Molecular cloning of cDNA of S100α subunit mRNA

Ryozo Kuwano, Toshinaga Maeda, Hiroshi Usui, Kazuaki Araki, Toru Yamakuni, Yuka Ohshima, Tadashi Kurihara and Yasuo Takahashi

Department of Neuropharmacology, Brain Research Institute, Niigata University, Asahimachi 1, Niigata 951, Japan

Received 28 April 1986

The primary structure of the bovine S-100α mRNA on the basis of molecular cloning and sequence analysis of the cDNA are described. The sequence is composed of 532 bp which include the 282 bp of the complete coding region, 89 bp at the 5′-noncoding region, 161 bp at the 3′-noncoding region, polyadenylation signal, ATTAAA and poly(A) tail. Northern blot analysis shows that the size of S-100α mRNA is about 700–800 bases long and a single mRNA occurs in bovine brain. Bovine brain contains both S100α and β subunits and their mRNAs. In contrast, the rat brain contains only S100β subunit and its mRNA.

S-100 protein *mRNA* *cDNA cloning* *Nucleotide sequence*

1. INTRODUCTION

S-100 protein is a brain specific protein discovered by Moore [1]. The electrophoretical heterogeneity of this protein was shown in many papers. Recently Isobe and Okuyama [2,3] isolated the α and β subunits of S-100 protein from bovine brain and determined that the heterogeneity of this protein depends on the different combination of two subunits; S-100a_o (αα), S100a (αβ), and S-100b (ββ). Further they demonstrated the structural relation between S-100 protein and Ca^{2+}-binding proteins of the EF-hand type.

S-100 protein is mainly localized in astrocytes in the central nervous system, although many investigators are still studying the problem of cellular localization [4]. In our previous studies it was found that rat brain contains exclusively S-100b composed of homodimer of the β subunit [5]. We succeeded in cloning of cDNA to S-100β mRNA in rat brain [6]. However, we have no information concerning whether α or β or both subunits of S-100 protein are specifically expressed in rat brain. For studies of the molecular mechanism that controls the expression of S-100α and β subunit genes, we attempted the cloning of S-100α cDNA as the next step.

2. MATERIALS AND METHODS

Restriction endonucleases were obtained from Takara Shuzo and New England Biolabs. DNA polymerase I, bacterial alkaline phosphatase, T_4 polynucleotide kinase and terminal deoxynucleotide transferase were from Takara Shuzo and Pharmacia P-L. [γ-^{32}P]ATP and [α-^{32}P]dCTP were purchased from Amersham. Avian myeloblastosis reverse transcriptase was from Bio Rad Laboratories. Nitrocellulose (BA85) was from Schleicher & Schüll.

2.1. *Preparation of RNA*

Total microsomes were prepared from adult rat brains and bovine cerebellum. Microsomal RNA was isolated by a phenol/chloroform/isoamyl alcohol extraction procedure. Poly(A) RNA was isolated from microsomal RNA by oligo(dT)-cellulose chromatography.

2.2. *Construction of rat brain and bovine cerebellar cDNA libraries*

Double-stranded cDNA was prepared from poly(A) RNA using reverse transcriptase with oligo(dT) as a primer and inserted into the *Pst*I site of pBR322, using the dG-dC tailing method as described by Land et al. [7].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2.3. *Oligodeoxynucleotide synthesis and colony screening*

Two different mixtures of 14-base-long oligodeoxynucleotide (3′-TGNCGNTACCT$^{T}_{C}$TG-5′) and (3′-AA$^{A}_{G}$GT$^{T}_{C}$CT$^{T}_{C}$AT$^{A}_{G}$CA-5′) complementary to mRNA deduced from amino acid residues (7–11) and (72–76) of bovine S-100α subunit, respectively, were synthesized. cDNA libraries were screened by colony hybridization using the above synthesized oligodeoxynucleotides as the probes.

2.4. *Restriction mapping and DNA sequence*

The cloned cDNA was digested with several restriction endonucleases and analyzed by electrophoresis on polyacrylamide and agarose gels. Restriction enzyme digested DNA was 5′-labeled using bacterial alkaline phosphatase, T_4 polynucleotide kinase, and [^{32}P]ATP. Their nucleotide sequences were determined by the chemical method of Maxam and Gilbert [8].

