ACTIVITY OF ENDONUCLEASE S_1 IN DENATURING SOLVENTS: DIMETHYSULFOXIDE, DIMETHYLFORMAMIDE, FORMAMIDE AND FORMALDEHYDE

James R. Hutton* and James G. Wetmur

*Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115 and Department of Microbiology, Mount Sinai School of Medicine, CUNY, New York, New York 10029

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Summary

The endonuclease S_1 from Aspergillus oryzae is shown to be active in aqueous solutions of greater than 60 percent formamide, 50 percent dimethylsulfoxide, 30 percent dimethylformamide and in 2 percent formaldehyde. The melting profile of T7 DNA is determined by S_1 digestion in 50 percent formamide and is found to agree with the optical melting profile. The single-strand specificity of S_1 is retained in 50 percent formamide at 37°C, 3°C below the DNA melting temperature. S_1 is shown to be active at temperatures greater than 5°C above the DNA melting temperature in this solvent.

INTRODUCTION

The single-strand specific endonuclease S_1 of Asperquillus oryzae, which was first isolated by Ando[1] and subsequently studied by others[2-4], has proven convenient and quite useful for monitoring the progress of a DNA renaturation experiment[2]. It has also been shown to be capable, under the proper conditions, of cleaving both strands of circular covalently closed, superhelical SV 40 DNA to generate unit length linear duplex molecules with intact single strands and to be capable of generating a duplex cleavage at a position containing a single-strand nick[5] or a mismatch[6]. It is also a potential tool for studying low melting regions in DNA as has been done with mung bean nuclease 1[7]. The convenience and usefulness of S_1 nuclease could be extended if it were shown to be active in solvents which destabilize

the DNA duplex while maintaining stringent specificity requirements for duplex formation[8,9]. This potential is demonstrated below.

MATERIALS AND METHODS

Preparation and Assay of Endonuclease S_1: Endonuclease S_1 was prepared from Taka-Diastase powder (Parke-Davis; Detroit, Michigan) as previously described (10). The reaction conditions used are given in appropriate figure legends.

DNA: The DNAs of T7 and λ were prepared as previously described (10,11). To obtain radiolabeled DNA, ^{32}P-labeled phosphate or ^{3}H-labeled thymidine was added to the E. coli minimal growth medium two replication cycles before infection or induction. Calf thymus and salmon sperm DNAs were obtained from Sigma Chemical Co.

Reaction Assay: The amount of DNA degradation was monitored either by measuring the fraction of DNA dialyzable as described in figure 2 or by measuring the fraction of radioactivity soluble in 10% TCA. For the TCA assay, an aliquot of the reaction solution was mixed with an equal (or larger) volume of 2.5 mg/l salmon sperm DNA; TCA was added to give 10% (v/v) final concentration; the solution was chilled at 0°C, and centrifuged for 10 min. at 12,000g. An aliquot of the supernatant was counted in a triton based fluor (15.0g PPO, 0.325g POPOP, 1.0 liters toluene, 950 ml Triton X-100, and 200 ml of H_2O).

Reagents: All chemicals used were reagent grade. DMSO was obtained from Fisher and Malinckrodt, DMF from Eastman and Fisher, and formamide and formaldehyde from Fisher. These were used without further purification.

Melting temperature: The melting temperature of T7 DNA in a solution of 50% formamide, 5% glycerol, 10^{-4}M $ZnSO_4$, 0.1M NaAc plus HCl to pH 4.25 was determined by S_1 digestion (see figure 5) and by absorbance. The fraction of DNA which is single stranded was assayed with S_1 in two ways: digestion with S_1 at the melting temperature or incubation of the DNA solution at the melting temperature followed by addition of S_1 and incubation at 37°C. The absorbance-temperature profile was determined using a Beckman Acta III spectrophotometer while increasing the solution temperature at a rate of 0.5°C per minute as previously described[11], or by using a water-jacketed cuvette and a recirculating Haake bath in conjunction with a Cary spectrophotometer to follow the hyperchromic shift at 275nm after a 10 min. equilibration time at each temperature measured.

