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Energy transfer in complexes of *E. coli* single-stranded DNA-binding protein with single-stranded poly-(2-thiouridylic acid)

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The complexes of point-mutated *Escherichia coli* single-stranded DNA-binding protein (**Eco** SSB) with poly-(2-thiouridylic acid) (poly S^2U) have been studied by optical detection of magnetic resonance spectroscopy (ODMR). Previous work has determined that two of four tryptophan (Trp) residues in **Eco** SSB undergo stacking interactions with nucleic acid bases. Selective photoexcitation of S^2U bases was performed and subsequent triplet→triplet energy transfer from S^2U to nearby Trp residues in the protein took place. The zero-field splitting (ZFS) parameters and sublevel kinetics were determined for each Trp residue sensitized by S^2U. The sublevel lifetimes of the two sensitized residues are similar to those of normal Trp. The ZFS parameters, on the other hand, show a dramatic reduction relative to those of the uncomplexed protein, implying a more polarizable environment for the sensitized Trp residues and/or charge transfer interactions with the S^2U bases.

Spectroscopy, ODMR; DNA binding protein, single-stranded; Energy transfer, triplet-triplet

1. INTRODUCTION

Single-stranded DNA binding proteins (SSB) bind to DNA with no sequence specificity and are believed to be involved in many important metabolic functions including repair, replication and recombination [1]. *Escherichia coli* SSB (**Eco** SSB) has been the object of extensive study and recently we have employed optical detection of magnetic resonance (ODMR) spectroscopy of the excited triplet state to study complexes of **Eco** SSB with single-stranded homopolynucleotides [2,3]. From these studies we learned that short range interactions between tryptophan (Trp) residues of the protein and nucleic acid bases play an important role in the stabilization of the complex. **Eco** SSB has 4 Trp residues (at positions 40, 54, 88 and 135) among its 177 amino acid residues, and based on studies done in complexes of point-mutated **Eco** SSB, where one Trp residue at a time is substituted by phenylalanine (Phe), it was determined that only Trp^{40} and Trp^{54} undergo close range interactions, and probably are stacked with nucleic acid bases [4].

In this study, we report further ODMR investigations of two single point-mutated **Eco** SSB proteins complexed with a unique polynucleotide, poly-(2-thiouridylic acid) (poly S^2U). The triplet state energy of poly S^2U is higher than that of Trp. Upon complex formation, we observe triplet-triplet energy transfer from S^2U bases to Trp, by selectively exciting the triplet state of S^2U and monitoring Trp emission. By using this method on the mutants **Eco** SSB W40F and **Eco** SSB W54F, we have obtained the triplet state zero-field splitting (ZFS) parameters, and the triplet state sublevel kinetics of each sensitized Trp, Trp^{54} in the former and Trp^{40} in the latter mutated SSB. The low temperature phosphorescence of 2-thiouracil has been studied previously [5], and the triplet state of 1-methyl-2-thiouracil (1-Me-S^2U) in 1-Me-U has been investigated by ODMR [6]. ODMR results for poly S^2U are presented in this study.

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2. MATERIALS AND METHODS

Poly S^2U was a generous gift from Professor Karl Scheit, Max-Planck-Institut für Biophysikalische Chemie, Göttingen. The point mutated **Eco** SSB proteins were prepared as described previously [7]. Both protein and poly S^2U were dialyzed against a 10 mM potassium phosphate buffer, pH 8.4, containing 20 mM NaCl. Poly S^2U forms a stable double helix with a melting point of 62.5°C in 50 mM NaCl, pH 7 [8]. At the lower NaCl concentration and higher pH used in this work, the melting point of the polymer (3.0×10^{-5} M) in the presence of **Eco** SSB (2.5×10^{-6} M) was lowered to ~44°C while in the absence of the SSB it melted at ~53°C. The complexes for ODMR studies were prepared with a final protein concentration of $\sim 10^{-4}$ M and a 12-fold molar excess of polynucleotide. All samples contained 25% (v/v) of ethylene glycol as cryosolvent. ODMR samples were incubated at 55°C for 30 min and then rapidly frozen by plunging the sample directly into liquid helium. The presence of **Eco** SSB/poly S^2U complexes in the chilled sample was verified by the observation of Trp phosphorescence via $T \longrightarrow T$ energy transfer upon selective excitation of S^2U.

