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recA Filaments in Solution†

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ABSTRACT: recA protein has been shown previously by electron microscopy to form long filaments. The extent of oligomerization in solution can be followed by light scattering since the filaments scatter light far more strongly than the constituent units. Filament formation is highly sensitive to the presence of various nucleotides and ions: ATP, ADP, CTP, UTP, TTP, and GTP all disrupt the filaments at concentrations of 1 mM while AMP at the same concentration has no effect. The presence of Na^+, K^+, Ca^{2+}, and Mg^{2+} at higher concentrations also causes filament disruption but in a highly cooperative manner with the midpoints for the transitions at 17, 18, 50, and 30 mM, respectively. The disruption is extremely fast; stopped-flow measurements indicate a half-life of <1 ms on the addition of 40 mM NaCl, 1.5 ms for 1 mM ATP, and 7.5 ms for 1 mM ADP. The rate constant for formation of filaments increases with increasing protein concentration. Cross-linking experiments on the protein at pH 8.1 indicate the presence of an oligomer of at least a hexamer. This degree of association persists on the addition of nucleotides and salts at concentrations that disrupt the larger filaments.

The recA protein of *Escherichia coli* is a relatively small protein, M_r 37 800 (Horii et al., 1980; Sancar et al., 1980), which catalyzes a wide range of activities. It has a central role in the "SOS" response to cell damage (Radman, 1975; Witkin, 1976) and is also vital for recombination and post-replicative DNA repair (Clark, 1973; Radding, 1978).

The cloning of the recA gene has facilitated the production of large amounts of pure protein and has led to rapid progress in characterizing its activities in vitro (McEntee & Epstein, 1977; Sancar & Rupp, 1979; Ogawa et al., 1979). It has been shown to be a single-stranded (ss) and double-stranded (ds) DNA-dependent ATPase (Ogawa et al., 1979; Roberts et al., 1979; Weinstock et al., 1979; McEntee et al., 1979a,b; Shibata et al., 1979a,b; West et al., 1980) and to catalyze D-loop formation, branch migration, and strand exchange in the presence of a nucleotide (Cassuto et al., 1981; Cox & Lehman, 1981; DasGupta et al., 1981; West et al., 1981). It has also been shown to be a polynucleotide- and ATP-dependent protease (Roberts et al., 1979; Craig & Roberts, 1980), cleaving the repressors lex, λ, and p22 at a specific Ala–Gly bond (Pabo et al., 1979; Horii et al., 1981; Sauer et al., 1981).

Much of the recent research on the recA protein has centered on the nature of the complexes formed on the binding of the protein to DNA. Electron microscopy has revealed long chains of recA molecules forming smooth filaments with

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ssDNA (Flory & Radding, 1982; Dunn et al., 1982) and helical structures with dsDNA (DiCapua et al., 1982; Dunn et al., 1982). In some cases, bundles of such filaments have been observed (DiCapua et al., 1982). Smooth filaments of recA protein in the absence of DNA or nucleotides at pH 7.5 have been observed by electron microscopy (Flory & Radding, 1982). The filaments, of about 100 nm in length, are destroyed by the addition of ADP, ATP, or GTP. This is in contrast with earlier studies using glycerol gradient sedimentation (Ogawa et al., 1979). At pH 7.5, the protein behaved as a mixture of oligomers, while at pH 6.2 on a sucrose gradient, mainly dimers were observed (McEntee et al., 1981). In both cases, aggregation was observed on the addition of ATP and other nucleotides. Kuramitsu et al. (1981) found that on gel filtration the protein behaved as an oligomer, but the exact degree of polymerization appeared to depend on the pH of the solutions and the percentage of glycerol present. In particular, high concentrations of glycerol lowered the state of aggregation.

One possible way to compare solution and electron microscopy results is the use of light scattering. Light scattering techniques have been used to study the polymerization of a number of different proteins, e.g., tubulin (Gaskin et al., 1974), and have provided useful kinetic and equilibrium data. Polymers scatter light much more than the smaller units of which they are composed since the intensity of scattering depends on the radius of gyration to the power of 2. The size of the recA polymers as seen under the electron microscope suggested that the oligomerization of this protein might also be amenable to study by such a method.

Here we present a basic study of the oligomerization of the recA protein in the absence of DNA, under a wide range of different conditions, by using light scattering as a simple probe of the state of polymerization. We also report studies on the aggregation of the protein and the effects of polynucleotide and nucleotides by cross-linking techniques using dimethyl suberimidate.

Materials and Methods

Materials

recA protein was prepared as described by Cotterill et al. (1982). The fraction used had been purified by S-200 and then phosphocellulose column chromatography.

