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Molecular cloning of cDNA coding for brain-specific 14-3-3 protein, a protein kinase-dependent activator of tyrosine and tryptophan hydroxylases

(amino acid sequence/protein phosphorylation/monoamine biosynthesis/enzyme regulation)

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ABSTRACT **The 14-3-3 protein is a family of acidic proteins present exclusively in the brain and is believed to have a function in monoamine biosynthesis because of its ability to activate tyrosine hydroxylase and tryptophan hydroxylase in the presence of Ca^{2+}/calmodulin-dependent protein kinase type II. In this study, we resolved bovine brain 14-3-3 protein into seven polypeptide components by means of reversed-phase chromatography and determined the amino acid sequence of one of these components (η chain) by cloning its cDNA from a bovine cerebellum cDNA library. The η-chain mRNA is 1.8 kilobases long and encodes a polypeptide of 246 amino acids and M_r 28,221. Computer-assisted analysis of the sequence indicates that the η chain exhibits no internal sequence repeats, nor does it have significant sequence similarity to other proteins with known amino acid sequence. However, the η chain appears to consist of two structural regions that are distinguishable in their clearly different charge characteristics: the almost neutral amino-terminal region and the strongly acidic carboxyl-terminal region. The structural features of the η chain and the domain organization of tyrosine and tryptophan hydroxylases suggest that the 14-3-3 protein binds to the regulatory domain of the phosphorylated hydroxylases through its acidic carboxyl-terminal region and activates the hydroxylases by inducing an active conformation.**

Nerve stimulation, neurotransmitters, and certain growth hormones have been shown to accelerate biosynthesis of catecholamines and of neurotransmitter serotonin in the target cells (1). This acceleration is thought to result from an increase in the activity of tyrosine hydroxylase (TyrOHase; tyrosine 3-monooxygenase, EC 1.14.16.2) or tryptophan hydroxylase (TrpOHase; tryptophan 5-monooxygenase, EC 1.14.16.4), the initial and rate-limiting enzymes in the pathway of monoamine biosynthesis. Various mechanisms appear to be involved in this regulatory process: the induction of enzyme molecules, the increased biosynthesis of the coenzyme tetrahydrobiopterin, and the phosphorylation of enzymes through the action of protein kinases (1). The phosphorylation is coupled with a series of cellular second messengers including cAMP, diacylglycerol, and Ca^{2+}, and three types of protein kinases, cAMP-dependent protein kinase, Ca^{2+}/phospholipid-dependent protein kinase, and Ca^{2+}/calmodulin-dependent protein kinase type II (kinase II), are thought to mediate the effects of these second messengers (2). The phosphorylation of TyrOHase by cAMP- or Ca^{2+}/phospholipid-dependent kinase leads to a direct increase in the catalytic activity (3, 4). But unlike these two protein kinases, kinase II requires an additional protein factor for the activation of TyrOHase and TrpOHase (5). Yamauchi *et al.* (5) isolated this regulatory factor from the rat brain and described it as an "activator" protein.

By means of biochemical and immunochemical techniques, we recently showed (6) that this kinase II-dependent activator protein is substantially identical to the 14-3-3 protein, a "brain-specific" protein originally isolated by Moore and Perez (7). No physiological function had been attributed to the 14-3-3 protein before this finding; however, the previous studies indicated that this protein is abundant in the brain (about 1% of total cytosolic proteins) (8), is localized exclusively in neurons (9), and is axonally transported to the nerve terminals in the retinal ganglion cells (10). The 14-3-3 protein is a dimeric molecule having a molecular weight of ≈55,000 and, like many cytosolic proteins, seems to have no oligosaccharides attached to the polypeptide chain (9). However, little is known about the structure of the 14-3-3 protein because the protein exhibits complex heterogeneity due to the presence of multiple polypeptides with similar molecular weights (M_r ≈ 29,000 and 32,000) and isoelectric points (pI = 4.6–5.2) (7, 9). No information on the amino acid sequence of the 14-3-3 protein was reported, except that the protein appears to have a blocked amino terminus.