Phosphorescence and ODMR measurements were conducted using the apparatus described previously [2]. As a control, the pure protein was also studied under the conditions mentioned above using a 16 nm wide excitation band centered at 295 nm. The protein/poly S^2U complexes, on the other hand, were excited at ~365 nm, selected using a $CuSO_4$ (12 cm, 50 g/l) filter, a CS-7-60 band pass filter and a WG-345-2 cut off filter. The excitation is in the $T_1 \longleftarrow S_0$ band of

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S^2U ensuring direct excitation of its triplet state. No phosphorescence from Trp was observed when the protein samples were excited under these conditions in the absence of poly S^2U, since Trp does not absorb significantly in the $T_1 \longleftarrow S_0$ band of S^2U. Poly S^2U emits short-lived phosphorescence in the same spectral region as Trp, so time resolved modifications were applied. A shutter routine was used to monitor Trp phosphorescence, in which the sample was excited for 100 ms, followed by a delay of 100 ms prior to the opening of the emission shutter for a period of 300 ms. In order to avoid interference from poly S^2U phosphorescence, the slow passage ODMR measurements were conducted [9] as follows. After cessation of optical pumping, the sample emission was allowed to decay, during which the microwave frequency was swept. This decay spectrum was then modified by subtracting from it the phosphorescence decay in the absence of a microwave sweep. All modified slow passage responses were corrected for transient effects by extrapolation to zero microwave sweep rate. For the sublevel kinetics measurements, the microwave induced delayed phosphorescence (MIDP) method was employed at 1.2 K, as described previously [2], except that a delay of 350 ms between the closing of excitation and opening of emission shutters was used in order to eliminate all detectable S^2U emission. The lifetime of the T_x sublevel (the only radiative sublevel of Trp) was obtained by fitting the decay tail of the MIDP response.

3. RESULTS AND DISCUSSION

An essential feature of these measurements is to ensure that the Trp residues which we observe are excited exclusively by energy transfer from S^2U. Excitation of S^2U in its $S_1 \longleftarrow S_0$ band, which extends to the red of the Trp absorption, could not be done without also exciting Trp. We found, however, that we could produce the Trp triplet state exclusively by energy transfer from S^2U using direct $T_1 \longleftarrow S_0$ excitation of the latter at ~365 nm.

The phosphorescence spectrum of poly S^2U is shown in fig.1A. The sensitized time-delayed spectrum of the **Eco** SSB W40F mutant complexed with poly S^2U is shown in fig.1B. The well resolved vibronic bands are characteristic of Trp phosphorescence. The experimental results obtained for the Trp residues of the SSB mutants and their complexes with poly S^2U are summarized in table 1. Also included are data for 1-Me-S^2U [7] and for poly S^2U. The uncomplexed protein, **Eco** SSB W88F,W135F, contains only the two Trp residues that are involved in close range interaction with nucleic acid bases upon complex formation. Data on this double mutated SSB and its complex with poly dT [2] are also given in table 1. The phosphorescence 0,0 band of the **Eco** SSB complexes with poly S^2U are red-shifted by over 2 nm relative to the uncomplexed protein, but in this case, only the Trp residues that are sensitized by the poly S^2U bases emit. The lifetimes of the sensitized Trp residues given in table 1 show a slight reduction relative to the uncomplexed protein.