Nucleotides and other reagents were obtained from Sigma Chemicals Ltd., except for adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS) (Boehringer). The concentration of nucleotides was determined spectroscopically and calculated from published figures for the extinction coefficients at the relevant pH. ssDNA was prepared from highly polymerized calf thymus DNA by boiling for 30 min and then plunging the mixture into ice.

Methods

All experiments were performed at 25 °C in a solution containing 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.1), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol (buffer A) except where indicated. Otherwise, the Tris-HCl was replaced by sodium maleate (10 mM), potassium phosphate (10 mM), triethanolamine hydrochloride (10 mM), or glycine hydrochloride (10 mM). The pH was altered as specified. Cross-linking reactions were performed in 100 mM triethanolamine hydrochloride, pH 8.1, 10 mM $MgCl_2$, and 5% glycerol (buffer B). Polynucleotides and nucleotides were added as indicated.

Light scattering measurements were performed by using a cuvette of 1.5-mL capacity in a thermostated cell holder. The intensity of light scatter was measured at 90° to the incident light. Excitation and emission slits were generally set at 300 nm, but control experiments were performed at 400 and 500 nm.

All experiments were initiated by addition of enzyme from a stock solution in 50 mM Tris-HCl–50% glycerol to 0.8 mL of buffer A in the cuvette. Mixing was achieved by inverting the stoppered cuvette several times. All additions were made by using Hamilton syringes. All glassware used had been previously treated with dimethyldichlorosilane, except for the Hamilton syringes. Those used for the addition of recA were previously washed with recA solution.

Stopped-flow experiments were performed by using the apparatus described by Fersht et al. (1975). Excitation was at 400 nm by using a tungsten halide lamp, and all scattered light at 90° was collected. One syringe held 1.38 μM recA protein in buffer A and the other syringe solutions of nucleotides or salt in the same buffer.

Cross-linking experiments were performed as described by Thomas (1978) at protein concentrations of 1 and 0.1 mg/mL. The final volume of the reaction solution was either 0.3 mL (for 1 mg/mL recA) or 3 mL (for 0.1 mg/mL recA). The reaction was initiated by the addition of dimethyl suberimidate (11 or 22 mg/mL to give a final concentration of dimethyl suberimidate of 1.1 or 2.2 mg/mL) to a solution of protein and additives as specified in buffer B. Dimethyl suberimidate solutions were prepared immediately prior to use, and the pH was adjusted to 8.1 by addition of NaOH. Samples of 0.15 or 0.45 mL were taken from the solutions containing 1 and 0.1 mg/mL protein, respectively, after 0, 30, 60, 120, and 180 min. The reaction was stopped by the addition to 40% trichloroacetic acid to precipitate the protein. The samples were allowed to stand on ice for 30 min, and the precipitate was collected by centrifugation for 15 min at 12000 rpm. The pellet was washed twice with cold acetone–5 mM HCl and then suspended in gel sample buffer [6.25 mM Tris-HCl (pH 6.50), 4% sodium dodecyl sulfate ($NaDodSO_4$), 20% glycerol, 10% 2-mercaptoethanol, and 0.1% bromophenol blue]. The protein was then subjected to a $NaDodSO_4$–polyacrylamide gel by using either slab gels [5.5 × 10 cm, running buffer of 0.1 M Tris–*N*,*N*-bis(2-hydroxyethyl)glycine (Bicine) and 0.1% $NaDodSO_4$ (Laemmli & Favre, 1973)] or tube gels (10% polyacrylamide, 9 cm long, with a stacking gel of 3% polyacrylamide, running buffer of 0.025 M Tris, 0.191 M glycine, and 0.01% $NaDodSO_4$, pH 8.3). After being stained with Coomassie blue G/250, the tube gels were scanned by using a Beckman DMS spectrophotometer equipped with a gel scanner and integrator.

Results

Light scattering increases with decreasing wavelength. Accordingly, measurements were made at 300 nm in order to achieve maximum sensitivity without entering into a region where the protein or added nucleotides have significant absorption. Some of the experiments reported here have also been repeated at 400 and 500 nm, and, apart from a decrease in sensitivity, no significant difference in the results could be seen. Sensitivity was optimized by viewing at 90° to the incident light so that scatter could be measured against a dark background.

