linearly with F-actin concentration, it should be kept in mind that this does not necessarily mean that the specific viscosity is a direct measure of the number of G-actin units in the polymeric state.

In the depolymerization experiments the observed viscosities of actin solutions were compared to those of the solvent, measured under the same conditions, according to the relationship

$$\eta_{rel} = \frac{\eta_{solution}}{\eta_{solvent}} \quad (1)$$

The results are presented as the specific viscosity of actin (η_{sp}) where

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

The densities of solutions containing 1 mg./ml. of actin are not significantly different from those of the corresponding solvent. It was shown that the viscosities of 1 *M* solutions of Na_2SO_4, $(NH_4)_2SO_4$, KF, $NaClO_4$, and urethan are not affected by the addition of 1 mg./ml. of bovine serum albumin to a greater extent than was accounted for by the viscosity of the protein. The viscosity changes observed upon depolymerization were large compared to the changes in viscosity of the solvent over the concentration range in which depolymerization occurred, so that the concentration at which half-depolymerization occurs is not sensitive to the choice of viscosity scale.

Results

The Effect of Depolymerizing Agents on F-Actin. The viscosity of F-actin in a series of RbI solutions is plotted as a function of the time after mixing actin and salt in Figure 2. The viscosities drop to a constant value

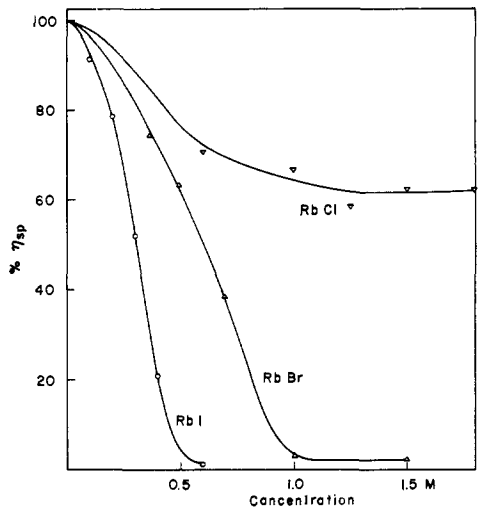

Figure 3. Effect of rubidium halides on the viscosity of F-actin solutions. Conditions as in Figure 2.

characteristic of a particular salt concentration in less than 5 min. and remain constant thereafter. At high salt concentration the depolymerization is complete and the viscosity drops to the viscosity of G-actin. The steady viscosities at lower salt concentration are indicative of the existence of a quasi-equilibrium, in which partial depolymerization has occurred, but in which all of the individual G-actin units are not rapidly and reversibly undergoing polymerization and depolymerization.[17] Depolymerization was shown to be reversible by dialysis at 4° for 12 hr. against two changes of 100 volumes of the polymerizing salt solution. After a small correction for dilution upon dialysis, reversibility was shown to be more than 80% for all of the depolymerizing solvents examined, unless noted otherwise.

In Figure 3 are shown the viscosities of F-actin in RbCl, RbBr, and RbI solutions, after quasi-equilibrium had been attained, as a function of salt concentration. For RbI and RbBr there is a sigmoid relationship between the viscosity and the concentration of added salt, and the viscosity at high salt concentrations approaches that of G-actin, indicating complete depolymerization. On the other hand, RbCl causes a smaller drop in viscosity, which levels off with increasing salt concentration and does not approach that of G-actin. Such a viscosity change does not represent true depolymerization, but results from a type of aggregation. A similar phenomenon is found with other alkali chlorides and with certain other compounds, as noted below. That F-actin exists in an aggregated state under these conditions is shown by the fact that the protein can be sedimented at 20,000*g* in 15 min., conditions which do not result in the sedimentation of normal F-actin. Furthermore, under these conditions there is no release of inorganic phosphate from the splitting of ATP, which is observed upon partial depolymerization of F-actin by effective depolymerizing agents. At higher concentrations, many compounds which give this type of behavior cause cloudiness, sometimes accompanied by an increase in viscosity, and eventually precipitation of the protein.

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Table I. Depolymerization of F-Actin by Simple Halides. Concentrations of Salt Required to Cause a 50% Decrease in η_{sp} at 25° and pH 7.6

Cation	Anion, *M*		
	Cl^-	Br^-	I^-
Li^+	1.00	0.25	0.25
Na^+	>2.7[a]	0.75	0.25
K^+	>3.20[a]	1.10	0.44
Rb^+	>3.00[a]	0.65	0.30
Cs^+	>3.90[a]	0.90	0.30
NH_4^+	1.10	0.45	0.12
$(CH_3)_4N^+$	2.30	0.75	0.30

[a] No depolymerization occurs up to the molarities listed.

The aggregation or precipitation of F-actin caused by inorganic halides was shown to be completely reversible upon dialysis.