2.5. *Northern blot analysis*

Poly(A) RNAs were treated with 50% formamide. Samples were electrophoresed on a 1.5% agarose gel containing 2.2 M formaldehyde and transferred onto nitrocellulose filters. The filters were hybridized with nick-translated [^{32}P]cDNA [9,10].

3. RESULTS

3.1. *Cloning of cDNA to S-100α mRNA*

We previously reported the cloning of cDNA to S-100β mRNA from rat brain. We then attempted the cloning of cDNA of S-100α subunit from the rat brain cDNA library by using nucleotide sequence homology between S-100α and S-100β since S-100 protein is one of the most conservative proteins and the amino acid homology of these subunits is 58%. However, we did not find any positive clones. Therefore, we constructed a cDNA library from bovine cerebellar poly(A) RNA. For screening of the bovine cerebellar cDNA library, we used two different mixtures of synthetic oligodeoxynucleotide as probes. Three positive clones were selected from 30000 transformants.

The restriction endonuclease map is shown in fig.1. The long inserted cDNA, pRSα-11, was further analyzed and the nucleotide sequence determined (fig.2). The cloned pRSα-11 has the 282 bp of the coding region, 89 bp at the 5′-noncoding region, 161 bp at the 3′-noncoding region, polyadenylation signal, ATTAAA and poly(A) tail. Since the size of mRNA is about 700–800 bases long (fig.3), pRSα-11 nearly covers the complete nucleotide sequence, assuming 100 bases of poly(A) tail. At position 65 Asn deduced from the nucleotide sequence is different from Asp determined by the amino acid sequence analysis [3]. There is no additional amino acid sequence at the carboxyl terminus of the mature protein. High homology was observed in not only the amino acid but also the nucleotide sequence between S-100α and β subunits. This extensive sequence homology may indicate that the S-100 protein subunits evolved from a common gene.

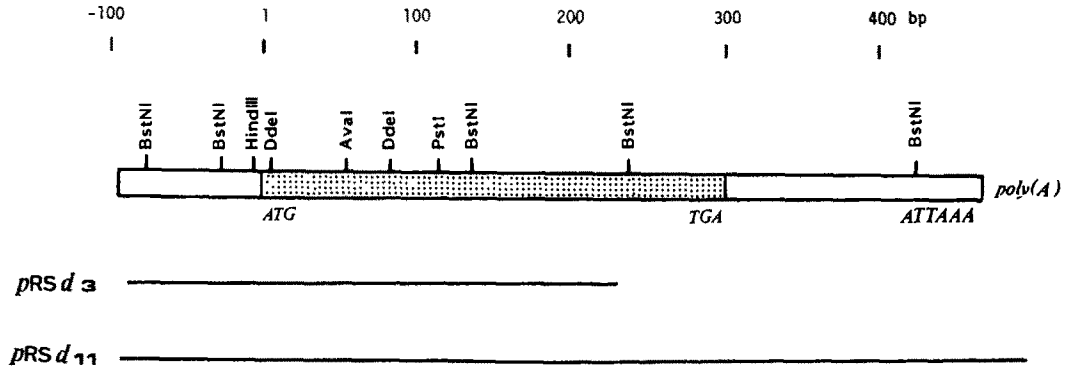


Fig.1. Restriction map of cDNA to S-100α mRNA. The coding nucleotide sequence for the α subunit of S-100 protein is indicated by the shaded box.