The crucial initial observation was that addition of recA protein in 50% glycerol to buffer A in a cuvette causes an exponential rise in scattered light from background to a high level (Figure 1). This was not observed on the substitution of either native bovine serum albumin (BSA) or recA protein that had been boiled for 30 min. This is consistent with the

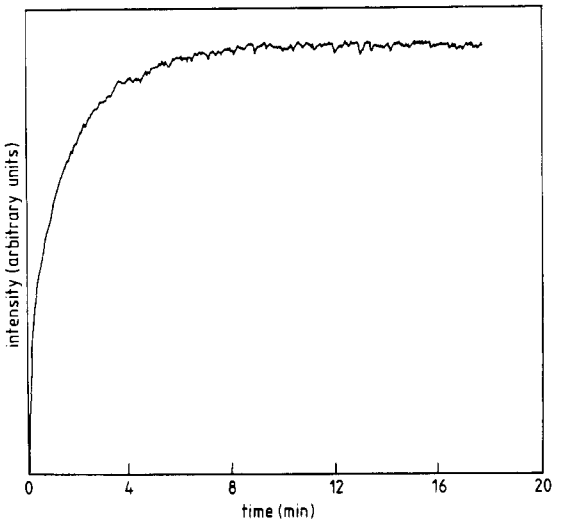

FIGURE 1: Increase in light scattering on addition of recA protein (2 μL of a 19 μM solution in 50% glycerol–50 mM Tris-HCl to 0.8 mL of buffer A).

observation of Kuramitsu et al. (1981) that recA protein is in a relatively low state of aggregation in high glycerol solution but when the glycerol is diluted out the long filaments found by Flory & Radding (1982) can form. The scatter remained constant for at least 2 h. First-order rate constants for the aggregation were measured from a semilogarithmic plot (Figure 2).

Effect of Protein Concentration. Electron microscopy indicates that the state of aggregation depends on the protein concentration. At concentrations (measured as the subunit) less than 0.25 μM, no aggregation could be seen, but as the concentration increases from 0.25 to 4 μM, the percentage of molecules greater than 100 nm in length increases from 15% to 35% (Flory & Radding, 1982).

The light-scattering measurements show that increasing the protein concentration causes increases in the rate constant for the aggregation (Figure 3). The final amplitude, however, is, within experimental error, linear with concentration. At a concentration of 0.12 μM, there was a lag of 10 min before any rise in scattering could be detected. Once the aggregation had started, however, it proceeded with good first-order kinetics although it was very slow. Between 0.25 and 1.8 μM monomer, good first-order kinetics were observed, but at concentrations greater than 1.8 μM, the aggregation showed a tendency toward a second slower phase after the usual rapid rise, and the approximation to first-order kinetics was not as good.

Table I: Stopped-Flow Measurement of Disaggregation of recA Filaments on Addition of ADP and ATP[a]

added nucleotide	final concn (mM)	k_{obsd} (s^{-1})	decrease in intensity[b] (%)
ATP	1	460	100
	0.1	92	100
ADP	1	92	100
	0.1	16	66

[a] Average of four to five different runs. Final concentration of recA = 0.7 μM. [b] Decrease in amplitude of signal relative to initial and buffer blanks.

Effect of Nucleotides on Light Scatter. ATP, GTP, and ADP (at 1 mM) were shown by electron microscopy to disrupt the long filaments of recA while AMP had no effect (Flory & Radding, 1982). The same was observed by light scattering in solution (Figure 4). Other nucleotides tested at the same concentration (UTP, CTP, and TTP) had a similar effect. The same decrease in light scattering was observed whether the aggregate had been formed for 5 min or 2 h, suggesting that no aging process occurred. The addition of ADP (1 mM) before recA completely inhibited the formation of filaments.

The rate of this decrease was too fast to be measured in the fluorometer, and so stopped-flow techniques were used to measure of the rates of disaggregation. First-order kinetics were observed (Table I). At a lower concentration of ATP (100 μM), there was a lag of 8–10 ms before the disaggregation commenced—indicating that the process is probably

