Biochimica et Biophysica Acta, 1202 (1993) 13–21

BBAPRO 34569

Characterization of *Serratia marcescens* nuclease isoforms by plasma desorption mass spectrometry

Jytte Pedersen [a], Maria Filimonova [b], Peter Roepstorff [c] and Kirsten Biedermann [a]

[a] *Department of Biotechnology. The Technical University of Denmark, Lyngby (Denmark),* [b] *Department of Microbiology, Kazan State University, Kazan (Russia) and* [c] *Department of Molecular Biology, Odense University, Odense M (Denmark)*

(Received 27 January 1993)
(Revised manuscript received 5 May 1993)

Key words: Nuclease; Enzyme isoform; Primary structure; Plasma desorption mass spectrometry; (*S. marcescens*); (*E. coli*)

Isoforms of *Serratia marcescens* nuclease found in the natural nuclease produced by *S. marcescens* and in recombinant nuclease produced by *Escherichia coli* were structurally characterized by peptide mapping using plasma desorption mass spectrometry. The nuclease isoforms produced and secreted from *S. marcescens* B10M1, which are present in much greater amounts than in *S. marcescens* W225 nuclease produced by *E. coli*, were characterized completely and the information used to facilitate characterization of the recombinant nuclease isoforms. After purification of the nuclease the isoforms were separated on a DEAE-cellulose anion-exchange column and then digested with endoproteinase Lys-C. The peptides generated were isolated by reverse-phase HPLC and their molecular masses determined by plasma desorption mass spectrometry. Comparison of the peptides from the native nuclease, Sm2, and the two isoforms, Sm1 and Sm3, revealed that they differed only in the N-terminus, the latter being found to lack three amino acids in Sm1 and one amino acid in Sm3. No interior post-translational changes were found in either of the three isoforms. Using this information we were able to confirm that Sm1, the isoform lacking three amino acids, was also present in very small amounts in recombinant *S. marcescens* W225 nuclease produced and excreted by *E. coli*.

Introduction

Escherichia coli is frequently used for producing heterologous proteins, including some that are secreted from the cells; however, it has recently been observed that the secreted products can be heterogeneous [1–3]. A greater understanding of the nature and origin of such heterogeneities is essential for evaluating product quality and production systems in general. Our standard laboratory model for production studies is nuclease originating from *Serratia marcescens* W225 but produced and secreted by *E. coli* [4,5]. Studies made using the very sensitive zymogram technique in conjunction with isoelectric focusing indicated that this recombinant nuclease contains small amounts of nuclease-active heterogeneities [6]. It was, therefore, attempted to isolate and purify the nuclease isoforms, and to characterize them by peptide mapping after proteolytic cleavage and mass determination of the resultant peptides by plasma desorption mass spectrometry; however, as the amounts of isoforms present were so small and varied from batch to batch, complete characterization was unsuccessful.

Isoforms have also been found in nuclease from *S. marcescens* B10M1; in this case, however, the amounts present are rather large, and therefore suitable for characterization. In addition, the two isoforms identified, named Sm1 and Sm2 after the order in which they elute during anion-exchange chromatography [7,8], seem also to be present in *S. marcescens* W225 nuclease produced by *E. coli*. Thus Sm2 has the same isoelectric point (p*I* 6.8) as the recombinant native nuclease (rSm2), i.e., that encoded by the *S. marcescens* W225 *nuc* gene, and Sm1 has the same isoelectric point (p*I* 7.4) as one of the other recombinant nuclease isoforms (rSm1) produced by *E. coli*. On this background we decided to isolate and characterize nuclease isoforms from *S. marcescens* B10M1 in order to facilitate further characterization of the *S. marcescens* W225 nuclease isoforms from *E. coli*. This was achieved by peptide mapping in combination with plasma desorption mass spectrometry. We present the complete characterization of isoforms found in nuclease preparations

Correspondence to: K. Biedermann, Department of Biotechnology, Building 223, The Technical University of Denmark, DK-2800 Lyngby, Denmark.