Volume 6 Number 6 1979 Nucleic Acids Research

Segmental flexibility in Escherichia coli ribosomal protein S1 as studied by fluorescence polarization

Y.Gloria Chu and Charles R.Cantor

Barth Laboratories, Departments of Chemistry and Biological Sciences, Columbia University, New York, NY 10027, USA

Received 14 December 1978

ABSTRACT

Ribosomal protein S1 covalently reacts with approximately one equivalent of iodoacetylethylenediamine(1,5)-napthol sulfonate (IAEDANS) or iodoacetylaminofluorescein (IAAF). The product AEDANS-S1 can bind to 30S ribosomal subunits lacking S1 as shown by polyacrylamide-agarose gel electrophoresis AEDANS-S1 and AAF-S1 when added back to S1-depleted 30S subunits modulate poly(U)-dependent polyphenylalanine synthesis in the presence of IF3 in a very similar way to unmodified S1. AEDANS-S1 also stimulates R17-dependent fMet-tRNA binding to 1.0M NH_4Cl washed ribosomes whereas AAF-S1 does not. Both static and nanosecond fluorescence polarization techniques were used to study the rotational motions of AEDANS-S1. Several previous studies had indicated that S1 is a highly extended protein which can be modeled by a prolate ellipsoid with an axial ratio of 10 to 1. However, the rotational correlation time we find is about half that expected for such a particle. This suggests that S1 is a flexible protein with at least two domains that can rotate independently.

INTRODUCTION

Several features of Escherichia coli ribosomal protein S1 make it uniquely interesting among the more than fifty ribosomal proteins. With a molecular weight about 68,000 it is by far the largest ribosomal protein (1,2). S1 is fairly weakly bound to the 30S subunit and in that sense may function more as a protein factor than as an intrinsic ribosomal protein (3). S1 is required for initiation when a natural mRNA is used (4) and also strongly modulates the protein synthesis stimulation by both synthetic and natural mRNA (4,5). S1 has been implicated by affinity labeling as involved in mRNA binding (6,7,8,9). In addition to its role in the ribosome S1 has a binding site specific for DNA (10) of still unknown biological function, is a subunit of Qβ replicase

(11,12), and is capable of acting as a helix unwinding protein (13,14,15).

All of these aspects of S1 made it an extremely attractive target for attempts at the preparation of specific fluorescent derivatives. The simplest approach was to react sulfhydryl-specific fluorescent dyes with the known reactive cysteine (16,17) of S1. Here we report the preparation and properties of two such derivatives. They maintain sufficient biological activity to allow many types of fluorescent studies of the function of protein S1.

MATERIALS AND METHODS

Preparation of ribosomes and components

Escherichia coli MRE600 cells were purchased from Microbiological Research Establishment, Porton, England. 70S ribosomes were isolated as described by Traut et al. (18) and were washed once with 1.0M NH_4Cl. 30S subunits containing about 0.4 copy of S1 per 30S subunit were isolated by layering these washed 70S ribosomes in 20mM Tris HCl (pH 7.5), 1mM $Mg(OAc)_2$, 100mM NH_4Cl and 6mM 2-mercaptoethanol onto a 10 to 25% linear sucrose density gradient in the same buffer and centrifuging for 24 hours at 17,000rpm in an SW27 rotor. 50S subunits were isolated from twice 1.0M NH_4Cl washed 70S ribosomes in a buffer of 10mM Tris HCl (pH 7.6), 0.25mM $Mg(OAc)_2$, 30mM NH_4Cl and 6mM 2-mercaptoethanol by zonal centrifugation (19). 30S subunits used to prepare 30S subunits free of S1 were isolated from 1.0M NH_4Cl washed 70S ribosomes by the method of differential centrifugation (18). 30S subunits free of S1 were prepared by dialysis against 1mM Tris HCl (pH 7.5) as described by Tal et al. (20)

Preparation of fluorescent conjugates of S1

Purified ribosomal protein S1 prepared from Escherichia coli A19 was a gift from Dr. Kuei Huang (21). It comigrated with a sample of S1 kindly provided by Dr. A. Wahba when subjected to $NaDodSO_4$ polyacrylamide gel electrophoresis and was estimated to be more than 85% pure. (^3H)IAEDANS was synthesized according to the procedure of Huang et al. (22) at a specific activity of 29.3 Ci/mole. AEDANS-S1 conjugates were made as follows: 0.2% (v/v) 2-mercaptoethanol was first added to an S1 solution and then was re-

moved by a Sephadex G-25 column equilibrated in a buffer of 10mM Tris HCl (pH 7.6), 30mM KCl. To the pooled peak fractions containing S1 a 100-fold excess of (^{3}H)IAEDANS in the same buffer was added and then the mixture was incubated at 37°C for one hour. 1% (v/v) 2-mercaptoethanol was added at the end of the incubation and then the unreacted dye was removed by a Sephadex G-25 column equilibrated in a buffer of 10mM Tris HCl (pH 7.6), 20mM $Mg(OAc)_2$, 250mM NH_4Cl and 6mM 2-mercaptoethanol. In order to remove any remaining noncovalently attached dye, the pooled peak fraction containing (^{3}H)AEDANS-S1 was dialyzed into the same buffer containing 6M urea overnight and then into the same buffer containing no urea. The protein concentration in the labeled protein was measured by the method of Lowry et al. (23) using crystalline bovine serum albumin as a standard. The concentration of the dye in the labeled protein was determined by scintillation counting using a solution of (^{3}H)IAEDANS as a standard. Absolute (^{3}H)IAEDANS concentration was determined from absorbance at 336nm, using a molar extinction coefficient of 6.1×10^3 as determined by Hudson et al. (24).