In this study we present the resolution of multiple components of 14-3-3 protein, partial sequencing of one of these components (η chain), cloning and sequencing of its cDNA, and the deduced amino acid sequence.‖

MATERIALS AND METHODS

Materials. The 14-3-3 protein was prepared from bovine brain as described (6). It was shown (6) to exhibit molecular characteristics very similar to those described by Moore and Perez (7) and Boston *et al.* (9). TrpOHase was purified from rat brainstem by pteridine affinity chromatography (11). Bovine brain kinase II was generously provided by S. Yamauchi (Tokyo Metropolitan Institute for Neurosciences).

Isolation of η Chain and Assay for Activator Activity. The η chain was isolated from 14-3-3 protein by reversed-phase HPLC under conditions described in the legend to Fig. 2. The eluate containing the η chain was immediately neutralized with aqueous ammonia and 50 mM Tris/HCl buffer (pH 7.5)

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Abbreviations: kinase II, Ca^{2+}/calmodulin-dependent protein kinase type II; TyrOHase, tyrosine hydroxylase; TrpOHase, tryptophan hydroxylase.

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‖The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03868).

and applied to a DEAE-5PW column (0.75 × 7.5 cm; Toso). After washing the column with 10 ml of 50 mM Tris/HCl buffer (pH 7.5), the adsorbed η chain was eluted with 0.4 M NaCl in the same buffer. The η chain thus obtained was assayed on the basis of its ability to activate TrpOHase in the presence of kinase II as described by Yamauchi *et al.* (5).

Amino Acid Sequence Analysis. The η chain (40 μg) was cleaved by cyanogen bromide (CNBr) in 70% formic acid and the fragments were separated on a reversed-phase phenyl-5PW RP column (0.46 × 7.5 cm; Toso) by elution with a linear gradient of acetonitrile (10–60%) in 0.1% trifluoroacetic acid. Where necessary, the fragments were further purified by rechromatography on an ODS-120T column (0.46 × 15 cm; Toso). The amino acid sequence of the purified fragments was determined with a model 470A automated sequenator (Applied Biosystems, Foster City, CA) according to the protocol provided by the supplier.

Synthesis of Oligodeoxynucleotide Probe and Screening of the Bovine Cerebellum cDNA Library. On the basis of the amino acid sequence Lys-Gly-Asp-Tyr-Tyr derived from one of the CNBr fragments and on the assumption that methionine should precede this sequence, the following oligonucleotide probe was synthesized: 5′-ATG-AAR-GGN-GAY-TAY-TA-3′. A bovine cerebellum cDNA library was constructed by the method of Land *et al.* (12), and replicate nitrocellulose filter blots of the cDNA library were hybridized to the oligonucleotide probe, which had been end-labeled by using [γ-^{32}P]ATP as described (13).

Restriction Endonuclease Mapping and DNA Sequence Analysis. Plasmid DNA was isolated from cloned bacteria by the method of Currier and Nester (14). Restriction endonuclease mapping of isolated plasmid DNA was carried out as described (13). DNA sequencing was performed by the method of Maxam and Gilbert (15).

RESULTS AND DISCUSSION

Heterogeneity. The 14-3-3 protein was reported to be heterogeneous, containing two or more molecular species that have different subunit compositions (9). Consistent with this observation, our 14-3-3 preparation was separated by NaDodSO$_4$/PAGE into two polypeptides of apparent M_r 27,000 and 30,000 (ref. 6 and Fig. 1, lane 1), which exist at a relative content of 9:1 and are both reactive to an antiserum against 14-3-3 protein (6). However, we found that the 14-3-3 protein was a more complex polypeptide mixture than previously thought; at least seven polypeptides were eluted from a reversed-phase column with a gentle gradient of acetonitrile in 0.08% heptafluorobutyric acid (Fig. 2). These polypeptides (designated α–η; Fig. 2) were estimated to have a M_r of 27,000 by NaDodSO$_4$/PAGE, except the ε chain, which had a M_r of 30,000. Subsequent analysis suggested that all these polypeptides share a common immunological determinant and have highly similar amino acid sequences, but no two of the sequences appear to be identical (T.I., unpublished data). We anticipated, therefore, that the 14-3-3 protein is a family of proteins that arose from a common evolutionary origin and are closely related in structure and function. To determine the amino acid sequence of 14-3-3 protein by molecular cloning, we selected one of the components (η chain) isolated by HPLC because its cDNA was found to be abundant in the cDNA library we used.

