Segmental flexibility of ribosomal protein S1 bound to ribosomes and Qβ replicase

Y. Gloria Chu*, Charles R. Cantor, Irene Sawchyn+ and Patricia E. Cole†

Department of Chemistry, Columbia University, New York, NY 10027, USA

Received 25 March 1982; revision received 18 June 1982

QB replicase *Protein S1* *Ribosome* *Polarization* *Fluorescence* *Anisotropy*

1. INTRODUCTION

Ribosomal protein S1 is a multidomain protein [1–4] which functions in two different settings. As a ribosomal protein it is required for the binding of mRNAs to the ribosome and the formation of a 30 S initiation complex with natural mRNA [5–10 and references within]. As a subunit of Qβ replicase it is essential for the initiation of transcription of Qβ RNA [11,12]. One of the S1 domains contains a sulfhydryl group which is reactive with *N*-ethylmalemide (NEM) and iodoacetylethylenediamine (1,5)-napthol sulfonate (AEDANS) [1,13–15]. This site is involved in the RNA binding properties of S1 since NEM modification of S1 inactivates the protein's helix unwinding ability [10,16]. Although NEM-modified S1 binds with comparable affinity as unmodified S1 to 30 S subunits [17], NEM-S1 is inactive in initiation complex formation with natural messengers [1,10]. In contrast, AEDANS-S1 is active in initiation complex formation, presumably because of a more flexible side chain [1]. Qβ RNA transcription is more tolerant to sulfhydryl modifications of S1 since Qβ replicase reconstituted with either NEM-S1 or AEDANS-S1 is 80% as active as Qβ replicase containing unmodified S1 [18].

Given these variations in the functional involvement of the reactive domain, we wanted to determine whether this domain makes direct contact with the ribosome or Qβ replicase or both. If it does, we would expect a substantial increase in the fluorescence anisotropy of the AEDANS-labeled S1 when the S1 is bound to replicase lacking S1 [R(-S1)] or ribosomes lacking S1. We show here in preliminary studies that the increase in anisotropy is much less than expected. The simplest interpretation of this result is that the labeled domain is still free to move when S1 is bound.

Present addresses: *Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720, USA; +Western Electric, Engineering Research Center, P.O. Box 900, Princeton, NJ 08540, USA; †Box 16A, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA–To whom requests for reprints should be sent

Abbreviations: NEM, *N*-ethylmaleimide; AEDANS, iodoacetylethylenediamine (1,5)-naphthol sulfonate; AEDANS-S1, protein S1 from *E. coli* derivatized with AEDANS; R-S1, Qβ replicase lacking the S1 subunit

2. EXPERIMENTAL PROCEDURES

2.1. *Chemicals*

Nucleoside triphosphates were purchased from P-L Biochemicals. Sucrose (density gradient grade) was obtained from Beckman Instruments. All other chemicals were reagent grade.

2.2. *Proteins, ribosomes and Qβ RNA*

The preparation of *E. coli* 30 S subunits and 70 S ribosomes lacking S1 and the labeling of S1 with [^{3}H]AEDANS (approximately 1.1 mol AEDANS/mol S1) were described previously [1]. Replicase lacking S1 and Qβ RNA were purified as detailed elsewhere [18]. The molecular weight of Qβ replicase was calculated using the sequence molecular weights of 30 257 for EF-Ts [19] and 43 225 for EF-Tu [20], 61 159 for S1 [21] and 65 317 for the viral subunit [22].

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

2.3. *Static fluorescence polarization measurements*

Static polarization was measured with a Schoeffel RRS-1000 spectrofluorimeter using a quartz polarizing prism for the 360 nm exciting beam and a polaroid sheet followed by a 1/4 wavelength scrambling plate for the emission beam. Emission was monitored at 480 nm and anisotropy calculated as previously described [23]. Where appropriate, ribosome, Qβ RNA, S1 and replicase blanks were prepared and their contribution to the polarized emission subtracted [23].

2.4. *Binding of [^{3}H]AEDANS-S1 to ribosomes and ribosomal subunits*

To analyze the binding of [^{3}H]AEDANS-S1 to 30 S subunits and 70 S ribosomes, 0.5 ml of each sample used in the polarization measurements was loaded onto a 5% to 20% linear sucrose gradient in the same buffer and centrifuged at 32 000 rev./min for 3 h at 4°C in a Beckman SW 50.1 rotor. The gradients were analyzed for 260 nm absorbance and for radioactivity by scintillation counting in Triton X-100.