IAAF was purchased from Molecular Probes and AAF-S1 was prepared in exactly the same manner described previously for AEDANS-S1. The fluorescence absorbance of the AAF-S1 remained unaltered when the protein was exposed to a variety of denaturing agents. Thus, we assume that the extinction coefficient of AAF in the conjugate is the same as that for IAAF reacted with excess 2-mercaptoethanol. This allows absorbance to be used to compute the amount of covalently attached AAF in the S1-conjugate.

Gel electrophoresis

Polyacrylamide-agarose gel electrophoresis was run as described by Dahlberg (25) in a Tris EDTA borate, pH 8.3 buffer using the Pharmacia gel electrophoresis apparatus GE-4. Gels were made 3% in acrylamide and 0.5% in agarose and were run at 70 volts at 4°C for 5.5 hours.

$NaDodSO_4$ polyacrylamide gel electrophoresis was carried out as described by Weber et al. (26). Samples were boiled for 3 minutes in 1% $NaDodSO_4$ and 1% 2-mercaptoethanol before loading on the gel. For radioactivity measurements, gels were sliced and put into scintillation vials and 0.4ml of 30% H_2O_2 was added to the

vials and then the slices were incubated overnight at 75-80°C. 0.6ml of water and 10ml of scintillation fluid containing Triton X-100 were added to the dissolved slices and the vials were counted in a Packard scintillation counter.

Poly(U)-dependent protein synthesis assay

Poly(U)-dependent poly(phe) synthesis in the presence of IF3 was performed as described by Sobura et al. (4) with some modifications. The reaction mixture in 0.175ml contained 0.5 A_{260} unit of 30S subunits free of S1, 1 A_{260} unit of 50S subunits, 5µg of IF3, 152µg S100, 4µg pyruvate kinase, 176µg tRNA, 10µg poly(U) and S1 as indicated. The final concentrations were 1.2mM ATP, 0.04mM GTP, 8.8mM PEP, 6µM (^{14}C)phenylalanine (91 mCi/mmole, 0.09 µCi per aysay), 59µM (^{12}C)amino acids except tyrosine which was 30µM, 62mM Tris HCl (pH 7.6), 15mM $Mg(OAc)_2$, 59mM KCl, 9.4mM NH_4Cl and 8.8mM 2-mercaptoethanol. S1 in the S100 enzymes was removed by a poly(C)-cellulose column. Purified IF3 was a gift from Dr. A. Wahba.

Initiation complex formation with R17 RNA as messenger RNA

fMet-tRNA binding assay with R17 RNA as messenger RNA was carried out as described by Kolb et al. (27) with some modifications. The reaction mixture in 50µl contained 1.0 A_{260} unit of 4-times 1.0M NH_4Cl washed 70S ribosomes, 15 pmole (^{3}H)fMet-tRNA (specific activity 11,000 dpm/pmole), 0.24 A_{260} unit of R17, 0.22 µg IF2, 0.42µg IF3 and S1 as indicated. The final concentrations were 0.2mM GTP, 56mM Tris HCl (pH 7.6), 5.0mM $Mg(OAc)_2$, 58mM NH_4Cl and 1mM dithiothreitol. R17 was a gift from Dr. P. Thammana (28). Purified initiation factors were gifts from Dr. A. Wahba. The reactions were carried out at 37°C for 15 minutes. At the end of incubation, samples were diluted with one ml of cold buffer which was 50mM Tris HCl (pH 7.6), 100mM NH_4Cl, 5mM $Mg(OAc)_2$, and quickly filtered through Millipore filters followed by two washes with the same buffer. Radioactivity retained on the filter paper was measured.

The counts from the sample containing 5µg of (^{3}H)AEDANS-S1 but no (^{3}H)fMet-tRNA were used to calculate the tritium counts contributed by (^{3}H)AEDANS-S1 of all the samples assuming that the amount of (^{3}H)AEDANS-S1 retained on the filter paper was proportional to the amount of (^{3}H)AEDANS-S1 originally present in the

reaction mixture. The error introduced by this assumption should be within experimental error. 5μg of (^{3}H)AEDANS-S1 was also loaded directly on a piece of dried filter paper to see the maximum possible contribution of tritium counts from (^{3}H)AEDANS-S1.