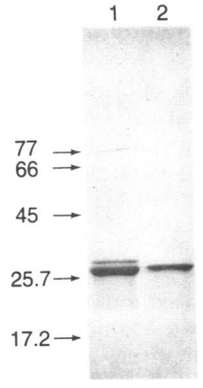

FIG. 1. NaDodSO$_4$/PAGE of bovine brain 14-3-3 protein (lane 1) and purified η chain (lane 2) (Coomassie blue staining). Electrophoresis was performed as described (6). The molecular weight markers used were ovotransferrin (M_r 77,000), serum albumin (M_r 66,000), ovalbumin (M_r 45,000) chymotrypsinogen (M_r 25,700), and myoglobin (M_r 17,200).

Characterization and Determination of Partial Amino Acid Sequence of η Chain. The η chain had a pI of 4.6 and appeared to be the most hydrophobic of the polypeptides isolated from 14-3-3 protein (Fig. 2). It comprised about 10% of the total 14-3-3 polypeptides. The η chain purified by reversed-phase HPLC showed a single band at M_r 27,000 in NaDodSO$_4$/PAGE (Fig. 1, lane 2), showed a single peak in rechromatography (Fig. 2), and had an ability to activate TrpOHase in the presence of kinase II (Fig. 3) after renaturation in an aqueous buffer solution. Thus, the η chain is a pure 14-3-3 polypeptide whose activity has been identified.

Because the η chain had a blocked amino terminus, sequence information was obtained from its fragments derived by CNBr cleavage. Thus, the sequence of a total of 117 residues was determined through the sequenator analysis. This corresponds to about half of the total η-chain sequence deduced by cDNA cloning (see Fig. 5).

Nucleotide and Deduced Amino Acid Sequence of η Chain. Screening of a bovine cerebellum cDNA library (30,000 transformants) with a 17-mer oligonucleotide probe (5′-ATG-AAR-GGN-GAY-TAY-TA-3′) yielded six positive clones (pAP62, -115, -141, -142, -144, and -149), four of which (pAP62, -141, -142, and -149) were derived from a single species of mRNA encoding the η chain. The nucleotide sequences of these four cDNAs were determined by the strategy shown in Fig. 4. Nucleotide sequencing of the other two clones (pAP115 and pAP144) as well as the partial amino acid sequence analysis of the polypeptides isolated by HPLC

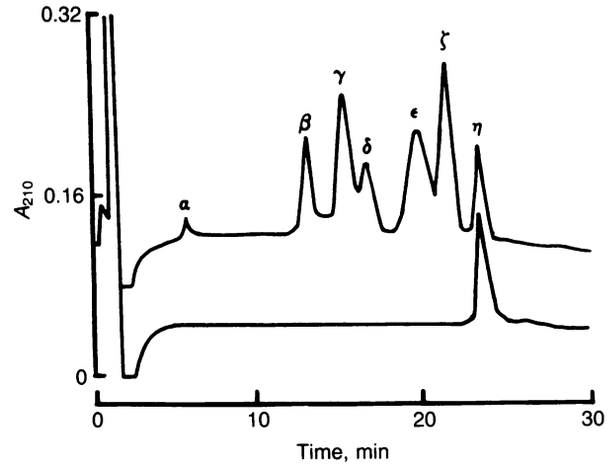

FIG. 2. Upper profile shows separation of bovine brain 14-3-3 protein by reversed-phase HPLC. The 14-3-3 protein (50 μg) was applied to a VP304-1251 column (0.46 × 5 cm; Senshu Kagaku, Tokyo) and eluted by a 30-min linear gradient of acetonitrile (50–60%) in 0.08% heptafluorobutyric acid at flow rate of 1 ml/min. The polypeptides detected are designated as α–η. Lower profile shows elution of the isolated η chain (5 μg) obtained by rechromatography under the same conditions.

