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The primary sequence of the PFK-1 inactivating zinc-binding protein as deduced from cDNA sequencing

Identity of the zinc-binding protein with rat parathymosin

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We have recently described the sequence of the Zn^{2+}-binding domain (43 amino acid residues) of a newly detected Zn^{2+}-binding protein which reversibly inactivates phosphofructokinase-1 in a Zn^{2+}-dependent manner [(1986) J. Biol. Chem. 269, 5895-5900; (1988) Eur. J. Biochem. 177, 561-568]. Here, we describe the primary sequence of this protein based on a full-length cDNA. A sequence comparison reveals the identity of the Zn^{2+}-binding protein with a protein called parathymosin-α.

Zinc-binding protein; Phosphofructokinase-1; Parathymosine-α

1. INTRODUCTION

Brand and Söling [1] have reported on a protein with an apparent M_r of 19000, which inactivates phosphofructokinase-1 (PFK-1; EC 2.7.1.11) in a Zn^{2+}-dependent manner. The inactivation of PFK-1 is reversible upon removal of the zinc ions. A peptide containing the four Zn^{2+}-binding domains of this molecule was identified and sequenced [2]. This peptide was still able to inactivate PFK-1 [2].

Based on the partial sequence obtained, oligonucleotides were synthesized and used for screening a λgt-11 rat liver library. Positive clones were identified and a full-length cDNA was sequenced. Based on this cDNA, the primary sequence of the Zn^{2+}-binding protein was deduced. A comparison of the obtained sequence with data in the literature led to the result that the Zn^{2+}-binding protein is identical with rat parathymosin-α, a recently identified protein, whose function had remained more or less unknown up to now [3–5].

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2. MATERIALS AND METHODS

2.1. *Materials*

The λgt11 rat liver cDNA library was obtained from Clontech, Heidelberg, FRG. T_4-polynucleotide kinase, T_4-ligase, *Eco*RI, bacterial alkaline phosphatase, [γ-^{32}P]ATP and [α-^{35}S]dATP as well as Hybond N nylon membranes came from the Amersham-Buchler Co., Braunschweig, FRG. The Sequenase-2.0 kit was from United States Biochemicals Co., Cleveland, OH, USA. Blots were performed using GeneScreenPlus from NEN-Dupont, Bad Homburg, FRG, autoradiography with Kodak XAR5 film.

2.2. *Methods*

Oligonucleotides were synthesized on an Applied Biosystems 381A oligonucleotide synthesizer. Following deprotection with conc. ammonia and desalting using Pharmacia NAP-10 columns, the oligonucleotides used were 5'-end labelled using [γ-^{32}P]ATP and T_4-polynucleotide kinase. Bacteriophage plaques were transferred onto Hybond N membranes according to [6] and hybridization with the labelled oligonucleotides was performed as in [7]. The hybridization temperatures were selected according to [7]. Positive plaques were picked and single positive plaques obtained by two additional rescreening cycles

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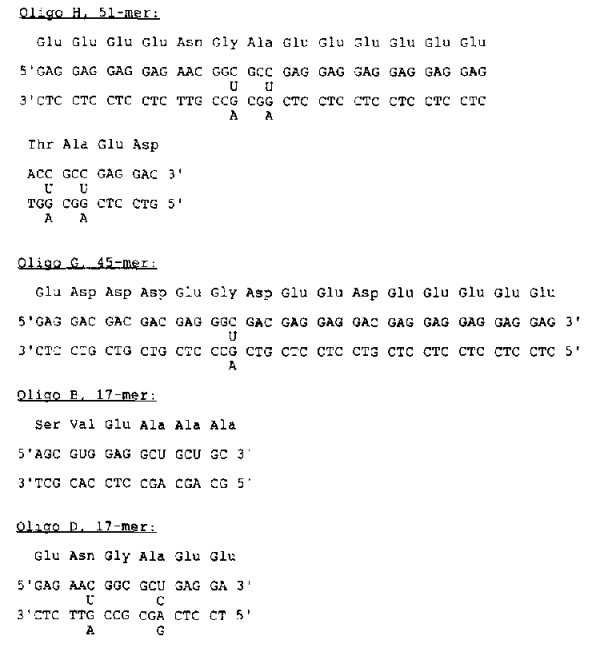