Static fluorescence polarization measurements

Static polarization was measured with a Schoeffel RRS 1000 spectrofluorimeter interfaced to a Tektronics E31 programable calculator. Temperature was controlled with a Lauda K2R circulating bath. The excitation wavelength was 360nm and the emission wavelength was 480nm. The difference in the monochromator transmittivity for the horizontal and vertical components of the emitted light was taken into consideration in the calculation of P as suggested by Paoletti et al. (29). The harmonic mean rotational relaxation time, τ_c, and the limiting polarization, P_o, were calculated from the Perrin plot of the data (30).

Samples in different concentrations of sucrose and glycerol were used. For each AEDANS-S1 sample, a corresponding sample containing non-labeled S1 was prepared and used as a control for the background. Values for the viscosity of sucrose solutions were taken from the Handbook of Biochemistry and those of glycerol from the Handbook of Physics and Chemistry. Sucrose was obtained from Beckman Instruments and glycerol from Amend Drug and Chemical Co. The two buffers used were Buffer A: 10mM Na_2HPO_4 (pH 7.7), 1mM EDTA, 100mM NaCl and 1mM 2-mercaptoethanol; Buffer B: 16.2mM Tris HCl (pH 7.4), 8.1mM $Mg(OAc)_2$, 81mM NH_4Cl, 0.4mM EDTA and 6mM 2-mercaptoethanol.

Excited-state lifetime and time-dependent emission anisotropy measurements

Lifetime and time-dependent emission anisotropy measurements were performed in the laboratory of Dr. C.-W. Wu by the single photon counting technique using an Ortec 9200 nanosecond fluorescence spectrofluorimeter as described by Bandyopadhyay et al. (31). The sample was in Buffer B. The exciting light was filtered through a Corning 7-37 filter and the emitted light was detected through a Kodak Wratten 65 filter.

The time dependence of the total emitted light was deconvoluted and analyzed in terms of two exponential decays by the method of moments (32). The final set of parameters chosen was

that which yielded the smallest sum of weighted squares of the residuals (33).

The anisotropy, A(t), was calculated according to the definition of Jablonski (34). Parameters which gave the smallest sum of square deviations were chosen to express A(t) as two exponential terms.

RESULTS

Stoichiometry of labeling

A covalent IAEDANS conjugate of protein S1 was prepared and purified as described in Materials and Methods. The ratio of AEDANS to S1 in the labeled protein was usually around 1.1 and was quite reproducible. Although the S1 samples contained small amounts of a lower molecular weight protein as shown on stained $NaDodSO_4$ polyacrylamide gels (Fig. 1a), the (^{3}H)AEDANS-labeled

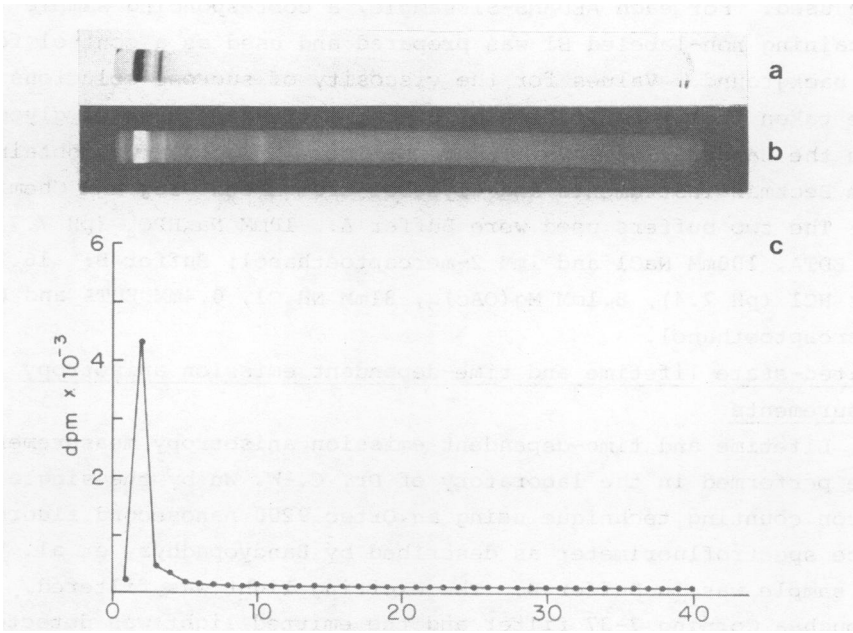

Figure 1
$NaDodSO_4$ polyacrylamide gel electrophoresis of (^{3}H)AEDANS-S1
(a) the gel stained with Coomassie brilliant blue
(b) fluorescence seen under UV illumination
(c) ^{3}H cpm of the sliced gel
The electrophoresis direction was towards the right.

protein when examined under ultraviolet illumination gave only one fluorescent band (Fig. 1b) which corresponded to the S1 band on the stained gel. Scintillation counting of the gel slices gave only one cpm peak which also corresponded to the S1 band on the stained gel (Fig. 1c).

A second fluorescent derivative was prepared by reacting S1 with IAAF as described in Materials and Methods. This contained about 1.2 AAF moieties per mole of S1. When AAF-S1 was subjected to $NaDodSO_4$ polyacrylamide gel electrophoresis only a single fluorescent band was seen which comigrated with the position of stained protein S1 (results not shown).