Table 1

Effect of ribosome binding on the fluorescence anisotropy of AEDANS-S1[a]

Measured, for a sample containing AEDANS-S1:	Anisotropy[b]
Alone	0.14
Plus 30 S subunits lacking S1	0.16
Plus 70 S ribosomes lacking S1	0.16
Assuming only 50% ribosome binding	0.18
Calculated:	
For AEDANS-S1 rigidly attached to ribosomes	0.23

[a] Concentrations of components were: [^{3}H]AEDANS-S1, 7.4 × 10^{-8} M; 30 S subunits lacking S1, 1.5 × 10^{-7} M; 70 S ribosomes lacking S1, 1.5 × 10^{-7} M. The buffer was 16.2 mM Tris-HCl (pH 7.4), 8.1 mM $Mg(OAc)_2$, 81 mM NH_4Cl, 0.4 mM EDTA and 6 mM 2-mercaptoethanol. The temperature was 20°C

[b] Calculated as described in Experimental Procedures; experimental error is estimated to be ± 0.01 for all these samples

3. RESULTS AND DISCUSSION

3.1. *Polarization measurements of AEDANS-S1 bound to ribosomes*

Fluorescence anisotropy measurements of AEDANS-S1 either free or bound to 30 S ribosomal subunits or 70 S ribosomes are summarized in table 1. The anisotropy, r, is related to the limiting anisotropy, r_0, by the Perrin equation which can be written as:

$$\frac{1}{r} = \frac{1}{r_0}(1 + \tau/\tau_c) \quad (1)$$

where τ is the fluorescence lifetime and τ_c is the rotational correlation time [24]. For AEDANS-S1, τ is 17.3 ns (1; the same for free protein or ribosome bound-S1 since there is no change in the AEDANS quantum yield upon binding). The limiting anisotropy of AEDANS-S1 has been determined by varying the temperature and the viscosity [1]. The average of four determinations was 0.23 ± 0.01; this is 64% larger than the anisotropy of free S1 in aqueous buffer at 20°C. For the 70 S ribosome, τ_c has been measured as 4.4 μs [25] and it will presumably be roughly one third of this for the 30 S particle. Both of these values are so much higher than τ that, if all the AEDANS-S1 is bound to ribosomes and the labeled domain is held rigid, equation (1) becomes essentially $r = r_0$. However, as shown in table 1, the anisotropy of AEDANS-S1 bound to either 30 S subunits or 70 S ribosomes is far less than r_0, and, within experimental error, is essentially the same as that for free S1. This indicates that the AEDANS is not held rigidly on the 30 S or 70 S ribosome.

According to Draper and von Hippel, the S1-30 S or 70 S association constant is 2 × 10^8 M^{-1} under buffer conditions similar to ours [26]. This implies that more than 90% of the AEDANS-S1 should be bound to 30 S subunits or 70 S ribosomes in our buffer. However, even if one makes the pessimistic assumption that only 50% of the AEDANS-S1 is bound, one can calculate [24] that the anisotropy of bound S1 is still far less than r_0 (table 1).

Note that the same anisotropy is seen when AEDANS-S1 is bound to either 30 S subunits or 70 S ribosomes (table 1), suggesting that 50 S subunits are unlikely to contribute to the binding site of S1 on 70 S ribosomes. This is consistent with the finding that S1 has the same association constant

with 30 S subunits and 70 S ribosomes [26] and the observation that 70 S ribosomes made by coupling 50 S subunits to 30 S subunits lacking S1 bind no more S1 than the same 30 S subunits in the absence of 50 S subunits [27].

3.2. *Polarization measurements of AEDANS-S1 bound to Qβ replicase and Qβ RNA*

The fluorescence anisotropy of AEDANS-S1 free or bound to R(-S1) is presented in table 2. The difference in the measured anisotropy reported for free AEDANS-S1 in tables 1 and 2 can be accounted for simply by the difference in the temperature (20°C versus 10°C) and the viscosity (aqueous versus 10% glycerol) of the two buffers [28]. Binding of S1 to R(-S1) causes no increase in anisotropy within experimental error.

The maximum anisotropy expected if all the AEDANS-S1 is bound to replicase and the labeled domain is held rigidly can be calculated from equation (1). Since the binding constant of S1 to R(-S1) is approximately 1×10^8 M^{-1} in this buffer [18], more than 90% of the AEDANS-S1 should be bound to R(-S1) under our conditions. Complex formation at 10°C was confirmed by the ability of AEDANS-S1 to restore Qβ RNA transcription activity to R(-S1) at 10°C (P.E. Cole and I. Sawchyn, unpublished results). The limiting anisotropy of AEDANS-S1 used is 0.23 ± 0.01. This value is insensitive to experimental conditions and is the same within experimental error as the measured r_0 of AEDANS-labeled nucleosomes [29]. The fluorescence lifetime τ can be taken as the same as that for free AEDANS-S1 (17.3 ns; 1) since there was no change in quantum yield upon binding to R(-S1). If the labeled domain is rigidly held, the rotational correlation time should correspond to that of complete four-subunit replicase. An estimate of the anisotropy expected for rigid replicase in 10% glycerol at 10°C was made as described in table 2. The result is that $r = 0.22$ (or greater if R(-S1) is not a sphere). This is significantly larger than the observed anisotropy (table 2) which indicates that the AEDANS is not rigid.