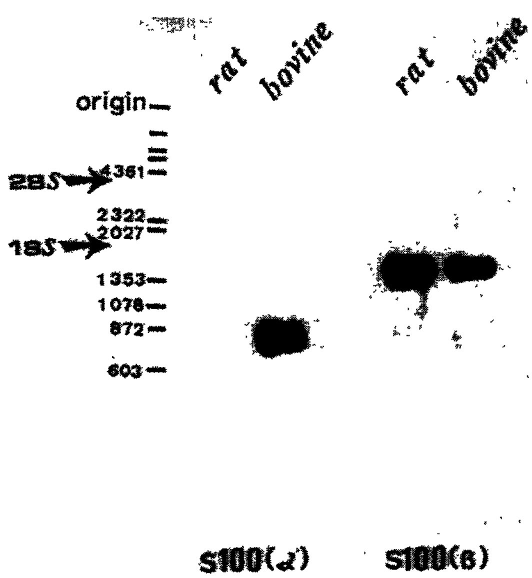
```
                                             -50                                                                     1
CGAGGTCTTCCTGCACAGCTCCAGGAGCCACATTTGCAGCCTCGTCTGCCTGTCCAGAACCTGCTCCCAGGCTGACAGTAAGCTTCGAG ATG GGC
                                                                                          Met Gly
                                                                                           1
                                                    50
TCT GAG CTG GAG ACG GCG ATG GAG ACT CTC ATC AAT GTG TTC CAC GCC CAC TCG GGC AAA GAG GGA GAC AAG TAC
Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val Phe His Ala His Ser Gly Lys Glu Gly Asp Lys Tyr
                           10                                       20
                   100                                                                    150
AAG CTG AGC AAG AAG GAG CTG AAA GAG CTG CTG CAG ACG GAG CTC TCC GGC TTC CTG GAT GCC CAG AAG GAT GCA
Lys Leu Ser Lys Lys Glu Leu Lys Glu Leu Leu Gln Thr Glu Leu Ser Gly Phe Leu Asp Ala Gln Lys Asp Ala
        30                                      40                                      50
                                                    200
GAC GCT GTG GAC AAG GTG ATG AAG GAG CTG GAT GAG AAC GGA GAT GGA GAG GTG GAC TTC CAG GAG TAT GTG GTG
Asp Ala Val Asp Lys Val Met Lys Glu Leu Asp Glu Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr Val Val
                                60                                      70
                   250                                                                 300
CTG GTG GCT GCC CTC ACG GTG GCG TGC AAC AAC TTC TTC TGG GAG AAC AGT TGAGGAGACAGCCCCGCGGCAGCGTTCCTCC
Leu Val Ala Ala Leu Thr Val Ala Cys Asn Asn Phe Phe Trp Glu Asn Ser
        80                                      90
                                      350                                                     400
CCTCCACCCTGCCATCCCTGCTCCTTCACCCTGCTTCCCCTCACCTTACTTTTCCCTCCCCACCAACAAGGGCGCAAGAATAGTGGGCCAGGCCTGCAAC

                                      450
TCCTCTTTCATTAAAGGCTTCTGTCTCACCAAAAAAAA
```

Fig.2. Nucleotide sequence of S-100α mRNA deduced from the cloned cDNA and the predicted amino acid sequence of the α subunit. Initiation codon ATG and termination codons TGA are underlined. The polyadenylation signal, ATTAAA, is double-underlined. The numbers below the line indicate positions of amino acid, and the numbers above the line indicate nucleotide positions, beginning with the initial codon. The nucleotides in the 5′-untranslated region are indicated by negative numbers. The synthetic oligodeoxynucleotide probes are indicated by the open box.

3.2. *Northern blot analysis*

We previously purified the S-100 protein from bovine and rat brain by the same procedure and then analyzed the subunit composition of the S-100 protein by high-performance liquid chromatography. Bovine brain S-100 consists of one α subunit and three molar excess of β subunit, however a peak at the retention time of the α subunit was not found in rat brain S-100 protein [5].



The nick-translated [^{32}P]cDNAs were used as probes to determine the size and molecular species of S-100 protein mRNA by Northern blot hybridization (fig.3). The size of mRNAs for S-100α and S-100β subunits was found to be about 700–800 and 1500 bases, respectively. Since the coding regions of both mRNAs are of almost the

Fig.3. Northern blot hybridization of brain poly(A) RNA. Poly(A) RNAs (4.5 μg) from adult bovine cerebellum and adult rat brain were electrophoresed on a 1.5% agarose gel containing 2.2 M formaldehyde. Samples were transferred onto nitrocellulose filters and hybridized with nick-translated [^{32}P]cDNA of S-100α and β subunits.

Table 1

(A) Domains	Percent homology		
	S100α/S100β	S100α/ICaBP	S100β/ICaBP
I/I	62	62	48
I/II	34	34	33
II/I	28	20	35
II/II	68	41	42

(B) Domains	Percent homology	
	S100α/CaM	S100β/CaM
I/I	38	38
I/II	31	32
I/III	32	39
I/IV	33	37
II/I	40	39
II/II	51	51
II/III	36	38
II/IV	53	49

same length, mRNA for the β subunit must have a much longer 3′-noncoding region than that for the α subunit. Fig.3 shows a discrete band for S-100α and β mRNA, respectively. S-100β mRNA occurs in both rat and bovine brain. In contrast, S-100α mRNA is absent in rat brain. This Northern blot analysis supported our previous data that S-100α and β subunits occur in a different manner in rat and bovine brain [5].

3.3. *Nucleotide sequence homology*

We examined the interdomain nucleotide sequence homology between S-100 protein and two Ca^{2+}-binding proteins of the EF-hand type (table 1). S-100 protein and the intestinal Ca^{2+}-binding protein (ICaBP) comprise two domains [11]. Calmodulin (CaM) consists of four domains [12]. High homology was observed between both subunits of S-100 protein and ICaBP; especially domain I and I, and domain II and II (table 1A). Somewhat high homology between S-100 protein and CaM was observed (table 1B).

4. DISCUSSION

We have described here the primary structure of the bovine S-100α mRNA on the basis of molecular cloning and sequence analysis of the cDNA. Since the cDNAs to both α and β subunits of S-100 protein were available, these cloned cDNAs were used as probes to examine mRNA in either rat or bovine brain by Northern blot analysis. The data from Northern blot analysis are in good agreement with those demonstrating that bovine brain contains both subunits and rat brain contains only S-100β subunit [5].

From the analysis of amino acid sequence homology the physiological roles of this protein in cellular function were recently proposed. According to Kligman and Marshak [13], the amino acid composition and sequence of the purified neurite-extension factor from bovine brain are nearly identical to those of the S-100β subunit. The regulatory chain (P11) in the 36 kDa protein, a major substrate of viral and growth-factor associated tyrosine specific protein kinases, showed high amino acid sequence homology with S-100α and β [14,15]. Further experiments are necessary to elucidate the role of S-100 protein in cellular function, including that as a trophic factor. Studies of the molecular mechanism that controls the expression of α and β subunit genes can provide information about cell differentiation and developmental or evolutional stage-specific expression of these genes.

ACKNOWLEDGEMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan to Y.T.

REFERENCES

[1] Moore, B.W. (1965) Biochem. Biophys. Res. Commun. 19, 739–744.
[2] Isobe, T. and Okuyama, T. (1978) Eur. J. Biochem. 89, 379–388.
[3] Isobe, T. and Okuyama, T. (1981) Eur. J. Biochem. 116, 79–86.
[4] Usuki, T., Iwanaga, T., Masuda, T., Takahashi, Y. and Fujita, T. (1984) Cell Tissue Res. 235, 509–514.
[5] Masuda, T., Sakimura, K., Yoshida, Y., Kuwano, R., Isobe, T., Okuyama, T. and Takahashi, Y. (1983) Biochim. Biophys. Acta 740, 249–254.

[6] Kuwano, R., Usui, H., Maeda, T., Fukui, T., Yamanari, N., Ohtsuka, E., Ikehara, M. and Takahashi, Y. (1984) Nucleic Acids Res. 12, 7455–7465.
[7] Land, H., Grez, M., Hauser, H., Lindenmaier, W. and Schütz, G. (1981) Nucleic Acids Res. 9, 2251–2266.
[8] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning, Cold Spring Harbor Laboratory, New York.
[10] Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.
[11] Desplan, C., Heidmann, O., Lillie, J.W., Auffray, C. and Thomasset, M. (1983) J. Biol. Chem. 258, 13502–13505.
[12] Putkey, J.A., Tsui, K.F., Tanaka, T., Lagace, L., Stein, J.P., Lai, E.C. and Means, A.R. (1983) J. Biol. Chem. 258, 11864–11870.
[13] Kligman, D. and Marshak, D.R. (1985) Proc. Natl. Acad. Sci. USA 82, 7136–7139.
[14] Gerke, V. and Weber, K. (1985) EMBO J. 4, 2917–2920.
[15] Glenney, J.R. jr and Tack, B.F. (1985) Proc. Natl. Acad. Sci. USA 82, 7884–7888.