Activity of fluorescent S1 derivatives

In order to examine the effect of labeling on the activity of the protein the following assays were carried out:

Ribosome binding: 30S subunits containing S1 and 30S subunits free of S1 can be separated on polyacrylamide gels into two bands (25). The faster migrating form on the gel is 30S subunits free of S1 and the slower migrating form is 30S subunits containing S1. 30S subunits which had been activated by incubation in a buffer of 10mM Tris HCl (pH 7.5), 20mM $Mg(OAc)_2$, 250mM NH_4Cl and 6mM 2-mercaptoethanol at 37°C for 20 minutes and contained about 0.4 copy of S1 per 30S subunit were used to examine AEDANS-S1 binding. When a 3.4 fold excess of (^{3}H)AEDANS-S1 (with respect to the 30S subunits free of S1) was added to the 30S subunits the faster migrating form which was originally present disappeared (Fig. 2) and radioactivity was found incorporated into the slower migrating form (results not shown). Therefore (^{3}H)AEDANS-S1 appears to bind in the normal manner to 30S subunits lacking S1 (Fig. 2).

Poly(U)-directed poly(phe) synthesis in the presence of IF3: S1 is known to modulate the protein synthesis directed by poly(U) in the presence of IF3 (4). The amount of (^{14}C)poly(phe) synthesized was measured as a function of the amount of S1 or (^{3}H)AEDANS-S1 present. The progressive stimulation and inhibition of protein synthesis caused by AEDANS-S1 closely paralleled that seen with S1 itself. Under a variety of conditions (^{3}H)AEDANS-S1 was about 85% as active as the unmodified protein (Fig. 3). The same assay was also carried out for AAF-S1 under slightly different conditions and this fluorescent derivative was about 80% as active as the

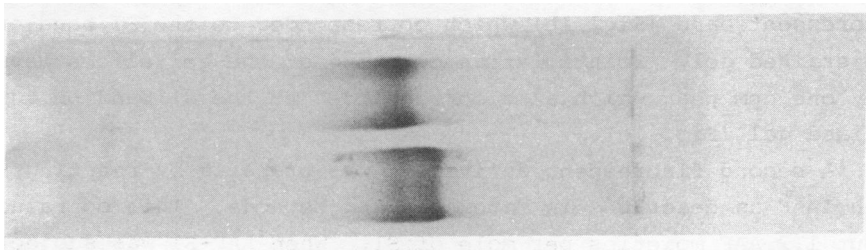

Figure 2
Polyacrylamide-agarose gel electrophoresis of 30S subunits reconstituted with (^{3}H)AEDANS-S1. Activated 30S subunits which contained about 0.4 copy of S1 per 30S subunit were used. The upper slot contained 0.3 A_{260} unit of 30S subunits and 3.2µg of (^{3}H)AEDANS-S1. The lower slot contained 0.3 A_{260} unit of 30S subunits only. The gel was stained with methylene blue. The electrophoresis direction was towards the right.

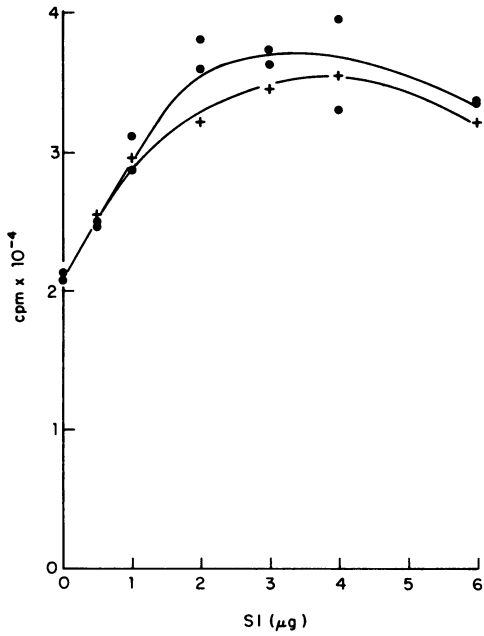

Figure 3
Poly(U)-dependent (^{14}C)poly(phe) synthesis in the presence of IF3 at various amounts of (^{3}H)AEDANS-S1 (+) or S1 (·). See Materials and Methods for the composition of the reaction mixture.

unmodified protein (data not shown).

R17 RNA-dependent (^{3}H)fMet-tRNA binding: The formation of an initiation complex with natural messenger RNA requires S1 (4). However, it has been reported that an N-ethylmaleimide (NEM) derivative of S1 does not stimulate the natural mRNA dependent (^{3}H)fMet-tRNA binding to ribosomes. The NEM-S1 was also incapable of unwinding RNA but was able to bind the 30S subunit lacking of S1 (15,27). This made it interesting to examine the activity of (^{3}H)AEDANS-S1 in promoting R17 RNA-dependent (^{3}H)fMet-tRNA binding.