Table 2 also shows that the anisotropy of free S1 increases slightly upon binding to Qβ RNA. Since S1 has an affinity of 5×10^6 to 10^7 M^{-1} for pyrimidine-rich sequences and a site size of 7–14 nucleotides [30–34] and the 1.5×10^6 dalton Qβ RNA [35] has several such binding sites for S1

Table 2

Fluorescence anisotropy of AEDANS-S1 in the Qβ replicase system[a]

Measured, for a sample containing AEDANS-S1:	Anisotropy[b]
Alone	0.17
Plus R(-S1)	0.18
Plus GTP	0.17
Plus GTP + Qβ RNA	0.19
Plus R(-S1) + Qβ RNA	0.19
Plus R(-S1) + Qβ RNA + GTP	0.19
Plus R(-S1) + Qβ RNA + GTP + ATP + CTP + UTP	0.18
Calculated:[c]	
For a rigid S1–R(-S1) complex	0.22
For a rigid replicase–Qβ RNA complex	0.23

[a] The final concentrations of components were [^{3}H]AEDANS-S1, 7.1×10^{-8} M; R(-S1), 1×10^{-7}; Qβ RNA, 4×10^{-8} M; and GTP, ATP, UTP and CTP, each 8×10^{-4} M. The buffer was 50 mM Tris-HCl (pH 7.5), 10 mM $Mg(OAc)_2$, 1 mM EDTA, 0.1 mM dithiothreitol, 10% (w/w) glycerol, 0.1 M NaCl and 3 mM NH_4Cl. The temperature was 10°C

[b] Calculated as described in Experimental Procedures; experimental error is estimated to be ± 0.01 for all of these samples

[c] Calculated considering that the sedimentation coefficients of replicase and R(-S1) are the same [11]; assuming that R(-S1) is a sphere and that both replicase and R(-S1) have typical protein specific volumes and hydrations of 0.73 cm^3/g and 0.32 g H_2O/g, respectively [24]; estimating a minimum Perrin shape factor for replicase and from that a minimum τ_c (251 ns) [1]; and then using equation (1)

[36,37], probably more than 50% of the AEDANS-S1 is bound to the Qβ RNA under our conditions. Assuming little internal flexibility in the RNA, one would expect the anisotropy of a rigidly bound RNA–S1 complex to be r_0, 0.23 ± 0.01. Thus the measured value in table 2 indicates that the AEDANS is still fairly flexible when AEDANS-S1 is bound to Qβ RNA. Similar results are obtained when AEDANS-S1 binds to poly rC (data not shown). Finally, table 2 shows that when S1 is bound to Qβ replicase and replicase in turn complexed with Qβ RNA, no further increase in an-

isotropy occurs. Addition of nucleoside triphosphate substrates also has no effect.

4. CONCLUSION

The AEDANS label of S1 is located in a flexible domain of the free protein [1]. This label shows no significant loss in flexibility when S1 is bound to 30 S subunits, 70 S ribosomes, Qβ replicase, Qβ RNA or the replicase–Qβ RNA complex. The simplest interpretation of these results is that the labeled domain remains flexible in all of these complexes. We cannot exclude the more complicated possibility that the domain becomes rigidly attached but local structural changes occur, allowing increased probe wiggle [24]. To exclude this possibility, complete Perrin plots or time-resolved anisotropy measurements of all the samples would be required. This will have to await future studies when larger amounts of labeled proteins are available. The apparent flexibility of the labeled domain is a puzzle. If the RNA melting function of this domain is actually used in replication or protein synthesis, it may act only in forming transient complexes too unstable or infrequent to be detected in these experiments.

ACKNOWLEDGEMENTS

This work was supported by PHS research Grants GM 26109, GM 21352 to P.E.C. and GM 19843 to C.R.C., NSF Grant PCM 8006354 to C.R.C. and NIH Research Career Development Award GM 00471 to P.E.C.