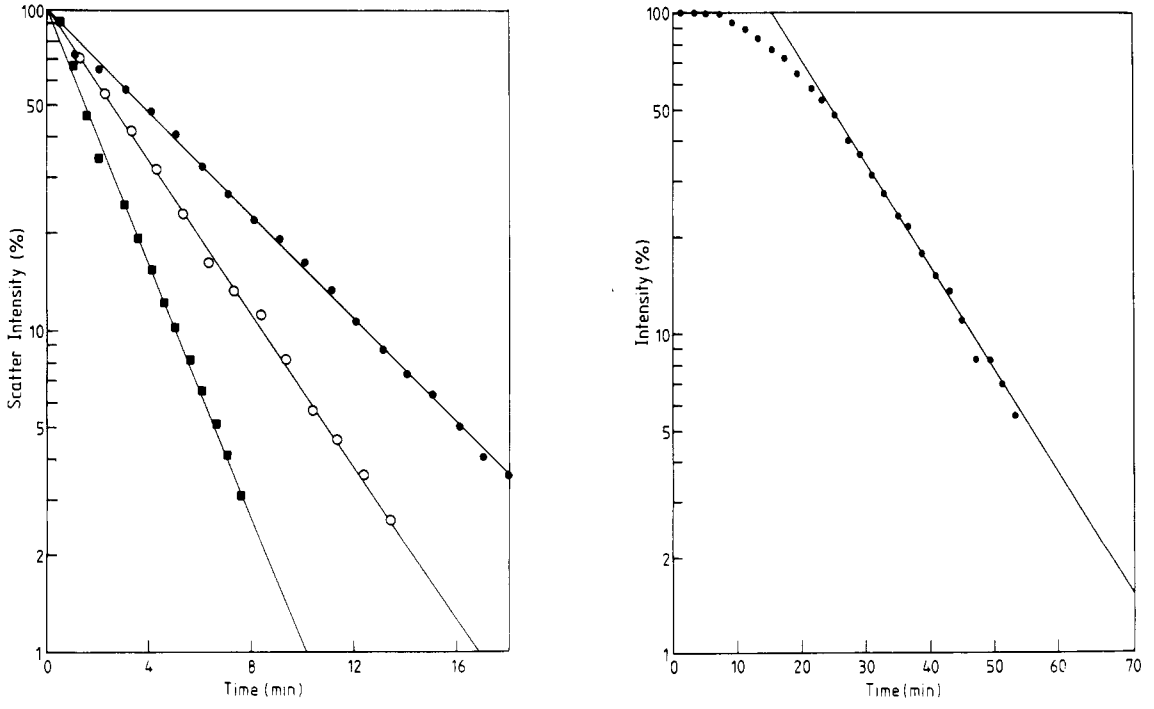

FIGURE 2: Determination of rate constants for oligomerization of recA protein at different protein concentrations in buffer A under standard conditions. (Left panel) (●) 0.24 μM; (○) 0.48 μM; (■) 0.96 μM; (right panel) 0.12 μM.

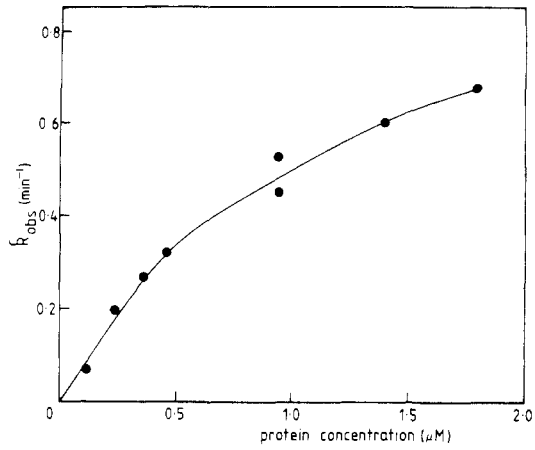

FIGURE 3: Variation of rate constant for aggregation of recA with protein concentration under standard conditions.

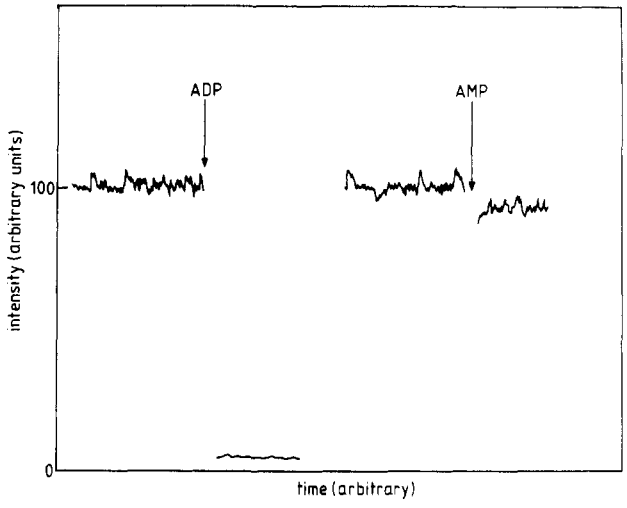

FIGURE 4: Detection of effects of addition of ADP (1 mM) and AMP (1 mM) to the preformed aggregate of recA (0.48 μM) under standard conditions.

cooperative with respect to ATP. Disaggregation was faster in the presence of ATP than ADP.

