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The amino-terminal sequence of the *Xenopus laevis* mitochondrial SSB is homologous to that of the *Escherichia coli* protein

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Two closely related forms of the single-stranded DNA binding protein purified from *Xenopus laevis* oocytes mitochondria have been identified. Their amino terminal sequences exhibit homology with the *Escherichia coli* SSB protein.

Amino acid sequence; Mitochondria; Sequence homology; single-stranded DNA-binding protein; (*Xenopus laevis, Escherichia coli*)

1. INTRODUCTION

The *E. coli* SSB, bacteriophage T4 gp32, fd gene 5 and adenovirus 72 K proteins are the better characterized single-stranded DNA-binding proteins (SSBs) and their role in DNA replication has been clearly demonstrated by both genetic and biochemical analysis [1,2]. In eukaryotic cells the general features of DNA replication are similar and it is likely that proteins bind to single-stranded parts of replicative intermediates, however several proteins previously identified as potential SSBs are actually involved in other biological processes and have probably a fortuitous affinity for ssDNA [3-6]. We have previously isolated a protein, from *Xenopus laevis* oocyte mitochondria, which is associated with the displaced strand of the replicative forms in vivo [7]. This protein, called mtSSB, binds preferentially and cooperatively to ssDNA [8] and it can under certain conditions stimulate the activity of DNA polymerase γ [9]. The mtSSB, which is probably homologous to the P16 protein found in rat liver mitochondria [10], appears as a single band corresponding to an apparent molecular mass of 15 500 Da on SDS-PAGE. Its sedimentation coefficient is 4 S which suggests that it is tetrameric in solution [7]. Its amino acid composition is close to that of other single-stranded nucleic acid-binding proteins [8]. In the present study, we report the amino-terminal sequence of this protein.

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Abbreviations: mt, mitochondrial; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of SDS; ssDNA, single-stranded DNA; SSB, ssDNA-binding protein; TFA, trifluoroacetic acid

2. MATERIALS AND METHODS

2.1. *Materials*

mtSSB was purified from highly purified mitochondria of 50 adult female *X. laevis* as described in [8]. Before amino-terminal sequencing, 1 nmol mtSSB was dialysed against 50 mM NH_4HCO_3, dried and dissolved in 10% acetic acid. It was then injected onto a C8 reversed-phase column (2.1 mm × 3 cm) RP300 Octyl equilibrated with 0.1% TFA. Elution (flow rate 0.3 ml/min) was performed with increasing concentrations of buffer (0.05% TFA, 80% acetonitrile); 0-30% buffer (15 min); 30-90% buffer (45 min).

2.2. *Preparation of tryptic peptides*

mtSSB (1 nmol) was digested with trypsin at a protein to enzyme mass ratio of 30 in 50 mM NH_4HCO_3 (pH 8) at 36°C for 24 h. After hydrolysis the tryptic peptides were dried and dissolved in 50 μl of 10% acetic acid. 25 μl of the sample were injected onto a C18 reversed-phase column (0.46 × 70 cm) ultra

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sphere X L-ODS. The peptides were eluted at a flow rate of 0.7 ml/min with a linear gradient from 2 to 90% (0.03% TFA, 80% acetonitrile) for 90 min.

2.3. *Preparation of cyanogen bromide peptides*

Dried mtSSB (1–1.5 nmol) was dissolved in 80% formic acid and a 100-fold molar excess of solid CNBr was added; the sample was incubated in the dark for 48 h, dried by a stream of N_2 and lyophilised. The CNBr fragments were dissolved in 50 μl of 10% acetic acid and injected onto a C18 column equilibrated with 0.1% TFA. The elution was performed at a flow rate of 0.5 ml/min with a linear gradient from 5 to 90% (0.085% TFA, 80% acetonitrile) for 45 min.

2.4. *Amino acid sequence determination*

Protein sequencing was performed as described in [11].

2.5. *Protein sequence similarity searches*

Searches were done on the computer of the CITI2 (Université René Descartes, Paris) using the algorithm of Lipman and Pearson [12] with the NBRF and the SWISSPROT protein sequences libraries.