30S subunits free of S1 prepared either by dialysis against 1mM Tris HCl (pH 7.6) or by salt wash did not give acceptable (^{3}H)fMet-tRNA binding activity when S1 was added to the reaction mixture. Therefore we used four-times 1.0M NH_4Cl washed 70S ribosomes which gave only one band on a polyacrylamide-agarose gel and showed about 60% conversion of the faster migrating form to the slower migrating form when a two-fold excess of S1 was added. The amount of (^{3}H)fMet-tRNA bound at different amounts of added S1 or (^{3}H)AEDANS-S1 was measured. The results in Fig. 4 indicate that the (^{3}H)AEDANS-S1 was about 82% as active as native S1. NEM-S1 was prepared in the same way as (^{3}H)AEDANS-S1 using a ten-fold excess of NEM to S1. The stoichiometry of NEM per S1 in this conjugate, by using (^{3}H)NEM, is around 1.0. A comparison of the (^{3}H)fMet-tRNA binding activity stimulated by (^{3}H)AEDANS-S1, NEM-S1 and AAF-S1 is given in Table 1. It is clear that among the three derivatives, only (^{3}H)AEDANS-S1 retained significant activity.

One possible explanation of the different activity of the various S1 derivatives is different sites of covalent modification. To test this, S1 was first treated with NEM and then reacted with (^{3}H)IAEDANS. The resulting ratio of (^{3}H)AEDANS to S1 was much less than 1. This suggests that (^{3}H)IAEDANS probably reacted with the reactive sulfhydryl group on the protein.

If we compare the chemical structure of IAEDANS, NEM, and IAAF, it appears that steric freedom near the sulfhydryl group is needed to retain good activity after modification: IAEDANS has a much longer side chain than IAAF and also fluorescein is much bigger in size than naphthalene. NEM does not have any side chain at all; the sulfur atom is attached to the maleimide ring directly.

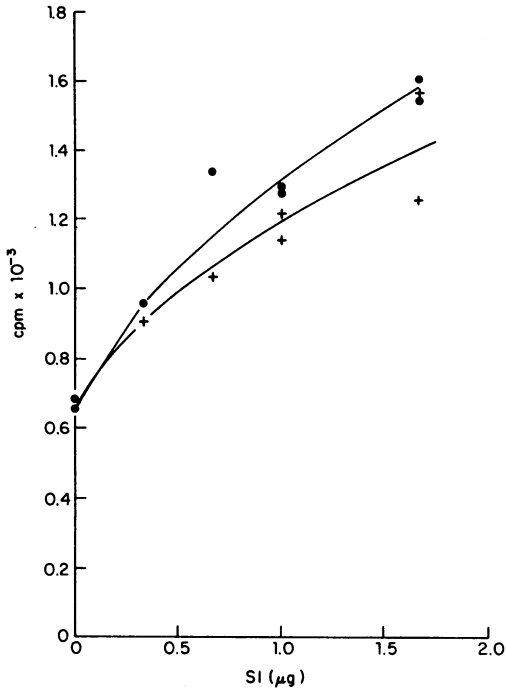

Figure 4
The amount of (^{3}H)fMet-tRNA bound to 70S ribosomes with R17 as messenger RNA vs. the amount of (^{3}H)AEDANS-S1 (+) or S1 (·) added. One pmole of (^{3}H)fMet-tRNA bound was equivalent to 2130cpm. The counts of controls were
1) minus R17, 290cpm
2) minus IF's, 120cpm
The ^{3}H counts contributed by (^{3}H)AEDANS-S1 were subtracted from the total counts as described in Materials and Methods. The counts of sample containing 5μg of (^{3}H)AEDANS-S1 but no (^{3}H)fMet-tRNA were 200cpm and those of 5μg of (^{3}H)AEDANS-S1 loaded directly on to a piece of filter paper were 300cpm. The background given by the filter paper was 30cpm.

Fluorescence lifetime of S1-AEDANS

In order to calculate the rotational correlation time from static fluorescent polarization data we need to know the lifetime of AEDANS-S1. The lifetime was measured by single photon counting techniques. The nanosecond emission kinetics of AEDANS-S1 (results not shown) could be fitted by two excited-state lifetimes, 11.8 nsec and 21.9 nsec, with the relative weight of 0.119 and

TABLE 1: The effect of the sulfhydryl reagents on the ability of S1 to stimulate (^{3}H)fMet-tRNA binding to ribosomes with R17 RNA as messenger RNA

		Additions				
	Experiment	None	S1	(^{3}H)AEDANS-S1[a]	NEM-S1	AAF-S1
(^{3}H)fMet-tRNA bound (cpm)	I	719	1606	1395	825	923
	II	840	1540	1558	884	929
Percent activity		0	100	88	9	18

Assays were carried out as described in the legend to Figure 4. The amount of S1 when present was 5µg.

[a] The ^{3}H counts contributed by (^{3}H)AEDANS-S1 were assumed to be 12% of the total counts and were subtracted.

0.140. In the calculation of the rotational correlation times for the static fluorescence polarization data the weighted average of the lifetimes, 17.3 nsec, was used.