Apparent Binding Constant of ADP. The extent of disruption of the aggregate on the addition of aliquots of ADP was used to estimate the binding of ADP by using a Scatchard plot (Figure 5):

$$[L_B]/[L_F] = n[E_0] - K[L_B]$$

where L = ligand, the subscripts B and F indicate bound and free, respectively, and n is the number of binding sites per monomer of recA protein (E). $[L_B]$ and $[L_F]$ were calculated by assuming that the decrease in light scatter is proportional to the fraction of enzyme disaggregated and that only the disaggregated protein is bound to ADP. We have previously shown by equilibrium dialysis that the stoichiometry of binding (n) is 1 (Cotterill et al., 1982). This procedure afforded a linear plot of the dissociation constant (15–20 μM). This value is slightly lower than that obtained by direct measurement by equilibrium dialysis (30 μM; Cotterill et al., 1982). At higher concentrations of enzyme, however, the dissociation constant appeared to be higher, but the use of concentrations comparable to those for equilibrium dialysis was impracticable for the light-scattering measurements.

Effect of Salt on Aggregation. It is seen in Figure 6 that at low concentrations of Mg^{2+} (less than 7.5 mM) no light scattering was observed above background. The scattering also decreased as the Mg^{2+} concentration was raised above 25 mM.

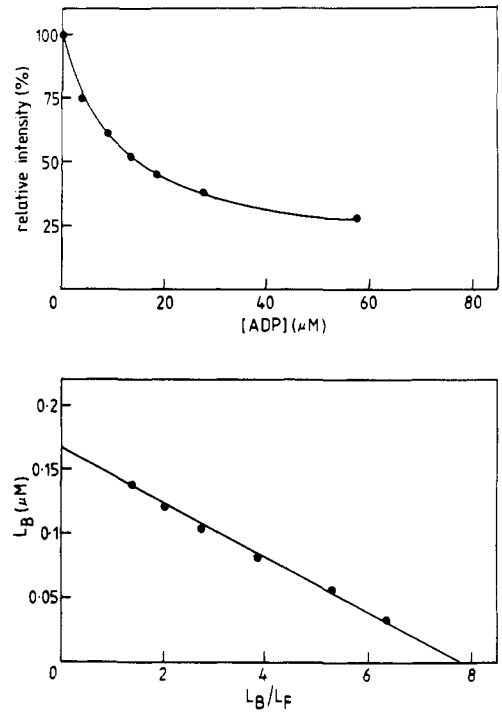

FIGURE 5: Determination of apparent binding constant for ADP and recA protein (0.48 μM). (Top panel) Decrease in amplitude of light scattering on addition of ADP. (Bottom panel) Data replotted as a Scatchard plot as described in the text.

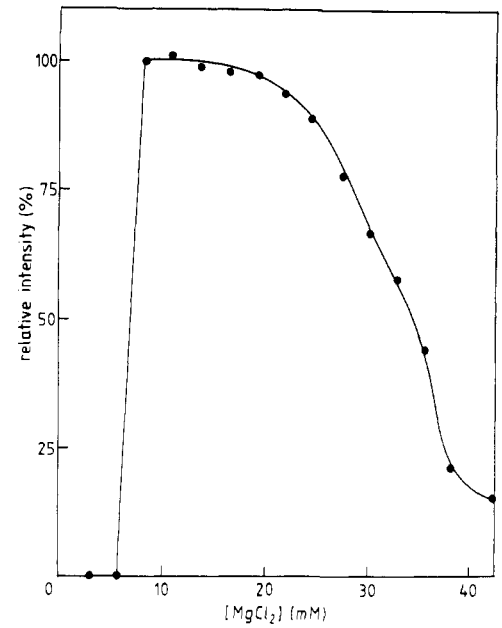

FIGURE 6: Effect of $MgCl_2$ concentration on the aggregation of recA protein (0.48 μM) under standard conditions.

Ca^{2+} (7 mM) or Mn^{2+} (3 mM) could replace the Mg^{2+} causing aggregation of recA, but Na^+ and K^+ could not. The rate constants for the aggregation with Ca^{2+} present were comparable to those with Mg^{2+}, but that with Mn^{2+} appeared slower.

Addition of salt to a preformed aggregate (in 10 mM Mg^{2+}) caused an extremely cooperative decrease in light scatter at a concentration dependent on the ion used (Figure 7). The disruption is not related to ionic strength but appears to be specific to the ion. The midpoints for the transitions were as follows: NaCl, 17 mM; KCl, 18 mM; $CaCl_2$, 50 mM; Mg^{2+}, 30 mM. The disruption by NaCl is very rapid. At 40 mM NaCl, disaggregation is complete in the dead time of the