RESULTS AND DISCUSSION

The dependence on the concentrations of formamide, DMF and DMSO of two solution parameters, buffer pH and DNA melting temperature (T_m), which are important for single strand specific enzymatic DNA hydrolysis are shown in figure 1. As seen from figure 1a, in the region of 0 to 40%, DMSO, formamide and DMF lower the T_m by approximately 0.68, 0.72 (8,9) and 0.87°C per

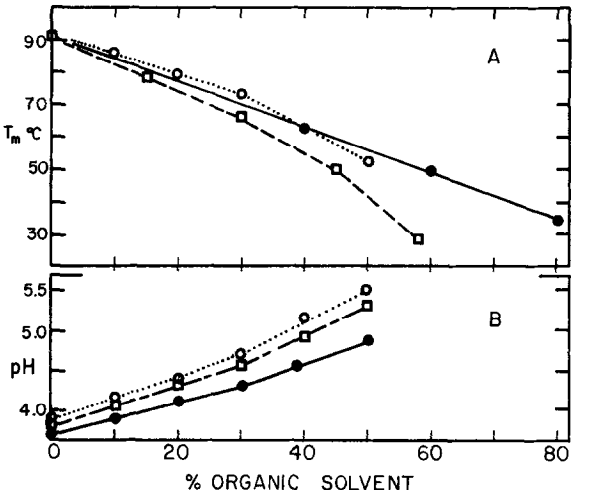

Figure 1: Effect of organic chemicals on solution pH and DNA duplex stability. Panel A. The dependence of the melting temperature, T_m, of λ DNA in 0.2 M NaCl on the percent (volume/volume) DMSO: O ; formamide: ● ; dimethylformamide: □ . Panel B. pH of Na $C_2H_3O_2$ buffer as measured with a Radiometer Copenhagen pH meter equipped with a type GK 2301c combination glass electrode as a function of percent (v/v) of organic solvent in the solution. Same symbols as panel A.

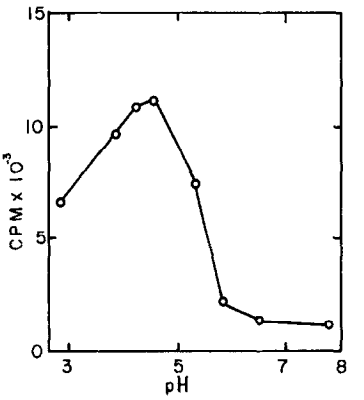

Figure 2: S1 activity as a function of measured pH in 40 percent formamide. Activity is expressed as the number of cpm rendered TCA soluble upon incubation of 37°C for 10 min using a reaction mixture consisting of 15,000 cpm of heat denatured ^3H-thymine labeled DNA, 40 percent formamide, 0.2 M Na $C_2H_3O_2$ buffer with aqueous pH to give the desired solution pH, and endonuclease S1 sufficient to solubilize 80% of the DNA at pH 4.5 under these reaction conditions with no formamide in a total volume of 0.11 ml.

percent (v/v), respectively. As indicated in figure 1b, in the region of 0 to 30% organic solvent, for acetate buffer, pH3.0-5.0, an increase of solution pH of 0.20, 0.25 and 0.27 pH units per 10% organic solvent was obtained for formamide, DMF and DMSO respectively. Above 30% organic solvent the dependence of the pH on organic solvent becomes slightly greater. S_1 displays the same activity-pH profile in 40% formamide, shown in figure 2, as was observed in 0% formamide by Ando[1]. The single strand nuclease activity has a reasonably flat pH response in the optimal region of pH, 4.0-5.0, and a sharp drop-off of activity is observed on the high pH side of the profile in the pH range 5 to 6.

The relative activity of nuclease S_1 as a function of the concentration of formamide, DMSO, DMF and formaldehyde (2% only) in the reaction mixture is

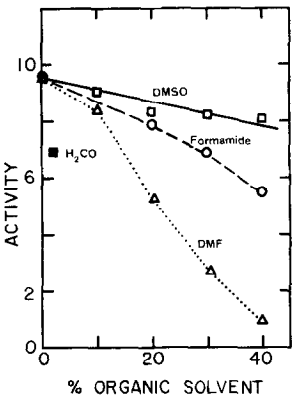

Figure 3: The relative activity of S_1 expressed as the number of A_{260} units of DNA nucleotides rendered dialyzable versus the percent (v/v) of organic solvent contained in the reaction medium. Each reaction mixture contained 11.0 A_{260} units of heat denatured calf thymus DNA, 0.1 M acetic acid, NaOH to give a final solution pH of 4.2, 2×10^{-4}M $ZnCl_2$ and 0.2 ml of S_1 preparation (A_{280} = 0.3) in a total volume of 2.2 ml, which was incubated at 37°C for one hour. After pH8 dialysis overnight, "A_{260}xml solution" was measured for samples and control.

indicated in figures 3 and 4. Figure 3 presents the relative activity of S_1 as measured by A_{260} units of heat denatured calf thymus DNA rendered dialyzable at various concentrations of each of these chemicals, and figure 4 presents similar data obtained for formamide and DMF using TCA solubilization of heat-denatured radio-labeled DNA to assay the enzymatic activity. S_1 has surprisingly high activity in all the solvent systems studied. These systems, therefore, appear to be potentially useful for studying secondary structure of DNA using S_1 digestion as a probe. Under the reaction conditions of figure 4, S_1 has essentially the same activity in 50% formamide at 37°C as in 0% formamide. These results are contrary to those reported by Case and Baker[12] in which they find S_1 activity much reduced in 30% and completely absent in 40% formamide solutions.