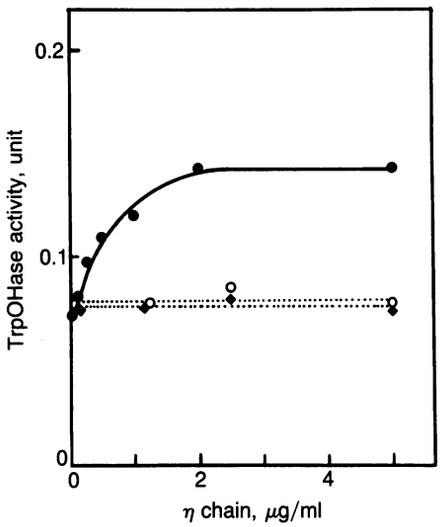

FIG. 3. Kinase II-dependent activation of TrpOHase by purified η chain. The activity of TrpOHase was assayed with various amounts of the η chain (●) under conditions described by Yamauchi *et al.* (5). Control experiments were performed with a complete assay system without kinase II (○) or without calmodulin (◆). One unit of TrpOHase is defined as the amount that catalyzes the formation of 1 nmol of 5-hydroxytryptophan per min at 30°C.

(Fig. 2) suggested that these cDNAs encoded the β and γ chains of 14-3-3 protein, respectively.

The nucleotide sequence of the cDNA for the η chain and the corresponding amino acid sequence are presented in Fig. 5. The cDNA sequence determined contains a total of 1793 bases including a single open reading frame starting with the first ATG codon at base 166 and ending with a TGA stop codon at base 904. The sequence contains a 165-base 5′ untranslated region (G+C content, 79%) and a 793-base 3′ untranslated region. In this 3′ untranslated region were found a consensus polyadenylylation signal (AATAAA) and a part of poly(A) tail (a stretch of 97 adenine residues). We estimated that the sequence determined represents almost the entire sequence of η-chain mRNA, because the size of η-chain mRNA was found to be about 1800 bases by blot hybridization analysis of electrophoretically fractionated RNA (data not shown).

The continuous open reading frame encodes a polypeptide of 246 amino acids with a M_r of 28,221. The amino acid composition and the M_r value calculated from the sequence account for the composition and the M_r of the η chain determined by amino acid analysis and NaDodSO$_4$/PAGE, respectively. The hexapeptide sequence designed for the synthetic oligonucleotide probe is perfectly matched with the sequence at positions 126–131, and all of the 117-residue sequence determined for the CNBr fragments is also found in this open reading frame (underlined in Fig. 5). Thus, we concluded that this sequence represents the complete sequence of the η chain.

Structural Features of η Chain. To examine the possible occurrence in other proteins of sequences homologous to the η chain, we used the SEARCH program to compare both the entire sequence and 30-residue segments of the sequence to the Protein Identification Resource database.** The η-chain amino acid sequence was found to be unique in that no proteins or their segments gave a significant score for homology. A graphic matrix plot of the η-chain sequence against itself, prepared by the DOTMATRIX program, suggested that the η chain has no internal homology in the amino acid sequence. A hydropathy plot of the sequence does not show any striking hydrophobic region that could serve as a signal peptide or a transmembrane segment (Fig. 6), indicating that the 14-3-3 protein is a typical intracellular, cytosolic protein.

The η chain contains three cysteine residues at positions 97, 112, and 194. Cys-194 appears to have a free sulfhydryl group, since the CNBr fragment spanning residues 166–223 was isolated directly from the unreduced chain. Cys-97 and Cys-112 were found in a single CNBr fragment spanning residues 72–126, and therefore we could not determine whether these cysteines have free sulfhydryls or form an intramolecular disulfide bridge. No interchain disulfide linkages should be present between η chains, because these chains behaved as monomers when dissociated from the 14-3-3 protein in gel electrophoresis without reducing agents. We found two asparagines (Asn-178 and Asn-229) in the tripeptide sequence Asn-Xaa-Ser/Thr that has a potential for N-glycosylation, but these residues seem not to be glycosylated: the 14-3-3 protein did not stain for carbohydrate (9) and no glucosamine or galactosamine was detected in amino acid analysis of the corresponding CNBr fragment. The η chain contains two serine residues at positions 59 and 64 that meet the consensus sequence proposed for cAMP- and/or calmodulin-dependent phosphorylation (17, 18). Although nothing has been reported about the phosphorylation of 14-3-3, we have noticed that Ser-59 is located in a hydrophilic environment (Fig. 6) that consists of a β-turn in the secondary structure predicted by the CHOFAS and PRPLOT programs.