The relative effectiveness of different compounds in depolymerizing F-actin was compared by determining the concentration of each compound which is required to cause a 50% decrease in the viscosity of F-actin solutions at quasi-equilibrium under the standard experimental conditions. Unless specifically noted otherwise, all compounds tested were shown to cause complete depolymerization to G-actin at high concentration. The results with a series of simple halides are summarized in Table I. For the anions, the order of depolymerizing activity is $I^- > Br^- > Cl^-$. The chlorides of Na^+, K^+, Rb^+, and Cs^+ cause aggregation rather than depolymerization. In marked contrast to the anions, variation in the nature of the cation generally has little or no effect on depolymerizing effectiveness. Salts of Na^+, K^+, Rb^+, Cs^+, and $(CH_3)_4N^+$ are remarkably similar in effectiveness. Salts of Li^+ and NH_4^+ are, however, more effective than those of the other monovalent cations.

It was shown previously that the optical rotatory dispersion of an actin preparation, which was contaminated with tropomyosin, is the same at low salt concentration and in the presence of 0.6 *M* KI.[12] Optical rotation measurements on the purified actin preparations used in the present study, after depolymerization in 0.8 *M* NaI, 1.5 *M* CsBr, and 1.5 *M* RbBr, gave b_0 values of −181, −178 and −182°, respectively, which are identical with those of purified G-actin at low ionic strength.[12] The values of $[\alpha]_D$ were found to be in the range of −44 to −50° for actin in the presence of NaI, NaBr, KI, LiI, LiBr, NH_4I, and $(CH_3)_4NI$ at concentrations which give complete depolymerization, according to the viscosity measurements. These results indicate that there is no detectable difference, as measured by optical rotation, in the conformation of actin at low ionic strength and after depolymerization by these salts at high ionic strength.

Under conditions in which actin is partially depolymerized, it catalyzes a slow hydrolysis of ATP, probably because of a reversible polymerization and depolymerization of G-actin molecules at the end of the F-actin chains.[17-21] Such a slow liberation of inorganic phos-

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phate from ATP was observed with actin in the presence of KI, NH_4Cl, NH_4Br, NH_4I, NH_4NO_3, urea, and imidazole at concentrations which cause a 50% decrease of specific viscosity. The rate of inorganic phosphate release was found to be 0.06 to 0.1 mole of phosphate per 60,000 g. of actin per hour, in good agreement with the previously reported values of 0.08 to 0.1 mole of phosphate per 60,000 g. actin per hour at low ionic strength in the presence of a limiting concentration of magnesium ion.[19,20] No inorganic phosphate was released by F-actin or by completely depolymerized actin at high concentrations of KI, NH_4Br, NH_4I, LiI, and urea.

The depolymerizing effectiveness of a number of other salts is compared in Table II. Salts of thiocyanate, perchlorate, and trichloroacetate are highly effective. Nitrate salts are effective and are unusual in that they exhibit a greater sensitivity to the nature of the cation than do other salts. Sodium salts of ATP, sulfite, sulfate, and phosphate do not cause depolymerization up to the highest concentrations examined. These salts do cause a decrease in F-actin viscosity without depolymerization, similar to that caused by RbCl, and probably would cause precipitation at higher concentrations. Such precipitation was observed with Na citrate, $(NH_4)_2SO_4$, KF, and KCH_3COO at the indicated concentrations. Monomethylammonium chloride causes depolymerization of F-actin at a slightly lower concentration than ammonium chloride, but with increasing methyl substitution the depolymerizing effectiveness decreases progressively in the series dimethyl-, trimethyl-, and tetramethylammonium chloride. On the other hand, cyclohexylammonium chloride is more effective than methylammonium chloride and tetraethylammonium bromide is more effective than tetramethylammonium bromide.

Table II. Depolymerization of F-Actin. Concentration of Salt Required to Cause a 50% Decrease in η_{sp} at 25° and pH 7.6

Salt	*M*	Salt	*M*
KSCN	0.26	KCH_3COO	~2.00[a,b]
$NaClO_4$	0.35	$(NH_4)_2SO_4$	~1.5[a,b]
$NaCCl_3COO$	0.45	KF	~1.50[a,b]
NH_4NO_3	0.40	CH_3NH_3Cl	0.80
$LiNO_3$	0.40	$(CH_3)_2NH_2Cl$	1.20
$NaNO_3$	1.80	$(CH_3)_3NHCl$	1.40
KNO_3	3.60	$(CH_3)_4NCl$	2.30
		$C_6H_{11}NH_3Cl$	0.40[c]
NaATP	0.25[a]	$(C_2H_5)_4NBr$	0.32
Na_2SO_3	0.60[a]	$(CH_3)_4NBr$	0.75
Na_2SO_4	0.93[a]	$C_6H_5CH_2NH_3Cl$	~0.2[b,c]
Na_2HPO_4	1.4[a]		
$Na_3C_6H_5O_7$	~1.3[a,b]		

[a] No depolymerization occurs up to the molarities listed. [b] Precipitation. [c] Depolymerization could not be reversed completely.

The effects of a number of organic compounds, many of which are denaturing agents for proteins, are compared in Table III. Formamide is an effective depolymerizing agent, but effectiveness is lost if the hydrogen atoms on the amide nitrogen are substituted, as in N-ethyl- and N,N-dimethylformamide, or if an alkyl group is added to the acyl moiety, as in acetamide and propionamide. Urea is effective, although less so than formamide. Effectiveness is maintained upon mono- or dialkyl substitution of urea, but is lost in tetramethylurea. Tetramethylguanidine and triethylenediamine hydrochloride are effective in a concentration range similar to that for other substituted ammonium chlorides. Guanidine hydrochloride and urethan cause precipitation. Pyridine and imidazole cause depolymerization.