Since S_1 activity appeared to be little affected by the presence of 50% formamide under the reaction conditions of figure 4, we decided to look at the substrate specificity and the activity-temperature profile in this system. Figure 5 presents the results of these studies. Curve A depicts the relative

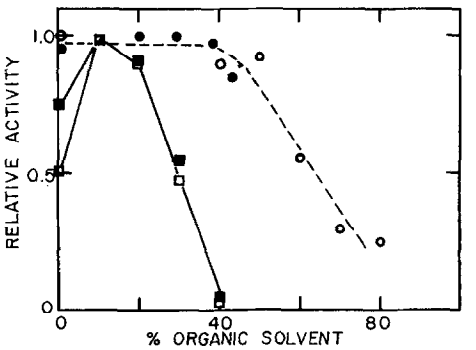

Figure 4: Dependence of S_1 activity on the concentration of formamide and DMF. Heat denatured 3H-thymidine labeled DNA was incubated with S_1 at 36°C in a reaction solution containing the indicated % of organic solvent, 5% glycerin, 10^{-4}M $ZnSO_4$, 0.1 M Na $C_2H_3O_2$ plus HCl to pH 4.25.

○, □ - data obtained using reaction conditions such that 38% of the counts were rendered TCA soluble with 0% formamide at 36°C.

●, ■ - data obtained using reaction conditions such that 70% of the counts were rendered TCA soluble with 0% formamide at 36°C.

●, ○: formamide; ■, □: DMF.

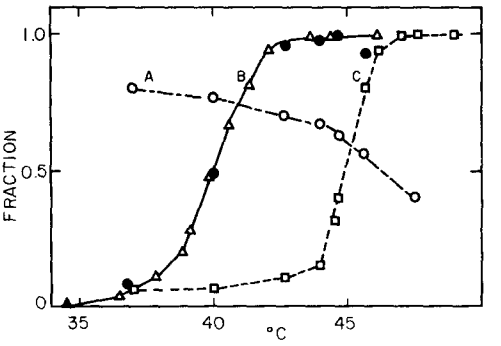

Figure 5: DNA melting profile measured with S_1, and S_1 activity in 50% formamide as a function of temperature.
Curve A (O) - Fraction of counts (2,100 cpm total for each data point) of denatured ^3H-labeled T7 DNA rendered TCA soluble in 15 min. at the indicated temperature in a solution of 50% formamide, 5% glycerol, 10^{-4}M $ZnSO_4$, 0.1 M Na $C_2H_3O_2$ plus HCl to pH 4.25.
Curve B - Fraction of hyperchromic shift (Δ) measured at 275 nm, and the fraction (●) of duplex DNA rendered susceptible to S_1 degradation in the reaction solution defined in curve A above (i.e. the fraction of ^{32}P-labeled double-stranded T7 DNA rendered TCA soluble, normalized to the amount of admired ^3H-labeled heat-denatured T7 DNA solubilized) on simultaneous denaturation and S_1 digestion by incubating for 15 min. at the indicated temperature.
Curve C - Fraction of double-stranded ^{32}P-labeled T7 DNA (□) rendered susceptible to S_1 digestion by preincubation for 15 min. in 50% formamide at the indicated temperature (solution same as defined for curve A, except that it contains no S_1 or glycerin). S_1 was then added to the solution and digestion carried out at 37°C. The data was normalized to the fraction of admixed single-stranded DNA TCA solubilized as described for curve B.

single-strand endonucleolytic activity of S_1 in this system. At several degrees above the melting temperature of T7 DNA, the enzyme retains considerable activity. Curve B depicts the melting profile obtained for T7 DNA by monitoring the hyperchromic shift of A_{275} and an identical profile obtained by S_1 digesting the DNA mixture at the temperature indicated. Curve C depicts the apparent melting profile obtained by preincubating the DNA in 50% formamide at the indicated temperature and then incubating with S_1 at 37°C to assay the fraction of duplex ^{32}P label rendered single stranded. As is obvious from curves B anc C, within the limits of the TCA solubility assay used, S_1 retains its single-stranded DNA specificity in 50%

formamide over the temperature range studied. Endonuclease S_1 in 50% formamide is potentially a very useful system for application in studies probing the secondary structure of DNA.

ACKNOWLEDGEMENTS

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