As expected from the acidic nature of 14-3-3 protein, the η chain contains 45 acidic (aspartic and glutamic) residues, compared to 32 basic (lysine and arginine) residues, which should produce an excess negative charge of 13 at neutral pH. However, these amino acids are not evenly distributed along the polypeptide chain (Fig. 6); the amino-terminal two-thirds of the polypeptide (residues 1–172) contains almost equal numbers of acidic and basic amino acids (net charge, −1), whereas the carboxyl-terminal third (residues 173–246) carries a high proportion of acidic amino acids (net charge, −12). Based on the amino acid composition computed from the sequence, the isoelectric points of the amino- and carboxyl-terminal regions were estimated to be 6.2 and 3.2, respectively, by the application of the Henderson–Hasselbalch equation (19). We propose, therefore, that the η chain is composed of two structural regions different in charge and that the acidic carboxyl-terminal region may be responsible for the kinase II-dependent activation of TyrOHase and TrpOHase, as discussed below.

Possible Activation Mechanism. Although the 14-3-3 protein has been shown to be an essential factor for the kinase II-dependent activation of TyrOHase and TrpOHase (5, 6), little information is available for its molecular mechanism of action. Yamauchi and Fujisawa (20) proposed a two-step mechanism, phosphorylation of the hydroxylases and activation of the phosphorylated hydroxylases, and showed that the latter step required the 14-3-3 protein.

According to structural studies (21–23), the aromatic amino acid hydroxylases TyrOHase, TrpOHase, and phenylalanine hydroxylase are all structural homologs and consist of two domains each having a catalytic or regulatory function. The catalytic domain, comprising approximately the carboxyl-terminal 300 residues, has highly homologous sequence among these hydroxylases (22) and exhibits a catalytic activity when it is isolated as proteolytic fragments (24, 25). The amino-terminal regulatory domain, on the other hand, is variable in length (100–160 residues) and less homologous in sequence (22) and carries the sites for the cAMP- and calmodulin-dependent phosphorylation (26). In the native

**Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 12.0. The programs SEARCH, DOTMATRIX, CHOFAS, and PRPLOT used were also provided by the Natl. Biomed. Res. Found.