Table III. Depolymerization of F-Actin. Concentrations of Organic Compounds Required to Cause a 50% Decrease in η_{sp} at 25° and pH 7.6

Compd.	*M*	Compd.	*M*
Formamide	1.3	Guanidinium Cl	0.28[a,b]
N-Ethylformamide	>3.0[a]	1,1,3,3-Tetramethylguanidinium Cl	0.75
N,N-Dimethylformamide	>3.0[a]	$(Guanidinium)_2SO_4$	0.5[c]
Acetamide	>2.5[a]	Triethylenediamine HCl	1.2
N-Ethylacetamide	>3.0[a]	Pyridine	0.7
N,N-Dimethylacetamide	>3.0[a]	Imidazole	1.14
Propionamide	>3.0[a]	Succinimide	>1.86[a]
Urea	3.4	Methanol	>4.0[a]
Methylurea	3.2	Ethanol	>4.0[a]
1,3-Dimethylurea	2.3	Propanol	>2.0[a,e]
Ethylurea	2.3	Acetone	>3.0[a,e]
Tetramethylurea	>3.0[a,d]	Phenol	>0.2[a,d]
Urethan	>2.0[a,d]		

[a] No depolymerization occurs up to the molarities listed. [b] Precipitation and denaturation at higher than 0.3 *M* concentration. [c] Irreversible depolymerization. [d] Precipitation and denaturation. [e] Precipitation.

Simple organic solvents, such as short-chain alcohols and acetone, are ineffective in causing actin depolymerization. Methanol and ethanol are ineffective up to 4 *M*, while propanol and acetone cause precipitation at 2 and 3 *M*, respectively. Phenol causes irreversible precipitation at 0.2 *M*. It has previously been reported by Kasai, *et al.*, that the birefringence of flow of F-actin is unaffected by acetone at concentrations up to 30%.[5] These authors also reported that at low salt concentrations organic solvents increase the flow birefringence of actin; however, salt-free organic solvents do not induce the G–F transformation of actin. Similar results were obtained by Mihashi.[22] However, Mihashi observed a decrease in the viscosity and flow birefringence of F-actin in 30% ethanol. We have confirmed the finding of these workers that organic solvents will cause an increase in the viscosity of actin at low salt concentration in the presence of magnesium ion (Table IV). However, it is difficult to ascribe this effect with certainty to an increase in the degree of polymerization, because small changes in viscosity may not accurately reflect changes in the degree of polymerization in these solvents and, in fact, methanol, ethanol, and acetone were shown to cause an increase in the viscosity of completely polymerized F-actin.

The reversibility of F-actin depolymerization by urea is concentration and time dependent. Even in the presence of excess free ATP there is an appreciable rate of irreversible denaturation at 25° in the presence of 5 or 6 *M* urea. These concentrations of urea are sufficient to cause complete depolymerization. The change in optical rotation at 550 mμ with time as denaturation proceeds is shown in Figure 4. Imida-

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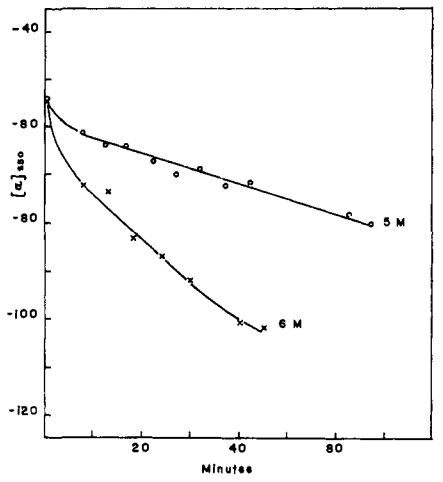

Figure 4. Specific rotation at 550 mμ of F-actin, 1.6 mg./ml., in urea solutions. Conditions as in Figure 2.

zole at a concentration of 1.5 *M* also causes denaturation at about the same rate as 5 *M* urea; a slow denaturation is also caused by pyridine. On the other hand, depolymerization by ethylurea and 1,3-dimethylurea occurs without a large change in conformation, compared to that of native G-actin. There is only a small change in optical rotation, from −55 to −60°, which is stable for at least 40 min., in the presence of these compounds. As is also the case with KI,[6,12] depolymerization by these compounds is not caused by denaturation of the protein. Actin depolymerization by substituted ureas is reversible.

Table IV. Effect of Organic Compounds on the Viscosity of Partially Polymerized Actin at 25° and pH 7.6[a]

Compd., *M*	η_{sp},[b] %
F-Actin[c]	(100)
G-Actin	0
G-Actin + Mg	56
G-Actin + Mg + methanol, 1	70
G-Actin + Mg + methanol, 4	77
G-Actin + Mg + ethanol, 1	66
G-Actin + Mg + ethanol, 4	85
G-Actin + Mg + propanol, 1	59
G-Actin + Mg + propanol, 4	Precipitate
G-Actin + Mg + acetone, 1	56
G-Actin + Mg + acetone, 4	99

[a] Protein concentration was 1.1 mg./ml. of actin in 10^{-3} *M* Tris buffer, pH 7.6, and 2×10^{-4} *M* ATP; $MgCl_2$ 10^{-3} *M* when added. [b] Compared to F-actin, taken as 100%. [c] In 0.1 *M* KCl and 10^{-3} *M* $MgCl_2$.