Fig.1. Sequences of oligonucleotides used for screening and cross-hybridization experiments. Peptide, mRNA and oligonucleotide sequences are shown. Probes H, G and D were deduced from the 43-residue peptide, B was constructed from an unpublished sequence of the zinc-binding protein.

using the same probe. Phage DNA was prepared from plate lysates, digested with *Eco*RI and separated by electrophoresis on 0.4% agarose gels. The DNA was blotted onto GeneScreenPlus-membranes and examined for insertion size and cross-hybridization with different oligonucleotides. The *Eco*RI cut inserts were ligated into M13 mp18 RF-DNA, which had been cut before with *Eco*RI and treated with bacterial alkaline phosphatase. The ligated DNA was transformed into competent *E. coli* CMK 603 cells. Sequencing of the recombinant M13 phage DNA was performed using Sequenase 2.0 according to the manufacturer's instructions. To prevent sequencing errors due to gel compressions, parallel Sequenase reactions were run with dGTP and dITP, respectively. The sequencing strategy followed the 'primer hopping' method as described in [8].

3. RESULTS

Based on the 43 residues peptide described by Brand et al. [2] and an additional unpublished sequence, four oligonucleotides were synthetized. The base sequences were selected according to a codon usage catalogue [9] as given in fig.1.

Screening of about 5×10^5 plaques of the λgt11 rat liver cDNA library with oligonucleotide H yielded about 120 positive signals. Ten clones were randomly selected and survived two rescreen cycles with the original probe. The *Eco*RI-cut phage DNA was blotted and examined for insert size and tested for cross-hybridization. Inserts of sizes between 0.5 and 1.2 kb were observed, but only clone H3 (about 600 bp insert length) and clone H9 (about 900 bp insert length) cross-hybridized with four different oligonucleotide probes. These two clones were selected for subcloning into M13 mp18. With clone H9, only five recombinant M31 plaques could be observed in several ligation-transformation experiments, whereas clone H3 did not yield recombinant plaques at all. Of the H9 recombinants, only one plaque provided single-stranded DNA which could be sequenced. This DNA contained, however, the complete 935 bp H9 insert. The sequence of this DNA is depicted in fig.2. It is evident that H9 contains an open reading frame for a 11471 Da protein. The amino acid composition of the protein derived from the sequenced cDNA fits with the previously published data [2], and the sequence of the Zn^{2+}-binding peptide reported earlier [2] can be completely recovered (underlined sequence in fig.2). The sequenced DNA contained also 109 bp of the 5′-untranslated and 506 bp of the 3′-untranslated regions including the 3′-poly-A tail.

As the calculated molecular mass of 11471 Da was not in line with the molecular mass deduced previously from SDS-polyacrylamide gel electrophoresis (19000 Da) [1], a sample of the protein was analyzed by laser-time-of-flight-mass spectrometry. A 'mother-peak' of 11470 Da was found by this method confirming the protein sequence deduced from the cDNA. The higher apparent molecular mass obtained by SDS-polyacrylamide gel electrophoresis results most likely from the very acidic character of the protein.

4. DISCUSSION

The recombinant full-length clone described here carries immediately upstream of the initiator codon the sequence CCC GGC ACC (positions 107–115 in fig.2). This sequence shows a strong homology with the consensus sequence (GCC GCC $\overset{A}{G}$CC) for which Kozak [10] assumes a positive regulatory effect on the translation rate.

Having obtained the full-length primary structure of the Zn^{2+}-binding protein, we undertook a search in a protein sequence data library in order to find possible homologies with other peptides or proteins. To our surprise, the Zn^{2+}-binding protein showed full identity with a protein of unknown function and properties called 'parathymosin-α'. The sequence of parathymosin-α had been deduced from the sequence of a partial cDNA clone and a N-terminal peptide sequence [4,5]. The designation 'parathymosin' was chosen by Haritos et al. [11] because it was initially found in the thymus together with prothymosin-α, the pro-form of the hormonal factor thymosin-α. However, in view of the fact that (in spite of a similar amino acid composition) there exists only a very limited sequence homology between parathymosin-α and prothymosin-α and that much more parathymosin-α is found in the liver than in the thymus [12,13], we feel that the name 'parathymosin' is misleading and should be replaced by the more informative designation '11.5 kDa Zn^{2+}-binding protein'. On

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GAATTCCGGC CGCCGCCACC GCGCCAAGTT CCGGCCGCGG CCACCTTCCG CCGTCCAGGG   60

CTCCTCCGCC TCGGCCCCGG GACCCCGGCT CCCCGCCAGC CCCGGCCCCG GCACC


 ATG TCG GAG AAG AGC GTG GAG GCA GCG GCC GAG CTA AGC GCC AAG       160
     Ser Glu Lys Ser Val Glu Ala Ala Ala Glu Leu Ser Ala Lys

 GAC CTG AAG GAA AAG AAG GAC AAG GTG GAG GAG AAG GCT GGC CGG       205
 Asp Leu Lys Glu Lys Lys Asp Lys Val Glu Glu Lys Ala Gly Arg

 AAA GAA CGG AAG AAA GAA GTA GTG GAG GAG GAG GAG AAT GGA GCT       250
 Lys Glu Arg Lys Lys Glu Val Val Glu Glu Glu Glu Asn Gly Ala

 GAG GAG GAG GAA GAA GAA ACT GCT GAG GAT GGA GAG GAT GAT GAT       295
 Glu Glu Glu Glu Glu Glu Thr Ala Glu Asp Gly Glu Asp Asp Asp

 GAA GGA GAC GAA GAA GAT GAG GAG GAA GAG GAG GAG GAG GAT GAA       340
 Glu Gly Asp Glu Glu Asp Glu Glu Glu Glu Glu Glu Glu Asp Glu

 GGC CCC GTG CGG AAG AGA ACT GCT GAA GAG GAG GAT GAA GCG GAT       385
 Gly Pro Val Arg Lys Arg Thr Ala Glu Glu Glu Asp Glu Ala Asp

 CCC AAG AGG CAG AAG ACA GAA AAC GGG GCG TCG GCT TGA               424
 Pro Lys Arg Gln Lys Thr Glu Asn Gly Ala Ser Ala ***


    GCCCCT GCCCGTGGGC TTGGGGATGG GAGGCCCCTC AGGTCCTGGA GGTGGGGCAG   480

 GAACACACAA ATCCAGCCCC CCTTCTCCTG GCTCCCTCGT CTGGCCCTGC CCCAGAGCTG   540

 TGACCCTTGC CCTTTGACCC AGCCTCTCAT TTCCATCTCT CCAGACACTG CTCCTTCACC   600

 CTCACTGCCA CAGGTCCAGC CCCCAACCCG CCTCATCCAA GCTCCCCAGC CGGCCCTCAC   660

 TTGCCCTAGC ATTCCTTGTT CTTCCCTGCC TTCCTCACCA TCGATCTGTT CCAGTCCTTG   720

 CGAAGCCTCT CCTTCCCCTC TGCACCCCGA GCCTCTCAGC CTGCCCTTCT CTCTCCTGCC   780

 TGACCCCTGG GTCTCCCTCA GATTCCCTCC TCTCAGACAG CGCCAGGCCG GGGTGGGGCT   840

 GGGGTTGGGG CCAAGCCCCG AAGCTGCCCC CTCCCCTTTT TGTATAATTT AATAAAGAAA   900

 CGGTCGCGCT TCAAAAAAAA AAAAAAAACG GAATTC                           936
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Fig.2. Sequence of a full-length cDNA coding for the rat liver Zn^{2+}-binding protein and the primary sequence deduced from the cDNA. The underlined part of the sequence corresponds to the peptide reported previously by Brand et al. [2].

the other hand, both prothymosin-α and the 11.5 kDa Zn^{2+}-binding protein share a common property, in that both contain several clusters of acid amino acids separated by 1–3 neutral amino acids (mostly glycine). Therefore, we do consider the possibility that prothymosin might also be a Zn^{2+}-binding protein. If this were the case, this property might affect its processing to the mature thymosin-α, the active form of the hormone.

We observed in our study that our H9 clone had poor ligation/transformation activity. This fits data from Horecker's group who reported that parathymosin recombinant Bluescript KS M13-plasmids carrying a cDNA, containing approx. 600 bp of the 3′-non-coding region exhibited very poor growth [4]. As a clone containing only 135 bp of the 3′-non-coding region did apparently not show this growth inhibitory effect [4], one can assume that the 3′-non-coding part of the cDNA is responsible for the inhibition, but that a sufficient length of the 3′-non-coding region is necessary to achieve it.

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