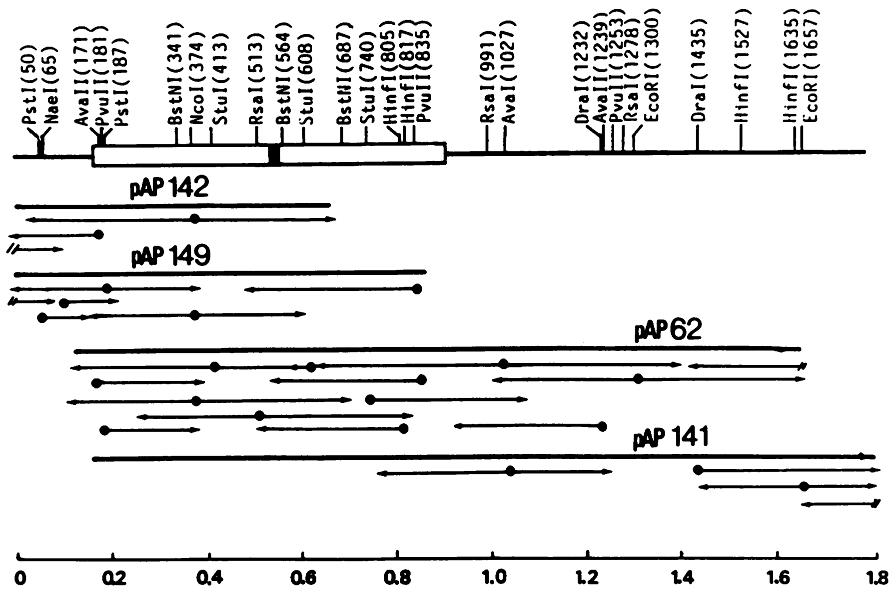

FIG. 4. Restriction map of the η-chain cDNAs and the sequencing strategy. The protein-coding region is indicated by the open box and the synthesized oligonucleotides by the filled box. The arrows indicate the direction and extent of sequence determination for each fragment analyzed. The sites of 5′-end labeling are indicated by filled circles at the ends of arrows. Slash marks at the ends of arrows mean that the sites of 5′-end labeling were located on the vector DNA. Most of the nucleotide sequence was determined with more than two clones. The sequences of all four clones were identical in their overlapping regions, except for the absence of a guanine at position 183 in pAP142, which probably was an artifact of reverse transcription of the mRNA. Scale at bottom is in kilobases.

enzymes TyrOHase and TrpOHase, the regulatory domain seems to inhibit an activity of the catalytic domain, because proteolytic removal of the regulatory domain activates the enzymes (27). Likewise, the binding of anions such as heparin, phospholipid, and polyglutamate activates the enzymes by a conformational transition that leads to an increase in the affinity of the enzymes for the cofactor tetrahydrobiopterin (27–29). The regulatory domain is notably basic, and an electrostatic interaction with these anions has been shown to induce this conformational transition. Therefore, from a view of the molecular organization of TyrOHase and TrpOHase, it appears likely that the acidic, carboxyl-terminal region of 14-3-3 protein binds to the basic, regulatory domain of the hydroxylase and activates the enzyme by a

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                                                                  1       5'···ACAGAGCGCCGGGCGAGCCAGCGAGAGGGCGAGAGGCGCTGCTTGC
  47 TGCCTGCAGCCTCGGCCGGCCGGCAAGCCAGTGCGCGTGCGCGGCGGCGGCCTCCGCGGCGACCGGAGAGGACGCGCGGGCGAGCGAGCGGAGCGGGAAGCGAGGCCGGGCTCAGCGAC

 166 ATG GGG GAC CGC GAG CAG CTG CTG CAG CGG GCG CGG CTG GCC GAG CAG GCG GAG CGC TAC GAC GAC ATG GCC TCC GCC ATG AAG GCG GTG
   1 Met Gly Asp Arg Glu Gln Leu Leu Gln Arg Ala Arg Leu Ala Glu Gln Ala Glu Arg Tyr Asp Asp Met Ala Ser Ala Met Lys Ala Val

 256 ACT GAG CTC AAT GAA CCT CTC TCC AAC GAA GAC CGA AAC CTC CTC TCC GTG GCC TAC AAG AAC GTG GTT GGT GCC CGG CGG TCT TCC TGG
  31 Thr Glu Leu Asn Glu Pro Leu Ser Asn Glu Asp Arg Asn Leu Leu Ser Val Ala Tyr Lys Asn Val Val Gly Ala Arg Arg Ser Ser Trp

 346 AGG GTC ATC AGC AGC ATC GAG CAG AAG ACC ATG GCT GAT GGG AAT GAG AAG AAG CTG GAG AAG GTG AAG GCC TAC CGG GAG AAG ATT GAG
  61 Arg Val Ile Ser Ser Ile Glu Gln Lys Thr Met Ala Asp Gly Asn Glu Lys Lys Leu Glu Lys Val Lys Ala Tyr Arg Glu Lys Ile Glu

 436 AAG GAG CTG GAG ACG GTG TGC AAC GAC GTG CTG GCG CTG CTG GAC AAG TTC CTC ATC AAG AAC TGC AAT GAC TTC CAG TAC GAG AGC AAG
  91 Lys Glu Leu Glu Thr Val Cys Asn Asp Val Leu Ala Leu Leu Asp Lys Phe Leu Ile Lys Asn Cys Asn Asp Phe Gln Tyr Glu Ser Lys