The explanation for the occurance of two lifetimes is unclear. Although S1 might have a second sulfhydryl group (35), since the stoichiometry of AEDANS labeling is always around one it is quite likely that only one sulfhydryl group on the protein is reactive. Even though the dye is covalently attached to the protein at only one site, however, the naphthalene ring may assume more than one orientation or placement on the protein surface. Alternatively, the protein may have two conformations in solution.

Static fluorescent polarization of AEDANS-S1

Polarization of AEDANS-S1 in 7.4 x 10^{-8}M in Buffer A was measured at 20°C as a function of sucrose concentration. A Perrin plot of the data is shown in Fig. 5.

To see if there were thermally activated rotations at 20°C the temperature was lowered to 10°C and polarization measurements were repeated. Additional polarization measurements of AEDANS-S1 were performed as a function of glycerol concentration in Buffer A, and as a function of sucrose concentration in Buffer B. All of

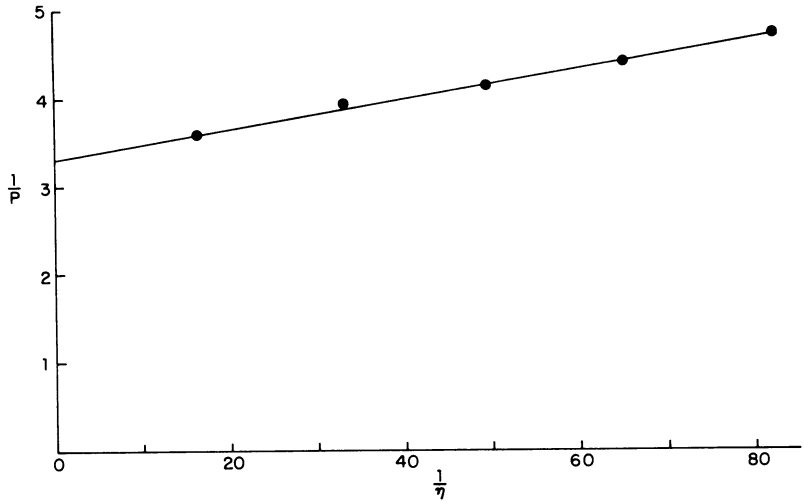

Figure 5
Perrin plot of AEDANS-S1 at different sucrose concentrations in buffer A. η is the viscosity of the solution. Temperature is 293°K which is not included in the abscissa.

these samples showed linear Perrin plots and the resulting limiting polarization and rotational correlation time values are summarized in Table 2.

A few features of this data merit further comment. Lowering the temperature from 20°C to 10°C did not change the rotational correlation time significantly, showing that thermally activated rotations are unlikely. Similarly, a substantial addition of magnesium (in Buffer B) caused no significant effect on the protein. Under all circumstances the limiting polarization of the dye is the same, suggesting that local motion and thus local environment of the AEDANS are relatively insensitive to conditions. However, the rotational correlation time measured in glycerol solution was much longer than those measured in sucrose solution. Thus glycerol may cause some changes in the overall conformation of S1.

Decay of anisotropy of AEDANS-S1

The disadvantages of the static fluorescence polarization

TABLE 2: Results of static fluorescence polarization measurements of AEDANS-S1 under different conditions

Buffer	Temperature °C	sucrose/glycerol	τ_c at 20°C	P_o
A	20	S	31	0.30
A	10	S	27	0.32
B	20	S	26	0.32
A	20	G	39	0.28

τ_c is the rotational correlation time and P_o is the limiting polarization.

Buffer A: 10mM Na_2HPO_4 (pH 7.7), 1mM EDTA, 100mM NaCl and 1mM 2-mercaptoethanol

Buffer B: 16.2mM Tris HCl (pH 7.4), 8.1mM $Mg(OAc)_2$, 81mM NH_4Cl, 0.4mM EDTA and 6mM 2-mercaptoethanol

measurements were that the lifetime of the excited state had to be determined independently and sucrose or glycerol had to be added to vary the viscosity with the risk that these might alter the conformation of the protein. To check these results we performed decay of anisotropy measurement of AEDANS-S1 in Buffer B. The result is shown in Fig. 6.

The best two exponential fit with parameters which yielded the smallest sum of squares gave two rotational correlation times equal to 26.1 nsec and 6.9 nsec with the relative weight of 0.108 and 0.068. The longer decay time is in excellent agreement with the results seen by static polarization. The shorter one may, in principle, represent some local motion of residues near the dye. However, it could easily be an artifact and we have chosen not to give it further consideration.