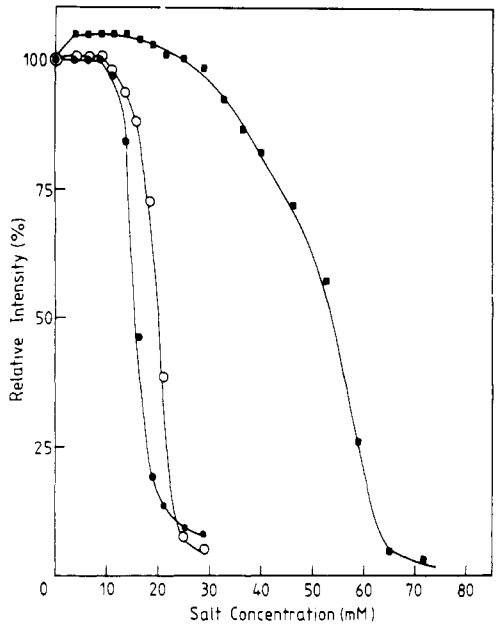

FIGURE 7: Effects of salts on the preformed aggregate of recA (0.48 μM) under standard conditions: (●) NaCl; (○) KCl; (■) $CaCl_2$.

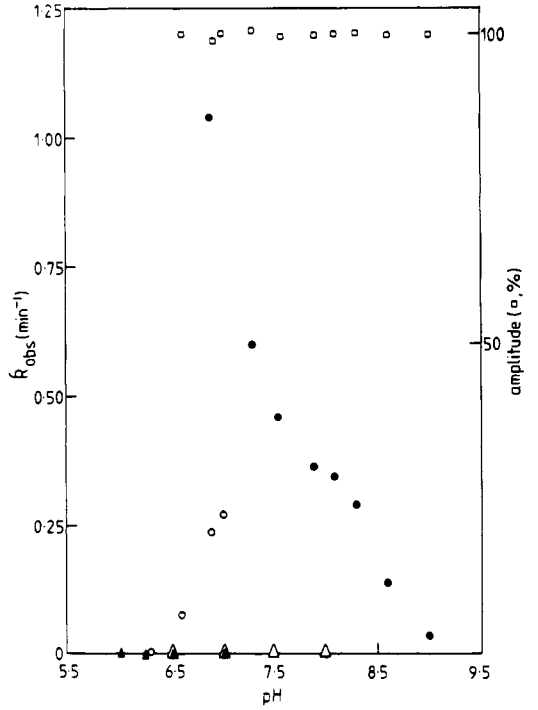

FIGURE 8: Effects of pH and buffer on the amplitude (□) and the apparent first-order rate constant for aggregation of recA (0.48 μM): (●) Tris; (○) Bis-Tris; (▲) sodium maleate; (△) phosphate.

stopped-flow spectrofluorometer (1.2 ms).

Effect of pH. Earlier reports had showed that the state of aggregation depends upon pH (Kuramitsu et al., 1981). The analysis of pH effects by light scatter was complicated by an apparent specific interaction of recA protein with the particular buffer used (Figure 8). Sodium maleate and phosphate buffers did not support any aggregation, even in pH regions where changes were observed with other buffers. The lack of aggregation in sodium maleate may be related to the high concentration of sodium ions present. Other buffers used—Tris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane (Bis-Tris), and triethanolamine—all supported aggregation to the same extent, but the observed rate constants measured at the same pH with different buffers were different.

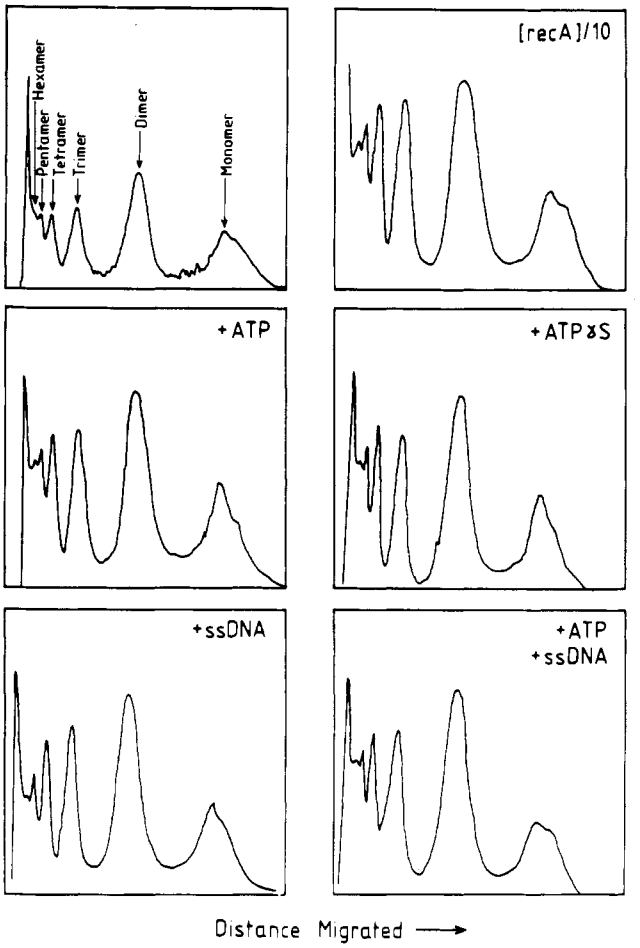

FIGURE 9: Cross-linking of protein under different conditions by dimethyl suberimidate, as described under Materials and Methods. recA concentration is 1 mg/mL; ATP (1 mM), ATPγS (100 μM), and ssDNA (ratio 10:1 ssDNA:recA) were added as indicated.

In the presence of 100 mM triethanolamine, no light scattering was observed at protein concentrations up to 20 μM, suggesting that structures of the size observed by light scatter are not present under the conditions used for cross-linking.

Cross-Linking. Triethanolamine was employed as buffer as it contains no amino groups that may interfere with the cross-linking. The high concentration was chosen to prevent pH changes as the reaction proceeded. recA protein has been shown to be equally as active under these conditions as with Tris-HCl (40 mM) buffer (unpublished results).

After 3 h (Figure 9), recA protein in the absence of DNA and nucleotides appeared to form many high molecular weight aggregates (much of the protein did not even enter the gel); bands as high as a hexamer could be distinguished.

It was not possible to detect any differences between the distribution of the bands under any of the conditions used, either by eye on the slab gels or from scans carried out on the tube gels (Figure 9). The addition of nucleotides and salts at concentrations which disrupted filaments as seen by light scattering had no effect on the cross-linking of the protein. Even under conditions of greatly reduced protein concentration (0.1 mg/mL as opposed to 1 mg/mL) all the high bands were visible, suggesting that these bands were not just due to nonspecific interactions of the proteins because of the high concentrations used.

Discussion

Light scattering provides a rapid and convenient probe of the aggregation state of recA under a variety of different conditions. It has advantages over electron microscopy and

sedimentation in that it can provide a continuous picture of the aggregation state, hence enabling transient changes to be observed, and more physiological conditions can be used. The interpretation of the light-scattering data, as used, is totally dependent on the results of electron microscopy, and so the two techniques are complementary.

The measurement of scattering at 90° is about 100 times more sensitive than measurement of the turbidity. However, with long molecules, not all of the scattered light is observed at 90° because of the destructive interference between light scattered from different parts along the same molecule—the larger the molecule and the shorter the wavelength, the greater the interference. It is possible to calculate the expected reduction [$P(\theta)$] for different length molecules at 90° and 300 nm (Tanford, 1969): under the conditions of these experiments, the deviation from unity should be within experimental error, except at the very high concentrations when there is a high proportion of molecules over 100 nm in length (first-order plots at very high concentrations do show some deviation from linearity). Further measurements at longer wavelengths of 400 and 500 nm gave very similar plots, apart from smaller amplitudes, suggesting that the reduction in light scattering due to the angle of viewing did not seriously affect the results.

The measured kinetics of recA aggregation showed similarity to those that have been obtained for tubulin polymerization by using light scattering (Johnson & Borisy, 1977). Their data fit a simple mechanism where polymers are built by addition or removal of a monomer (or small aggregate) unit at a time from either end on the polymer:

$$[M] + x \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} x \qquad (1)$$

where [M] = monomer concentration and x is the concentration of polymeric chains where x is assumed to be constant after initiation. This equation may be integrated to give an observed first-order rate constant for polymerization (k_{obsd}):

$$k_{obsd} = k_1 x + k_{-1} \qquad (2)$$

An important facet of the Johnson–Borisy model is that nucleation is slow compared with the subsequent polymerization, and so x remains constant. Consistent with this model, first-order kinetics for recA polymerization were observed during most runs. The number of chains is dependent on the protein concentration, and so as [M] is altered, x should alter. If there is a preexisting number of initiated chains in the stock solutions of recA protein in glycerol, then k_{obsd} should increase linearly with [M]. This is not observed (Figure 3), and so initiation must also be occurring during the experiments. The lag obtained at low concentrations also suggests that there is a nucleation phase following the dilution of glycerol in the recA stock solutions. This lag may also explain why at low concentrations no polymers were observed under the electron microscope since reaction mixtures for electron microscope studies were preincubated for only 15 min, by which time very little polymerization should have occurred at low protein concentrations.

Although recA polymerization as measured by light scattering fits the above conditions quite well, it is clearly an oversimplified view of the process. The deviation from the expected results at higher protein concentrations also suggests that a more complicated mechanism occurs. Unlike tubulin, it is likely that breakage in the center of chains occurs and x is not constant since the filaments are so readily disrupted.