In contrast with the phosphorescence lifetimes which show little change upon interaction of **Eco** SSB with poly S^2U, the ZFS of the sensitized Trp residues undergo a pronounced reduction relative to their values in the uncomplexed protein. Both |D| and |E| are greatly reduced, indicating that the average separation of the unpaired electrons increases for the triplet states

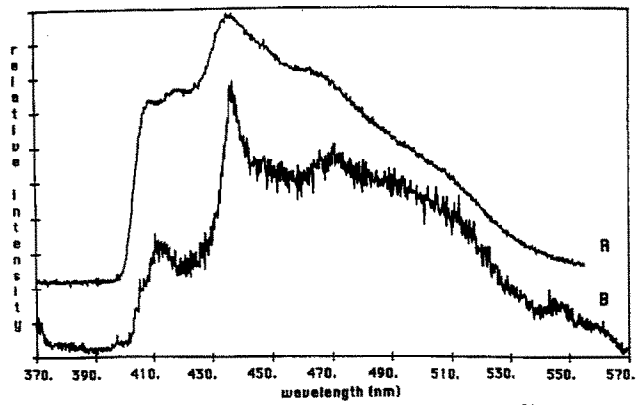

Fig.1. A: Poly S^2U phosphorescence spectrum. B: Trp^{54} sensitized phosphorescence in **Eco** SSB W40F + poly S^2U. The shutter routine described in the text was used and the sample was excited at ~365 nm in the S^2U $T_1 \longleftarrow S_0$ absorption band. For both A and B, the spectra were obtained at 4.2 K with a scan rate of 20 nm/min.

of both Trp^{40} and Trp^{54} upon complex formation with poly S^2U (table 1). Similar effects observed for Trp 54 upon complex formation of **Eco** SSB with poly dT [2,10] have been attributed to the effects of stacking interactions with thymine. The ZFS reductions are considerably larger with poly S^2U than with poly dT. Stacking interactions with nucleic acid bases, especially those containing polarizable atoms such as S, lead to an increase in the polarizability of the environment and a corresponding expansion of the triplet state wavefunction. This effect leads to a reduction of |D| and |E|. Charge transfer contributions to the triplet state of Trp can also lead to a reduction in the ZFS, particularly in

Table 1

Triplet state properties of point-mutated **Eco** SSB/poly S^2U complexes and related systems

Sample	$\lambda_{0,0}$ (nm)	Lifetimes[e] (s)	|D| (GHz)	|E| (GHz)
1-Me-S^2U[a]	390.0	0.007 0.041 0.153	8.679	2.182
Poly S^2U[b]	410	0.008 0.026 0.122	8.26	2.18
Eco SSB W88F, W135F[c]	410.9	6.1	2.99	1.26
Eco SSB W88F, W135F + poly dT[d]	415.1			
Eco SSB W40		5.1	3.03	1.29
Eco SSB W54		2.6	2.75	1.18
Eco SSB W54F + poly S^2U	414	5.5	2.70	1.10
Eco SSB W40F + poly S^2U	413	5.4	2.64	1.11

[a] Polycrystalline sample in 1-Me-U host, except for the lifetimes, which were measured in an ethyl ether/isopentane/ethanol (5:5:2) glass. Data from [7]

[b] In aqueous buffer, 25% ethylene glycol

[c] Measurements for the protein reported in [2] were repeated in this work in the solvent system reported in the text, because of changes in the microenvironment of Trp

[d] Data from [2]

[e] Measurements in this work were made at 4.2 K

the magnitude of |D|. The fact that both |D| and |E| are reduced, and the effect is larger for the more polarizable poly S^2U than for poly dT, suggests that local polarizability effects are important. Further evidence for the importance of local polarizability in determining |D| and |E| is provided by the complexes of **Eco** SSB with poly Br^5U [2] in which the ZFS parameters of Trp^{40} and Trp^{54} also are reduced relative to their values in the uncomplexed protein.

The triplet state sublevel kinetics of Trp obtained from MIDP measurements in the poly S^2U complexes are compared with those of Trp [11] in table 2. The sensitized Trps have a relatively long normal lifetime; the sulfur in S^2U does not produce a noticeable external heavy atom effect (HAE). This is in contrast with complexes of **Eco** SSB with poly Br^5U and poly Hg^5U which exhibit large external HAE [2], providing direct evidence for stacking interactions. The magnitude of the HAE, especially with poly Hg^5U is consistent only with an *out of plane* location of the heavy atom. The sublevel lifetimes of Trp^{40} and Trp^{54} in the mutant complexes with S^2U are very similar, as are the average lifetimes. The phosphorescence decay lifetimes measured at 4.2 K (table 1), when rapid spin-lattice relaxation averages the properties of all 3 sublevels, are in good agreement with the calculated average lifetimes (table 2), obtained from the individual sublevel kinetics measured at 1.2 K.