DISCUSSION

Both static and nanosecond fluorescence polarization measurements gave a rotational correlation time of AEDANS-S1 in Buffer B equal to 26 nsec. Previous studies have suggested that S1 is highly elongated and can be modeled by a prolate ellipsoid with an axial ratio of 10 to 1 (3). Most proteins studied so far by de-

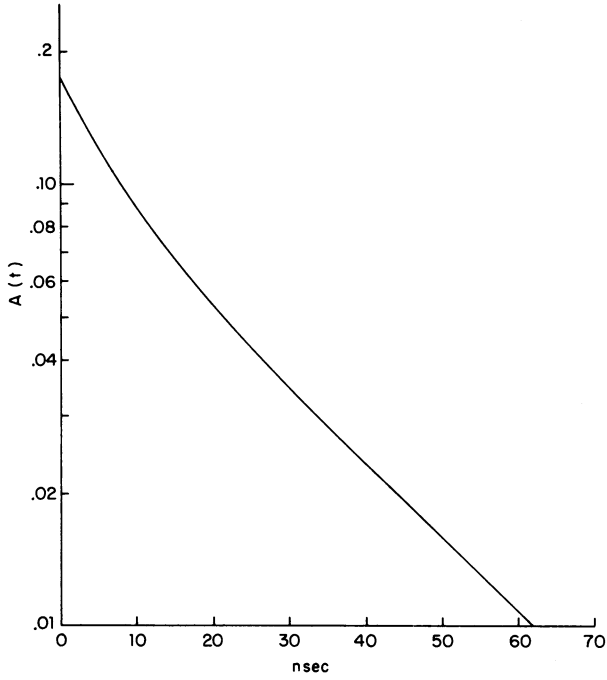

Figure 6
Time-dependent emission anisotropy of AEDANS-S1 in buffer B. The best two exponential fit for the curve is $A(t) = 0.108\exp(-t/26.1) + 0.068\exp(-t/6.9)$.

cay of anisotropy polarization techniques exhibit a single rotational correlation time, τ_c, sufficiently large to correspond to the motion of a rigid particle. See Table 1 in the paper by Yguerabide et al. (36). This measured correlation time is usually larger than the rotational correlation time, τ_o, expected for a hydrated spherical protein assuming a typical partial specific volume of $0.73 cm^3 g^{-1}$ and a typical degree of hydration $0.32 gH_2O/g protein$. When the ratio τ_c/τ_o observed for a series of proteins is plotted versus the ratio f (the observed frictional coefficient obtained by classical hydrodynamic measurements) to f_o (the frictional coefficient expected for an anhydrous sphere) for the same proteins a smooth curve is obtained (37). Typical results are shown in Fig. 7.

From sedimentation studies and molecular weight determination

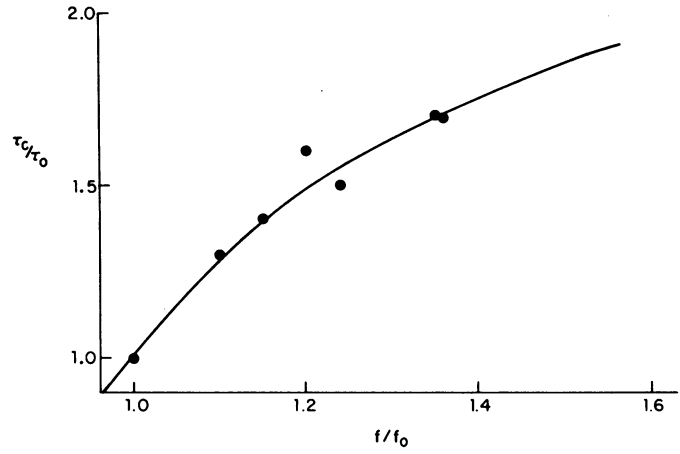

Figure 7
Comparison of the effect of shape on measured rotational or translational frictional properties. (This graph was adapted from one shown in Cantor and Timasheff (37)).

by $NaDodSO_4$ polyacrylamide gel electrophoresis the f/f_o ratio of S1 is 1.7 (36). Thus even a crude extrapolation of the results of Fig. 7 indicates that for S1 a ratio of τ_c/τ_o of 2 is expected. Taking the molecular weight of S1 as 68,000 the calculated τ_o for S1 is 30 nsec at 20°C. Therefore the expected τ_c for S1, if it is a rigid protein, is around 60 nsec. The measured rotational correlation time we observe, 26 nsec, is much shorter. This strongly suggests that S1 is not a rigid protein. It has at least two domains which can move fairly independently. One domain to which AEDANS is attached can rotate freely with respect to the rest of the molecule. The size and the shape of the domain is model dependent. However, for a prolate ellipsoid with half the molecular weight of S1 and an axial ratio of 5 to 1, τ_c is expected to be around 24 nsec which agrees fairly well with what we observed. By entirely independent techniques Moore and Laughrea have come to the same conclusion that S1 is a multi-domain protein (38).

S1 has two nucleic acid binding sites and it unwinds RNA and DNA (13,14,15). Our finding that S1 has at least two flexibly attached domains suggests interesting relationships between the

structure and the function of S1.

ACKNOWLEDGEMENTS

We thank Dr. K. Huang for the generous gift of S1 protein and Dr. P. Thammana for the gift of R17 and Dr. A. Wahba for the gift of initiation factors. We greatly appreciate the help of Mr. P. Bandyopadhyay and Dr. C. Wu in the nanosecond fluorescence measurements. We thank Drs. W. Szer and R. Fairclough for many useful discussions and Ms. A. Beekman and C. Soto for their technical assistance.