REFERENCES

[1] Chu, Y.G. and Cantor, C.R. (1979) Nucleic Acids Res. 6, 2363–2379.
[2] Moore, P.B. and Laughrea, M. (1979) Nucleic Acids Res. 6, 2355–2361.
[3] Labischinsky, H. and Subramanian, A.R. (1979) Eur. J. Biochem. 95, 359–366.
[4] Suryanarayana, T. and Subramanian, A.R. (1979) J. Mol. Biol. 127, 41–54.
[5] Szer, W. and Leffler, S. (1974) Proc. Natl. Acad. Sci. USA 71, 3611–3615.
[6] Szer, W., Hermoso, J.M., and Leffler, S. (1975) Proc. Natl. Acad. Sci. USA 72, 2325–2329.
[7] Van Dieijen, G., Van der Laken, C.J., Van Knippenberg, P.H. and Van Duin, J. (1975) J. Mol. Biol. 93, 351–366.
[8] Sobura, J.E., Chowdhury, M.R., Hawley, D.A. and Wahba, A.J. (1977) Nucleic Acids Res. 4, 17–29.
[9] Steitz, J.A., Wahba, A.J., Laughrea, M. and Moore, P.B. (1977) Nucleic Acids Res. 4, 1–15.
[10] Kolb, A., Hermoso, J.M. Thomas, J.O. and Szer, W. (1977) Proc. Natl. Acad. Sci. USA 74, 2379–2383.
[11] Kamen, R., Kondo, M., Römer, W. and Weissmann, C. (1972) Eur. J. Biochem. 31, 44–51.
[12] Carmichael, G.G., Landers, T.A and Weber, K. (1976) J. Biol. Chem. 251, 2744–2748.
[13] Moore, P.B. (1971) J. Mol, Biol. 60, 169–184.
[14] Acharya, A.S. and Moore, P.B. (1973) J. Mol. Biol. 76, 207–221.
[15] Subramanian, A.R. (1980) J. Biol. Chem. 255, 3227–3229.
[16] Thomas, J.O., Kolb, A. and Szer, W. (1978) J. Mol. Biol. 123, 163–176.
[17] Laughrea, M. and Moore, P.B. (1978) J. Mol. Biol. 121, 411–430.
[18] Cole, P.E., Sawchyn, I. and Guerrier-Takada, C. (1982) J. Biol. Chem. in press.
[19] An, G., Bendiak, D., Mamelak, L. and Friesen, J. (1981) Nucleic Acids Res. 9, 4163–4172.
[20] Arai, K., Clark, B.F.C., Duffy, L., Jones, M.D., Kaziro, Y., Laursen, R.A., L'Italien, J., Miller, D.L., Nagarkatti, S., Nakamura, S., Nielsen, K.M., Petersen, T.E., Takahashi, K. and Wade, M. (1980) Proc. Natl. Acad. Sci. USA 77, 1326–1330.
[21] Kimura, M., Foulaki, K., Subramanian, A.R., Wittmann-Liebold, B. (1982) Eur. J. Biochem. 123, 37–53.
[22] Mekler, P. (1981) Ph.D. Thesis, Universität Zürich, pp. 77–88.
[23] Fairclough, R.H. and Cantor, C.R. (1979) J. Mol. Biol. 132, 587–601.
[24] Cantor, C.R. and Schimmel, P. (1980) Biophysical Chemistry Part II: Techniques for the Study of Biological Structure and Function, pp 454–466, W.H. Freeman and Company, San Francisco, California.
[25] Lavalette, D., Armand, B. and Pochon, F. (1977) Proc. Natl. Acad. Sci. USA 74, 1407–1411.
[26] Draper, D.E. and von Hippel, P.H. (1979) Proc. Natl. Acad. Sci. USA 76, 1040–1044.
[27] Laughrea, M. and Moore, P.B. (1977) J. Mol. Biol. 112, 399–421.
[28] Segur, J.B. and Oberstar, H.E. (1951) Ind. Eng. Chem. 43, 2117–2120.
[29] Eshaghpour, H., Dieterich, A.E., Cantor, C.R. and Crothers, D.M. (1980) Biochemistry 19, 1797–1805.
[30] Yuan, R.C., Steitz, J.A., Moore, P.B. and Crothers, D.M. (1979) Nucleic Acids Res. 7, 2399–2418.

[31] Draper, D.E. and von Hippel, P.H. (1978) J. Mol. Biol. 122, 339–359.
[32] Draper, D.E., Pratt, C.W. and von Hippel, P.H. (1977) Proc. Natl. Acad. Sci. USA 74, 4786–4790.
[33] Lipecky, R., Kohlschein, J. and Gassen, H.G. (1977) Nucleic Acids Res. 4, 3627–3642.
[34] Mülsch, A., Colpan, M., Wollny, E., Gassen, H.G. and Riesner, D. (1981) Nucleic Acids Res. 9, 2367–2385.
[35] Boedtker, H. and Gesteland, R.F. (1975) in RNA Phages (Zinder, N. ed) pp. 1–28, Cold Spring Harbor Laboratory, New York.
[36] Goelz, S. and Steitz, J.A. (1977) J. Biol. Chem. 252, 5177–5179.
[37] Senear, A.W. and Steitz, J.A. (1976) J. Biol. Chem. 251, 1902–1912.