3. RESULTS

Automated Edman degradation of mtSSB (purified on HPLC as described in section 2), revealed that the amino-terminal sequence of the protein is heterogeneous. For most degradation cycles, two amino acids were found in approximately equimolar amounts. This result suggested that two variants of mtSSB could be present. This hypothesis was supported by the observation of two close spots on bidimentional polyacrylamide gel electrophoresis (not shown) and by the presence of two overlapping peaks obtained by chromatography on a C8 column (fig.1). From this chromatographic analysis we obtained two fractions containing differential amounts of the two variants, then we were able to determine the amino-terminal sequence (24 residues) of one

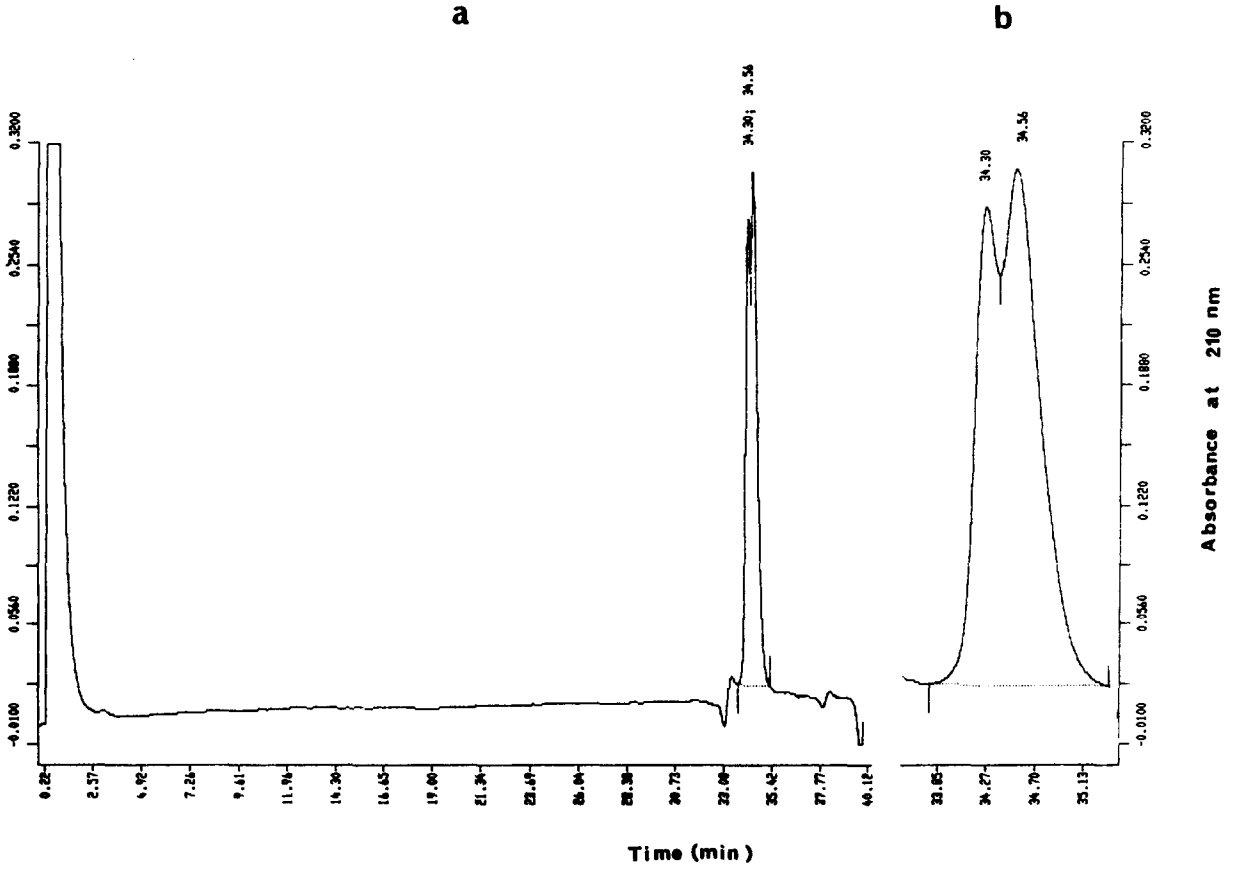

Fig. 1. Reverse-phase chromatography of mtSSB. mtSSB was chromatographed onto a C8 column as described in section 2. (a) Complete chromatogram, (b) expanded view of the peaks.

polypeptide, and by deduction most of that of the second polypeptide. This result and the determination of the sequence of two tryptic and one cyanogen bromide peptides from this region allowed us to establish the sequence of the first 24 amino acids of one variant and of 21 amino acids for the other (fig.2a). These sequences, which agree with the results obtained when the two polypeptides were degraded together, show that the two polypeptides are homologous. It should be noted that the methionine residue at position number 13 in mtSSB-2 had not been detected in the global amino acids composition which had been done, with a mixture of the two polypeptides, in the absence of a reducing agent [8]. The sequence of the first 24 residues of mtSSB-1 was used to search sequence similarities with the NBRF and SWISSPROT protein sequences libraries. In both cases the better score (54/104) was obtained with the *E. coli* SSB. In fact the sequence of the residues