 526 GTC TTC TAC CTG AAG ATG AAG GGC GAC TAC TAC CGC TAC CTG GCC GAG GTG GCT TCT GGC GAG AAG AAG AAC AGT GTG GTG GAG GCC TCA
 121 Val Phe Tyr Leu Lys Met Lys Gly Asp Tyr Tyr Arg Tyr Leu Ala Glu Val Ala Ser Gly Glu Lys Lys Asn Ser Val Val Glu Ala Ser

 616 GAG GCG GCC TAC AAG GAA GCC TTC GAG ATT AGC AAG GAG CAC ATG CAG CCC ACA CAC CCC ATC CGG CTG GGC CTG GCC CTC AAC TTC TCC
 151 Glu Ala Ala Tyr Lys Glu Ala Phe Glu Ile Ser Lys Glu His Met Gln Pro Thr His Pro Ile Arg Leu Gly Leu Ala Leu Asn Phe Ser

 706 GTG TTC TAC TAC GAG ATC CAG AAT GCG CCT GAG CAG GCC TGC CTC CTA GCC AAA CAA GCC TTC GAC GAC GCC ATA GCC GAG CTG GAC ACA
 181 Val Phe Tyr Tyr Glu Ile Gln Asn Ala Pro Glu Gln Ala Cys Leu Leu Ala Lys Gln Ala Phe Asp Asp Ala Ile Ala Glu Leu Asp Thr

 796 CTA AAC GAG GAT TCC TAT AAG GAC TCC ACG CTC ATC ATG CAG CTG CTG CGA GAC AAC CTC ACC CTC TGG ACG AGC GAC CAG CAG GAC GAG
 211 Leu Asn Glu Asp Ser Tyr Lys Asp Ser Thr Leu Ile Met Gln Leu Leu Arg Asp Asn Leu Thr Leu Trp Thr Ser Asp Gln Gln Asp Glu

 886 GAA GCC GGA GAA GGC AAC TGA GCGCCTTGGCCCGCCCCGCCCCCGCCCCATCACCACCAGTCCCCTCTCGCCACACTCACTAAGTATCCAGTGCTCAACCTATCTGTACGGC
 241 Glu Ala Gly Glu Gly Asn ===

 998 AGCACAGCTACTCAGACCTGCTGTCCGCCCCCGGGAAGCAGTTCCAGATAAATTAATTCATGGGCATCGCTGGACTGACGGTTGCTTTGAGCCCACAGGAGCTCCCTTTTTGGATCGTG
1117 CAGACAGGTGCGTTCTGAAGGAGGCATTGTCGTTTGCTTGCCTGTCTAGGTGAATTGCAGGCGAAAGCCTCAGAAAGTTAGAGAGGAGAATTAGCCACACAGGCTACAGTTGGTATTTA
1236 AATGGTCCACTTCAAACCAGCTGCTAGTGTTTTGTTAAAGCAGTACATCTGTGCATGCAAAAGTGAATTCACCCCTCCCTCTTCTTTCTTAGCTAATGGAAAACCATTAAGGGAAGCTG
1355 GAACGAGAGACCACTTGCTCCTTTCCATCAGCTTAATAATTAACTTTAACGTGAGGTTTCAGTAGCACCTTGTTCGCCTCTTTAAATTATGACGTGCACAAACCTTCTTTTCAATGCAA
1474 TGCATCTAAAGTTTTGATACCTGTAACTTTTTTTTTTGGTTGCAATTGTTTAAGAATCATGGATTTATTTTTTGTAACTCTTTGGCTATCGTCCTTGTGTATCCTGACAGCGCCATGTG
1593 TGTCAGCCCATGTCAATCAAGATGGGTGATTATGAAATGCCAGACTCCTAAATTAAATGTTTTGGAATTCAGTGGGTAAATAAAAATGCTGCTTTGGGGATATTAAAAAAAAAAAAAA
1712 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA··· 3'
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FIG. 5. Complete nucleotide sequence and deduced amino acid sequence of the 14-3-3 η chain. The nucleotides are numbered at the left of each line, beginning with the first nucleotide of the most-5′ cDNA clones (pAP142 and pAP149). The deduced amino acid sequence is also numbered at the left. