Activation of S_1 nuclease at neutral pH

José A. Esteban, Margarita Salas* and Luis Blanco

Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

Submitted July 17, 1992

S_1 nuclease is a single-strand-specific endonuclease that degrades DNA and RNA to nucleoside 5'-monophosphates. It has an acid pH optimum (4.0–4.5) and requires Zn^{2+} or Co^{2+} for maximal activity (1). This enzyme is widely used in DNA manipulation, mainly for the characterization of mRNAs (S_1 -mapping) (2) and the study of the tertiary structure of DNA (3, 4). A distinct disadvantage of the S_1 nuclease is that it is active only at low pH.

Here we report that the metal ion requirements of S_1 change at neutral pH and that this nuclease can be used at pH 7.5 with Mg^{2+} as metal activator (S_1 is essentially inactive in the presence of Mg^{2+} at acid pH; 1). As an example, we describe the degradation of a partially duplex DNA molecule with a 3' ssDNA tail. The longest strand is labeled at the 5'-end (see Figure 1). S_1 cleavage is expected to shorten the 5'-labeled strand yielding a blunt duplex molecule with the length of the non-protruding strand. This assay allows a quantitative estimation of the S_1 activity in different conditions of pH and metal activation, and also, a direct inspection of its ability to discriminate ss versus dsDNA. Lanes 1–5 in Figure 1 show the S_1 -catalyzed degradation of the partial duplex at different S_1 doses in standard conditions (1 mM Zn^{2+} , pH 5.0). Using intermediate S_1 concentrations (lane 3) most of the original substrate was made blunt-ended, although the ssDNA positions closest to the double-stranded region were more resistant to S_1 cleavage. Zn^{2+} -activation using neutral pH conditions was much more inefficient (lanes 6–10), S_1 being unable to fully degrade the single-stranded portion of the molecule at the highest concentration tested (lane 10). Lanes 11–15 show the degradation obtained with 20 mM Mg^{2+} (this concentration was shown to be the optimal one). Comparing lanes 1–5 with lanes 11–15 it can be concluded that S_1 is 10-fold more efficient when activated with Zn^{2+} at acid pH than when activated with Mg^{2+} at neutral pH. Nevertheless, in neutral conditions, Mg^{2+} -activation represents a 100-fold stimulation over Zn^{2+} -activation. Moreover, the degradation pattern obtained with 1 mM Zn^{2+} at pH 5.0 was very similar to the one obtained with 20 mM Mg^{2+} at pH 7.5 (compare lanes 2 and 3 with lanes 13 and 14). These results have been confirmed using different oligonucleotides as well as M13 ssDNA (not shown).

Despite the 10-fold reduction of activity, we have shown that S_1 can be used in neutral pH conditions for its most usual application: the specific removal of ssDNA. The possibility of using S_1 at neutral pH will enlarge the usefulness of this enzyme, allowing the study of protein-DNA interactions that generally occur at neutral pH. The suitability of S_1 over other

cleaving agents is based on its lack of sequence-specificity and harmful effects on proteins (as other chemical agents potentially have). The theoretical interest of this finding concerning the metal-assisted mechanism of S_1 cleavage remains open.

ACKNOWLEDGMENTS

This investigation has been aided by research grant 5R01 GM27242-13 from the National Institutes of Health, by grant no. PB90-0091 from Dirección General de Investigación Científica y Técnica, by grant BIOT CT91-0268 from European Economic Community and by an institutional grant from Fundación Ramón Areces.

REFERENCES

1. Vogt, V.M. (1973) *Eur. J. Biochem.* **33**, 192–200.
2. Berk, A.J. and Sharp, P.A. (1977) *Cell* **12**, 721–732.
3. Beard, P., Morrow, J.F. and Berg, P. (1973) *J. Virol.* **12**, 1303–1313.
4. Lilley, D.M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6468–6472.

Figure 1. Cleavage of a partially duplex DNA molecule by S_1 nuclease. A 5'-labeled (indicated with a dot) 48-mer oligonucleotide (0.5 ng, 6000 cpm), hybridized with a 29-mer oligonucleotide complementary to the 5' end, was used as substrate. The incubation mixture contained 50 mM NaCl, 5% glycerol, 50 mM NaOAc pH 5.0 (lanes 1–5) or 50 mM Tris-HCl pH 7.5 (lanes 6–15), 1 mM $ZnCl_2$ (lanes 1–10) or 20 mM $MgCl_2$ (lanes 11–15) and S_1 nuclease as follows: 3.5×10^{-3} u (lanes 1, 6 and 11), 3.5×10^{-2} u (lanes 2, 7 and 12), 0.35 u (lanes 3, 8 and 13), 3.5 u (lanes 4, 9 and 14) or 35 u (lanes 5, 10 and 15). S_1 unit, as defined by the manufacturer (Pharmacia). Reactions were carried out for 5 min at 25°C, stopped with EDTA to 20 mM and analyzed by PAGE (8% polyacrylamide, 8 M urea).

* To whom correspondence should be addressed