The triplet states of Trp^{40} and Trp^{54} in **Eco** SSB, known to undergo close-range interactions with polynucleotide bases upon complex formation, were populated selectively via triplet-triplet energy transfer in the complex with poly S^2U by direct excitation of the triplet state of S^2U. Using **Eco** SSB mutants W54F and W40F, it was possible to obtain the triplet state ZFS |D| and |E| parameters, as well as the sublevel kinetics for Trp^{40} and Trp^{54} individually. The ZFS of these residues were found to be reduced significantly below their values in the uncomplexed protein. Triplet-triplet energy transfer involves the direct transfer of energy from the excited donor to the acceptor via an exchange mechanism [12]. This requires an overlap of their electron clouds. The effective range for this process is ~0.5 nm, showing that a close range interaction probably involving stacking exists between the Trp^{40} and Trp^{54} residues and polynucleotide bases.

Table 2

Sublevel triplet state kinetics of Trp^{40} and Trp^{54} sensitized by poly S^2U complexed with single point-mutated **Eco** SSB proteins

Sample	k_x	k_y (s^{-1})	k_z (s^{-1})	k_{av}[a]	τ_{av} (s)
Eco SSB W54F + poly S^2U	0.322	0.112	0.077	0.170	5.9
Eco SSB W40F + poly S^2U	0.333	0.116	0.093	0.180	5.6
Trp[b]	0.24	0.119	0.038	0.134	7.5

[a] $k_{av} = \frac{(k_x + k_y + k_z)}{3} = \frac{1}{\tau_{av}}$

[b] In ethylene glycol/water (1:1, v/v), pH = 7. From [11]

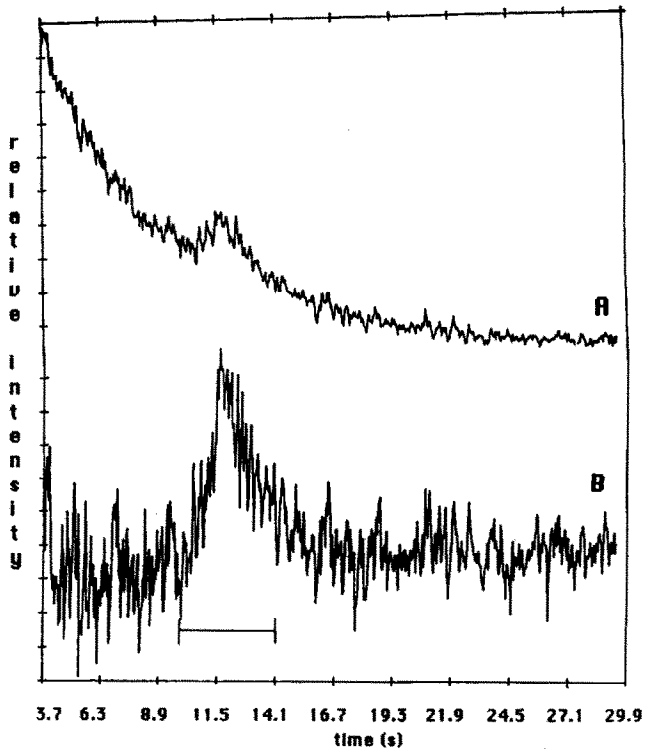

Fig.2. Delayed slow passage of |D|-|E| ODMR transition of Trp^{40} in **Eco** SSBW54F + poly S^2U. A: Microwaves are swept from 1.50 to 2.00 GHz (shown by horizontal bar) as the sample phosphorescence decays. B: Same ODMR transition after subtraction of the free decay. The sweep rate was 125 MHz/s, averaged over 22 scans.

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