REFERENCES

1 Giri, L. and Subramanian, A.R. (1977) FEBS Lett 81, 199-203.
2 Wittmann, H.G. (1974) In Ribosomes (Nomura, M., Tissieres, A. and Lengyel, P., eds.) pp. 93-114, Cold Spring Harbor Laboratory, New York.
3 Laughrea, M. and Moore, P.B. (1977) J. Mol. Biol. 112, 399-421.
4 Sobura, J.E., Chowdhury, M.R., Hawley, D.A. and Wahba, A.J. (1977) Nucleic Acids Res. 4, 17-29.
5 Van Dieijen, G., Van der laken, C.J., Van Knippenberg, P.H. and Van Duin, J. (1975) J. Mol. Biol. 93, 351-366.
6 Fisher, I., Scheit, K.H., Stöffler, G. and Kuechler, E. (1974) Biochem. Biophys. Res. Commun. 60, 1112-1118.
7 Fisher, I., Scheit, K.H., Stöffler, G. and Kuechler, E. (1975) FEBS Lett. 56, 226-229.
8 Lührmann, R. and Gassen, H.G. (1976) Eur. J. Biochem. 66, 1-9.
9 Pongs, O., Stöffler, G. and Bald, R.W. (1976) Nucleic Acids Res. 3, 1635-1646.
10 Draper, D.E. and Von Hippel, P.H. (1978) J. Mol. Biol. 122, 321-338.
11 Wahba, A.J., Miller, M.J., Niveleau, A., Laners, T.A., Carmichael, G.C., Weber, K., Hawley, D.A. and Slobin, L.I. (1974) J. Biol. Chem. 249, 3314-3316.
12 Inouye, H., Pollack, Y. and Petre, J. (1974) Eur. J. Biochem. 45, 109-117.
13 Szer, W., Hermoso, J.M. and Boublik, M. (1976) Biochem. Biophys, Res. Commun. 70, 957-964.
14 Bear, D.G., Ng, R., Van Derveer, D., Johnson, N.P., Thomas, G., Schleich, T. ans Noller, H. (1976) Proc. Natl. Acad. Sci. 73, 1824-1828.
15 Thomas, J.O., Kolb, A. and Szer, W. (1978) J. Mol. Biol. 123, 163-176.
16 Moore, P.B. (1971) J. Mol. Biol. 60, 169-184.
17 Acharya, A.S. and Moore, P.B. (1973) J. Mol. Biol. 76, 207-221.

18 Traub, P., Mizushima, S., Lowry, C.V. and Numura, M. (1971) In Methods in Enzymology (Moldave, K. and Grossmann, L., eds.) Vol. XX, pp. 391-392, Academic Press, New York.
19 Eikenberry, E.T., Bickle, T.A., Traut, R.R. and Price, C.A. (1970) Eur. J. Biochem. 12, 113-116.
20 Tal, M., Aviram, M., Kanarek, A. and Weiss, A. (1972) Biochim. Biophys. Acta 281, 381-392.
21 Huang, K.-H. and Cantor, C.R. (1975) J. Mol. Biol. 97, 423-441.
22 Huang, K.-H., Fairclough, R.H. and Cantor, C.R. (1975) J. Mol. Biol. 97, 443-470.
23 Lowry, O.H., Rosebrough, N.J., Farr, L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
24 Hudson, E.N. and Weber, G. (1973) Biochemistry 12, 4145.
25 Dahlberg, A.E. (1974) J. Biol. Chem. 249, 7673-7678.
26 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
27 Kolb, A., Hermoso, J.M., Thomas, J.O. and Szer, W. (1977) Proc. Natl. Acad. Sci. 74, 2379-2383.
28 Thammana, P. and Cantor, C.R. (1978) Nucleic Acids Res. 5, 805-823.
29 Paoletti, J. and Le Pecq, J.-B. (1969) Anal. Biochem. 31, 33-41.
30 Weber, G. (1966) In Fluorescence and Phosphorescence Analysis (Hercules, D.M., ed.) Interscience Publishers, New York.
31 Bandyopadhyay, P.K. and Wu, C.-W. (1978) Biochemistry 17, 4078.
32 Yguerabide, J. (1972) in Methods in Enzymology (Moldave, K. and Grossmann, L., eds.) Vol. XXVI, pp. 498-578, Academis Press, New York.
33 Brochon, J.C., Wahl, P.H., Charlier, M., Maurizot, J.C. and Helene, C. (1977) Biochem. Biophys. Res. Commun. 79, 1261.
34 Jablonski, A. (1962) In Luminescence of Organic and In-Organic Materials (Kallman, H.P. and Spruch, G.M., eds.) p. 110, Wiley, New York.
35 Moore, P.B. (1975) J. Mol. Biol. 98, 439-444.
36 Yguerabide, J., Epstein, H.F. and Stryer, L. (1970) J. Mol. Biol. 51, 573-590.
37 Cantor, C.R. and Timasheff, S.N. In The Proteins (Neurath, H. and Hill, R., eds.) Vol. 5, 3rd. Ed., Academic Press, New York, book in press.
38 Moore, P.B. and Laughrea, M. (1978) accompanying paper.