The effects observed with ADP, ATP, and GTP correlated well with results obtained under the conditions of electron microscopy. The more rapid disruption of filaments by ATP than ADP and at several orders of magnitude faster than the hydrolysis of ATP in the steady-state implies that the disruption by ATP does not involve the prior hydrolysis to ADP.

The effects of the salts are quite dramatic, but there is no correlation with their effects on binding of recA and DNA (either ss or ds) or on the ATPase activity, suggesting that these phenomena are not dependent on the very high aggregation state as observed under electron microscopy. The ability of Ca^{2+} and Mn^{2+} to substitute for Mg^{2+} to give aggregation may be compared with the ability of these ions to enable DNA binding and also for Mn^{2+} to be able to substitute for Mg^{2+} in the ATPase activity (Weinstock et al., 1981). The depolymerization caused by NaCl may also explain why Ogawa et al. (1979) did not see large aggregates as the lowest concentration of NaCl they used was 50 mM. The effect of sodium maleate also suggests why McEntee et al. (1981) observed dimers rather than filaments at pH 6.2.

The sensitivity of the aggregation to the nature of the salt rather than ionic strength suggests specific interactions. The same could be true of the effect of buffers although it is possible that in some cases the failure to support aggregation could be caused simply by the counterion of the buffer (e.g., Na^+ in sodium maleate).

The results of the cross-linking experiments suggest that aggregates higher than tetramers are formed under all conditions examined at pH 8.1. These aggregates observed by this technique are much smaller than those observed by light scattering since, under the reaction conditions for cross-linking, significant light scatter is not observed.

It is thus seen that under certain conditions recA protein may form very long polymers. However, the disruption of these structures with nucelotides and ions casts doubt on the significance in vitro for the activity of the protein. The concentrations of Mg^{2+} (1.5 mM), K^+ (50 mM), Na^+ (165 mM) (Lawrence et al., 1973), and ADP (1.2 mM; Ugurbil et al., 1982) in vivo are far higher than those required to disaggregate the protein, unless there are factors present in the cell that stabilize the filaments. Therefore, although filaments of recA may be important under some of the conditions used for experiments in vitro, the significance of such structures in vivo is questionable.

Registry No. ATP, 56-65-5; ADP, 58-64-0; CTP, 65-47-4; UTP, 63-39-8; TTP, 365-08-2; GTP, 86-01-1; Na, 7440-23-5; K, 7440-09-7; Ca, 7440-70-2; Mg, 7439-95-4; ATPγS, 35094-46-3.

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Phosphorus-31 Nuclear Magnetic Resonance Studies of Single Muscle Cells Isolated from Barnacle Depressor Muscle†

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ABSTRACT: ^{31}P NMR spectra (145.7 MHz) were recorded from single muscle cells isolated from the depressor muscle of the barnacle *Balanus nubilis*. The single cells, mounted in a small (2.2-mm) diameter solenoidal receiver coil, were scanned by using 45° pulses with delay times ranging from 1 to 10 s; 400 scans (10–15-min total accumulation time) were sufficient to obtain spectra with good signal/noise ratios. Spectra obtained within 30 min after dissection reveal that phosphoarginine and ATP are the only phosphometabolites present in appreciable (>1 mM) concentration in these cells under resting conditions. As the postdissection time is increased, the phosphoarginine content decreases 6–8%/h. This depletion is matched by concomitant increases in the concentrations of sugar phosphate esters and inorganic phosphate; ATP is maintained at constant levels during these shifts in phosphometabolite pools. The rates of sugar phosphate and inorganic phosphate accumulation are increased by exposing the cell to iodoacetate and cyanide (cyanide alone has no effect). Quantitative analysis of both the in vivo NMR data and the in vitro chemical analysis of single-cell extracts indicates that these cells contain about 60 mM phosphoarginine and 7 mM ATP. The in vivo data suggest that sugar phosphate and inorganic phosphate contents are within the 0.1–1.0 mM range. While the ADP content of the cell extracts is approximately 1 mM, the near-equivalence of the three ATP resonance signals observed in situ suggests that the sarcoplasmic content of ADP is <0.2 mM. Furthermore, if it is assumed that arginine kinase operates near equilibrium in these cells, a sarcoplasmic [ADP] of about 0.04 mM can be calculated.

^{31}P NMR in increasingly being used as a noninvasive probe of phosphometabolite content and turnover and of intracellular ionic conditions in intact muscle; such studies have been performed by using skeletal (Dawson et al., 1977; Gadian et al., 1981; Meyer et al., 1982), cardiac (Ingwall, 1982; Wu et al., 1981), invertebrate (Barrow et al., 1980), and smooth (Dillon et al., 1983) muscle types. The NMR spectra of such multicellular muscle preparations result from an averaging of the phosphometabolite levels or ionic parameters within the various component cells. Important differences between cells

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