The effect of guanidine hydrochloride on F-actin is complex. At a concentration of 0.1 *M* it increases the viscosity several-fold to a value which does not change for several hours. This effect is completely reversible by dialysis. At higher concentration, above 0.2 *M*, the viscosity falls and the actin precipitates with irreversible denaturation. At concentrations above 1 *M*, guanidine hydrochloride dissolves the denatured actin.

The quasi-equilibrium state of partially depolymerized actin shows little further change between 30 min. and 20 hr. in the presence of compounds which do not cause irreversible denaturation (Table V). The small decrease in the concentration of reagent required to give a 50% decrease in viscosity in the longer time period is probably caused by a small amount of denaturation and, possibly, by a limited amount of further slow depolymerization or rearrangement of the polymer actin. Depolymerization caused by the compounds listed in Table V was shown to be between 70 and 100% reversible. It is noteworthy that compounds which are ineffective in causing depolymerization in the short time period, such as substituted amides, potassium chloride, and alcohols, are still ineffective after 20 hr.

Table V. Time Dependence of the Depolymerization of F-Actin. Concentrations of Compounds Required to Cause a 50% Decrease in η_{sp} at 25° and pH 7.6

	M	
	30 min.	20 hr.
KI	0.44	0.28
KBr	1.1	0.90
KCl	>3.2	>3.0
KF	1.5[a]	1.2[a]
CH_3NH_3Cl	0.8	0.6
$(CH_3)_2NH_2Cl$	1.2	1.0
$(CH_3)_3NHCl$	1.4	1.2
$(CH_3)_4NCl$	2.3	1.5
$(CH_3)_4NBr$	0.75	0.4
1,1,3,3-Tetramethylguanidinium chloride	0.75	0.6
Ethylurea	2.3	1.1
Formamide	1.3	1.3
N-Ethylformamide	>3.0	>3.0
N,N-Dimethylformamide	>3.0	>3.0
Acetamide	>3.0	>3.0
Propionamide	>3.0	~2.8
Methanol	>4.0	>4.0
Ethanol	>4.0	>3.0
Propanol	>2.0[a]	>2.0[a]
Acetone	>3.0[a]	>2.0[a]

[a] Precipitation.

The depolymerization of F-actin by KI, LiI, or urea may be prevented or significantly reversed by sulfate or fluoride ions. The depolymerizing effect of urea is largely prevented or reversed if Na_2SO_4 is added, to a concentration of 0.6 *M*, either at the same time or after the addition of urea (Figure 5). Similar results are obtained with KF (Table VI). A smaller effect of questionable significance is observed with Na_2HPO_4 and sodium citrate. Potassium acetate has no effect. The depolymerizing effect of urea and of LiI is also reversed by Na_2SO_4.

The depolymerization of actin induced by urea and KI may also be partially reversed by 4 to 10×10^{-3} *M* ATP.[23] However, it is probable that this is a specific effect of ATP.

The degree of polymerization of partially depolymerized actin in urea solution is not affected significantly by the addition of ethanol. In the presence of 3.5 *M* urea the η_{sp} of F-actin was found to be reduced to 46, 52, and 47% of the values for F-actin in water after the addition of 0, 1, and 2 *M* ethanol, respectively; in 4 *M* urea the corresponding values were found to be 35, 37, and 28% after the addition of 0, 1, and 2 *M* ethanol.

(23) (a) M. Bárány, J. Spiró, G. Köteles, and E. Nagy, *Acta Physiol. Acad. Sci. Hung.*, 10, 159 (1956); (b) A. G. Szent-Györgyi, Proceedings of a Conference on Muscle as a Tissue, K. Rodahl and S. M. Horvath, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 68.

Table VI. Reversal of the Depolymerizing Effect of KI, LiI, and Urea on F-Actin at 25° and pH 7.6

Compd. added to F-actin, M	η_{sp},[a] %
KI, 0.5	27
KI + Na_2SO_4, 0.5 + 0.5	69
KI + KF, 0.5 + 1	50
KI + KF, 0.5 + 2	85
KI + Na_2HPO_4, 0.5 + 0.25	37
KI + Na_2HPO_4, 0.5 + 0.5	41
KI + $Na_3C_6H_5O_7$, 0.5 + 0.25	37
KI + $Na_3C_6H_5O_7$, 0.5 + 0.5	39
KI + $KC_2H_3O_2$, 0.5 + 2	27
LiI, 0.4	13
LiI + Na_2SO_4, 0.4 + 0.5	40
Urea, 4	26
Urea + Na_2SO_4, 4 + 0.6	71

[a] F-Actin specific viscosity, 100.

On the other hand, the depolymerizing effects of urea and KI are additive. The η_{sp} of a solution of F-actin was reduced to 50% in 3.3 M urea and to 85% in 0.33 M KI. In 3.3 M urea containing 0.33 M KI the actin was completely depolymerized.

Although it is well known that actin is depolymerized at strongly alkaline pH, the degree of polymerization is not sensitive to pH over a limited range of variation. The specific viscosity of actin in 3.5 M urea was reduced to 46 and 48% at pH 6.8 and 8.4, respectively; in 0.45 M KI the η_{sp} of actin was reduced to 42% at pH 6.8 and 43% at pH 8.2.

Discussion

A schematic representation of the different physical states of actin is shown in Figure 4 of ref. 24. The different states are characterized by a progressive increase in the exposure of the protein to the solvent, as one goes from precipitated F-actin to dissolved F-actin to monomeric G-actin and to unfolded, denatured actin. If the solvent is changed so as to make an energetically more favorable interaction with that part of the protein which becomes exposed to the solvent in a particular step, that step will be favored. In other words, each step may be regarded as the dissolving of a portion of the protein which was not previously in contact with the solvent, and a better solvent will facilitate this process.

The precipitation and aggregation of F-actin by sulfate, citrate, acetate, and fluoride may be ascribed to a less favorable interaction with the solvent of portions of the soluble protein in the presence of these known protein precipitants. The results of activity coefficient measurements on model compounds in the presence of these salts suggest that this may be accounted for by effects of the salts on peptide and amide groups and, possibly, on hydrophobic groups which are no longer exposed to solvent in the precipitated or aggregated state.[25] The fact that aggregation or precipitation caused by compounds which increase the activity coefficient of amide groups (*e.g.*, 2.0 M potassium acetate) is prevented by compounds which decrease the activity coefficients of such groups (*e.g.*, 0.5 M KI) is consistent with this view. Precipitation

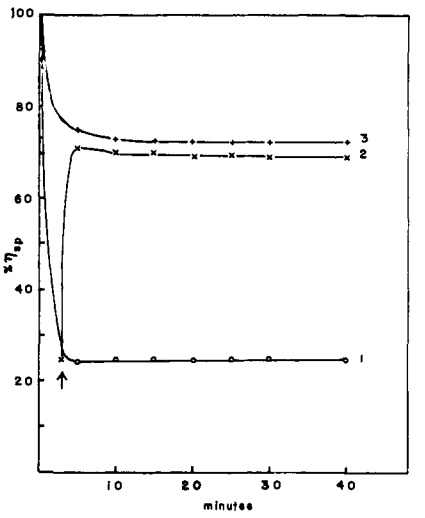

Figure 5. Reversal by Na_2SO_4 of the urea-induced depolymerization of F-actin: (1) in 4 M urea; (2) in 4 M urea; after 3 min. Na_2SO_4 was added to obtain a concentration of 0.6 M; (3) in 4 M urea containing 0.6 M Na_2SO_4.

may also be brought about by a decrease in the net charge of the protein, and it is probable that precipitation by magnesium[4] and guanidinium (Table III) ions is due to a decrease in the negative charge of actin caused by binding of these cations. An excess of either magnesium or guanidinium ions causes a redissolving of the protein as it acquires a net positive charge.

Depolymerization of F-actin involves exposure to the solvent of those parts of the actin molecule which were in contact with other actin molecules in the polymer and will be favored by changes in the solvent which decrease the activity coefficients of these groups. As indicated above, depolymerization is not a true equilibrium process under most experimental conditions, because the polymerization step may be coupled to the splitting of ATP and all of the monomeric G-actin units are not involved in a rapid and reversible conversion to F-actin. Asakura, *et al.*, have described the polymerization process in terms of a phase change.[26] In order to compare the effects of different solvents, we have considered the polymerization–depolymerization process as a "quasi-equilibrium" and have compared the concentrations of different solutes required to cause a 50% decrease in the specific viscosity of F-actin under standard experimental conditions.

At high concentrations of urea, actin becomes denatured, with a further exposure of the peptide chain to the solvent. Optical rotation measurements suggest that this denaturation occurs subsequent to a rapid depolymerization (Figure 4). Although it is possible that a partial, reversible denaturation takes place first, the denaturation of actin by urea is irreversible by the time that major conformational changes have taken place. In solutions of potassium iodide[2] and other salts, complete depolymerization can occur without any detectable change in the conformation of actin, compared to G-actin at low ionic strength, as measured by optical rotatory dispersion.

The following types of interaction between protein and solvent may be considered in attempting to explain

(24) D. R. Robinson and W. P. Jencks, *J. Am. Chem. Soc.*, 87, 2462 (1965).
(25) D. R. Robinson and W. P. Jencks, *ibid.*, 87, 2470 (1965).
(26) S. Asakura, M. Kasai, and F. Oosawa, *J. Polymer Sci.*, 44, 35 (1960).

the depolymerization of actin by the solvents examined here. Although a consideration of such interactions does not provide direct evidence for the nature of the forces maintaining F-actin in the polymer state, it may provide an indication of the type of forces which are available on the actin molecule and which may contribute to the stability of the polymer.

Covalent bonds of appreciable stability do not appear to be directly involved in the depolymerization process, because the salts and organic compounds which are effective in causing depolymerization do not cleave stable covalent bonds.

Binding of ions to charged groups has frequently been invoked to explain the polymerization and depolymerization of actin. This is probably the correct explanation for the polymerization of G-actin brought about by dilute salt solutions. The net negative charge of actin monomers in the absence of salt at neutral or slightly alkaline pH will be decreased by the binding of cations such as sodium, potassium, magnesium, or calcium, and this decrease in charge will facilitate polymerization. It has been shown that calcium and magnesium are bound to actin.[4,27] However, other factors may be involved, even at low ionic strength, in view of the facts that there is no detectable binding of potassium to actin between pH 7.7 and 9[28] and actin remains in the polymerized state even after the removal of bound calcium.[29]

The order of effectiveness of anions in depolymerizing F-actin is similar to the order of anion binding to proteins,[30] and it might be expected that in concentrated salt solutions binding of these anions would counteract the effect of bound cations and cause depolymerization by increasing the negative charge on the actin molecules.[31] The depolymerization of actin at alkaline pH is in accord with this view.[4] Although it is quite possible that simple electrostatic effects of this kind make a significant contribution, they cannot, by themselves, completely explain the observed effects of salts and denaturing agents on the depolymerization of actin. If the effects of concentrated salt solutions were due entirely to anion binding, then it would be expected that the addition of sulfate would promote depolymerization. In fact, sulfate and fluoride ions do not promote depolymerization and even cause a partial reversal of the depolymerizing effect of iodide and urea. Furthermore, a simple electrostatic mechanism does not explain the depolymerizing effectiveness of urea and other uncharged organic molecules. Finally, the extent of depolymerization in urea and potassium iodide is insensitive to pH between pH 6.8 and 8.2.

Hydrophobic Forces. If the actin polymer were held together by hydrophobic forces originating from the nonpolar side chains of amino acids, it would be expected that the addition of solvents which are better solvents than water for such nonpolar groups would favor depolymerization.[32] The observation that actin is depolymerized by deoxycholate is consistent with such a mechanism, but could also be explained by a gross denaturation of actin which involves exposure to the solvent of nonpolar groups in the interior of the native protein.[33] The depolymerizing activity of partially alkyl-substituted ureas and guanidinium salts and of pyridine and imidazole may reflect a contribution of solvent effects on hydrophobic groups, but the results obtained with other solvents suggest that such effects do not play a major role in actin depolymerization. Alcohols and acetone do not cause depolymerization and, in fact, cause an increase in the viscosity and double refraction of flow of partially polymerized actin solutions at low salt concentration (ref. 5 and 22 and Tables III and IV). The replacement of hydrogen atoms by alkyl groups in ammonium salts or in the amide or acyl portion of formamide, which increases the solvent power of solutions of such compounds toward nonpolar groups, decreases or eliminates the depolymerizing effect of the compound. The inactivity of tetramethylurea is difficult to reconcile with a large contribution of a hydrophobic effect. Furthermore, alcohol has no effect on the viscosity of actin solutions which have been partially depolymerized by urea.

If there were a large exposure of hydrophobic groups upon actin depolymerization, then salts which decrease the activity coefficient of hydrophobic groups should favor depolymerization and salts which increase such activity coefficients should hinder it. However, sodium perchlorate and sodium iodide, which salt *out* benzene, are effective depolymerizing agents for actin, and tetramethylammonium ion, which strongly salts *in* benzene,[34] is less effective in depolymerizing actin than unsubstituted ammonium ion.

Effects on the structure of water are not correlated in any simple manner with depolymerizing effectiveness toward F-actin. Two measures of the effect of solutes on the structure of liquid water are the viscosity, especially the viscosity B coefficient, and the unitary partial molal entropy of solution of a salt.[35] Both of these parameters are affected at least as much in the series sodium, potassium, rubidium, and cesium, as in the series chloride, bromide, and iodide,[35] yet these cations all have a very similar effect on actin depolymerization, while the anions show large differences in their depolymerizing effectiveness. Lithium iodide is a "structure-forming" salt with a positive B coefficient of 0.067, and cesium iodide is a strong "structure-breaking" salt with a negative B value of -0.125, but both salts depolymerize F-actin effectively. Similarly, lithium nitrate, with a positive B value of 0.101, has the same effectiveness as potassium iodide, which has a negative B value of -0.087. Even in the series of anions there is not a good correlation between effectiveness in actin depolymerization and effect on the

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(35) R. W. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., Inc., New York, N. Y., 1953, p. 170.

Table VII. Comparison of the Effects of Salts and Organic Compounds on the Salting Out of Acetyltetraglycine Ethyl Ester (ATGEE) and on the Depolymerization of F-Actin

Compd.	ATGEE, K_s[a]	η_{sp},[b] 50%	Compd.	ATGEE, K_s[a]	η_{sp},[b] 50%
$NaClO_4$	−0.33	0.35	KCl	+0.046	>3.00
LiI	−0.28	0.25	CsCl	+0.054	>3.90
$NaCl_3CCOO$	−0.27	0.45	$NaHSO_3$	+0.16	>0.60
NaI	−0.23	0.25	KF	+0.23	>1.50[c]
KI	−0.21	0.44	NaH_2PO_4	+0.36	>1.40
LiBr	−0.17	0.25	$(NH_4)_2SO_4$	+0.45	>1.50[c]
$C_6H_{11}NH_3Cl$	−0.13	0.40	Na_2SO_4	+0.48	>0.93
$(Et)_4NBr$	−0.11	0.32			
$NaNO_3$	−0.075	1.80			
KBr	−0.023	1.10			
NaBr	0.000	0.75			
Me_4NBr	+0.018	0.75	N,N-Dimethylformamide	+0.016	>3.00
LiCl	+0.021	1.00	Ethanol	+0.023	>4.00
NH_4Cl	+0.035	1.10	N,N-Dimethylacetamide	+0.032	>3.00
Phenol	−0.48	0.20[d]	Tetramethylurea	+0.043	>3.00[c]
Guanidinium chloride	−0.168	0.30[d]	1,1,3,3-Tetramethyl-guanidinium chloride	+0.074	0.75
Urea	−0.089	3.40			
Ethylurea	−0.075	2.30			
1,3-Dimethylurea	−0.050	2.30			
Formamide	−0.032	1.30			
Acetamide	−0.026	>2.50			

[a] Salting out constant for acetyltetraglycine ethyl ester[24]; K_s values for urea, amide, and guanidinium compounds were calculated from the data of D. R. Robinson and W. P. Jencks, *J. Biol. Chem.*, **238**, PC 1558 (1963). [b] Concentration of solute required to cause a 50% decrease in the viscosity of F-actin. [c] Precipitation. [d] Precipitation with denaturation.

viscosity of water. Both sodium iodide, which decreases the viscosity of water, and sodium trichloroacetate, which increases the viscosity of water, are effective depolymerizing agents for F-actin.

Interactions with Peptide and Amide Groups. The depolymerizing effectiveness of salts and organic compounds for F-actin is compared with the effects of the same compounds on the activity coefficient of acetyltetraglycine ethyl ester, a model for the peptide groups of proteins, in Table VII. Although a precise quantitative comparison is not possible, there is a significant correlation between the effects of these compounds on the model peptide and on the depolymerization of actin. Those salts which cause a large decrease in the activity coefficient of the model peptide, as shown by a negative salting-out constant (K_s), are highly effective depolymerizing agents for F-actin. Those salts which cause a considerable increase in the activity coefficient of the model peptide do not cause depolymerization of F-actin, and, in the case of sulfate and fluoride, may even reverse the depolymerizing effect of other salts. The salts in an intermediate group, which have a small effect on the activity coefficient of acetyltetraglycine ethyl ester, depolymerize F-actin only at high concentrations. A similar parallelism between effects on the activity coefficient of the model peptide and on the depolymerization of F-actin is observed for organic denaturing agents, with the single exception of tetramethylguanidinium ion.

It may be concluded that the depolymerization of F-actin in solutions of concentrated salts and denaturing agents could be largely accounted for if a number of peptide and amide groups on the protein are exposed to the solvent in the depolymerized, but not in the polymerized state. Effective depolymerizing agents decrease the activity coefficient of peptide and amide groups and will, therefore, favor the depolymerization process. It would be premature to speculate on the number of amide or peptide groups which would have to become exposed to account for the observed effects, but the number would not have to be large. At the concentrations which depolymerize F-actin, several of the effective depolymerizing agents decrease the free energy of acetyltetraglycine ethyl ester by approximately 150 cal./mole. The exposure of amide and peptide groups equivalent to about nine such peptides would, therefore, cause a tenfold change in the apparent equilibrium constant for actin depolymerization.

The existence of an effect of depolymerizing solvents on the activity coefficient of amide and peptide groups does not, of course, rule out the possibility of a significant contribution from other types of solvent–protein interaction as well. In particular, it is probable that, at least under some experimental conditions, electrostatic forces play an important role in determining the physical state of actin.

Dubuisson has reported that one to two protons/mole are taken up when crude F-actin is depolymerized by potassium iodide.[36] This result is difficult to interpret in the absence of information regarding the effect of potassium iodide on the activity coefficients of the ionizable groups of the protein; however, it could be accounted for by an increase in the net negative charge in the vicinity of the protein caused by anion binding to charged groups or amide groups or simply an increase in anion concentration brought about by a decrease in the anion activity coefficients in the neighborhood of amide groups.

The nature of the interaction of salts and organic compounds with amide groups has been discussed elsewhere.[24,25] It was concluded that salting out by certain concentrated salt solutions is similar to salting out of other organic molecules and could be described in terms of the effect of salts on the internal pressure of the

(36) M. Dubuisson, *Biochim. Biophys. Acta*, 5, 426 (1950).

solvent. The salting-in effect of large anions and of denaturing and depolymerizing agents of the urea–guanidinium class could be explained by a direct interaction of the salt or organic molecule with amide groups. Similar effects may be suggested for the interaction of these compounds with F-actin.

Communications to the Editor

Molecular Structure of Methyl Ethylene Phosphate

Sir:

The five-membered cyclic phosphate esters, intermediates in the hydrolysis of ribonucleic acids, have been the subjects of recent detailed kinetic studies in the form of model compounds.[1-3] One of these, methyl ethylene phosphate (MEP), undergoes alkaline hydrolysis, which splits the P–OCH_2 bond, at a rate some 2×10^6 times faster than the rate of hydrolysis of trimethyl phosphate.[3] A similar increase relative to an acyclic analog is found in the rates of hydrolysis and of ^{18}O exchange during acid-catalyzed hydrolysis of ethylene hydrogen phosphate. Furthermore, the ^{31}P n.m.r. peak shows a shift of −17 p.p.m. relative to that of 85% phosphoric acid.[4] Since most explanations of these phenomena have involved the bond angles in the strained five-membered ring and the P–OC bond lengths, low temperature single crystal X-ray diffraction methods have been employed in the present study in order to establish these angles and distances.

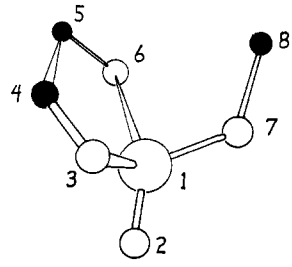

Figure 1. Molecular structure of methyl ethylene phosphate. Molecular parameters are P_1–O_2 = 1.44, P_1–O_3 = 1.57, P_1–O_6 = 1.57, P_1–O_7 = 1.57, O_3–C_4 = 1.41, C_4–C_5 = 1.52, C_5–O_6 = 1.45, O_7–C = 1.44 Å.; $O_2P_1O_3$ = 116.0°, $O_2P_1O_6$ = 117.3°, $O_2P_1O_7$ = 108.7°, $O_3P_1O_6$ = 99.1°, $O_3P_1O_7$ = 105.7°, $O_6P_1O_7$ = 109.2°, $P_1O_3C_4$ = 112.0°, $O_3C_4C_5$ = 107.8°, $C_4C_5O_6$ = 106.0°, $C_5O_6P_1$ = 112.0°, and $P_1O_7O_8$ = 118.8°. Standard deviations of distances are ±0.01 Å. for bonds to P_1, and ±0.02 Å. otherwise, while those of angles are ±0.6° about P_1, and ±0.9° otherwise. Dihedral angles are 10.9° between $O_3P_1O_6$ and $O_3C_4O_6$ plane normals, and 1.8° between $O_3P_1O_6$ and $O_3C_5O_6$ plane normals.

A total of 533 independent diffraction maxima were obtained from a single crystal grown from a melt at −5° and maintained at −40°. Six self-correlating levels were collected with the use of a Weissenberg camera and Cu Kα radiation on a crystal mounted on the [110] axis. The reciprocal lattice symmetry of C_{2h}, extinctions of *hkl* when $h + k$ is odd and *h0l* when *l* is odd, led to the possible space groups Cc and C2/c. The latter was ruled out by the fact that there are four molecules of low symmetry in a unit cell, whose parameters are a = 11.29, b = 5.96, c = 9.09 Å., and β = 113°, yielding an X-ray density of 1.47 g. cm.$^{-3}$. The structure was solved from the Patterson function. Full-matrix least-squares procedures, including anisotropic temperature factors, have yielded a value (H atoms omitted) of $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.10$, which is uniformly low for all classes of reflections and rises only to R = 0.16 for the outermost 121 reflections. Bond distances, angles, and standard deviations are shown in the legend of Figure 1, while atomic parameters and errors are listed in Table I.

Table I. Final Structure Parameters[a]

Atom	*x*	*y*	*z*
P_1	0.2500[b]	0.0236 ± 0.0003	0.2500[b]
O_2	0.2441 ± 0.0010	0.1794 ± 0.0013	0.3858 ± 0.0007
O_3	0.1318 ± 0.0009	0.1190 ± 0.0011	0.1061 ± 0.0009
O_4	0.3772 ± 0.0007	0.0891 ± 0.0012	0.2288 ± 0.0011
O_5	0.2518 ± 0.0013	0.2137 ± 0.0011	0.2795 ± 0.0011
C_6	0.1293 ± 0.0012	0.3030 ± 0.0024	0.3357 ± 0.0012
C_7	0.0718 ± 0.0014	0.3062 ± 0.0017	0.1536 ± 0.0011
C_8	0.3936 ± 0.0017	0.3136 ± 0.0021	0.1892 ± 0.0014

[a] Coordinates are given in fractions of a unit cell edge. [b] The *x* and *z* coordinates of one atom are arbitrary in the space group Cc.

Molecular structural aspects of particular interest are (a) the O_3PO_6 bond angle of 99° which is 10° less than the tetrahedral angle and 5° less than that in dibenzylphosphoric acid[4]; (b) the reduction of the POC angle from 119° in the unstrained part to 112° in the five-membered ring of methyl ethylene phosphate; (c) the pucker of the five-membered ring, presumably because of H···H interactions between the CH_2 groups, in such a way that the normal of the $O_3C_4O_6$ plane is about 11° from the normal of the O_3PO_6 plane; (d) the equality of all three esterified P–O bonds apparently independent of angle strain; and (e) the good agreement of the P–OC bond lengths of 1.57 Å. with that of 1.56 Å. in dibenzylphosphoric acid,[5] which are significantly shorter than ring P–OC bonds of 1.60 and 1.76 Å. in a pentaoxyphosphorane.[6] The difference

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