Table 1

Comparison of the properties of the *X. laevis* mitochondrial SSB with those of the *E. coli* SSB

Property	*E. coli* SSB	mtSSB
Molecular mass (SDS-PAGE)	19.5 kDa	15.5 kDa
Sedimentation coefficient	4.7 S	4 S
Oligomeric state	tetramer	likely tetramer
Relative affinity	DNA > RNA ssDNA > dsDNA	DNA > RNA ssDNA > dsDNA
Cooperativity	yes	yes
Electron microscopic appearance of the complexes of protein and ssDNA	beads	beads
[NaCl] for elution from ssDNA-Cellulose	1.0–2.0 M	1.5–2.5 M
Amino acid composition (%)		
non polar	30.6%	32.2%
polar uncharged or acidic	61.2%	50.2%
basic	10.6%	17.3%

a

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                    ──────────────────────────── N ────────────────────────────
                    ──────────────── T23 ────────────────
mtSSB-1      1 Ser Thr Asp Ser Val  -   -  Ile Leu Glu Arg Glu Ile Asn Lys Val 14
                *   *   *   *              *   *   *   *           *   *   *
mtSSB-2      2 Ser Thr Asp Ser Leu Xxx Ala Ile Leu Glu Arg Xxx Met Asn Lys Val 16
                                                                  ─── C1 ───

                    ──────────────── N ────────────────
                                ──── T17 ────
mtSSB-1     15 Gln Leu Leu Gly Arg Val Gly Gln Asp Pro 24
                *   *   *   *   *
mtSSB-2     17 Gln Leu Leu Gly Arg 21
                ──── C1 ────
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b

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mtSSB-1      9 Arg Glu Ile Asn Lys Val Gln Leu Leu Gly Arg Val Gly Gln Asp Pro 24
                 *       Δ   *   *   *       *       *   Δ       *   *   *   *
E. Coli SSB  3 Arg Gly Val Asn Lys Val Ile Leu Val Gly Asn Leu Gly Gln Asp Pro 18
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Fig. 2. Amino-terminal sequence of the *X. laevis* mtSSB. (a) Sequence of the two variants of mtSSB. N, T23, T17 and C1 show portions of sequence determined from native protein, tryptic peptides (number 23 and 27) and cyanogen bromide peptide, respectively. (Xxx) An undetermined amino acid; (-) a gap introduced to maximize the sequence alignment; (*) the presence of an identical amino acid in the polypeptides. (b) Alignment of the *X. laevis* mtSSB and *E. coli* SSB in their amino-terminal region A. (*) The presence of an identical amino acid in the two proteins; (Δ) an amino acid which is not identical to that found in *E. coli* SSB but to the one found in the F sex factor protein.

9 to 24 of mtSSB-1, shows 63% of identity with that of the residues 3 to 18 of the *E. coli* SSB (fig.2b). The amino acids 11 and 19 which are different from those of the *E. coli* SSB are identical to those of the F sex factor SSB which is itself highly homologous to the *E. coli* SSB [13]. Furthermore 2 of the 4 other amino acids that are not identical to those of the *E. coli* SSB correspond to a conservative change (residues 17 and 20). Table 1 shows that mtSSB and the *E. coli* SSB share other similar properties. The major difference is the percentage of basic amino acids which is slightly higher for mtSSB.

4. DISCUSSION

The amino-terminal sequences of two variants of mtSSB protein have been determined. The two forms are repeatedly found in approximately equimolar amounts which suggests that they are not produced by the presence of two allelic genes in the *X. laevis* population, but this observation is in agreement with the hypothesis of a genome duplication during the evolution of *X. laevis* [14]. However, it is not yet clear if the protein can exist as heterologous tetramers or if only two kinds of homologous tetramers can be found.

The amino-terminal sequence and the physicochemical properties of mtSSB are very similar to those of the *E. coli* SSB supporting the idea that, unlike several proteins previously identified as eukaryotic SSBs [3–6], mtSSB is actually an SSB. However, the carboxy-terminal sequence of mtSSB is as yet unknown and this region, possibly involved in protein-protein interactions, could show specific properties. The identification and the study of the gene(s) coding for mtSSB, which can now be undertaken, might allow the determination of the complete sequence of the native protein and of its cytoplasmic precursor.

In conclusion, we have described two closely related forms of the *X. laevis* mtSSB, which are homologous to the *E. coli* protein.

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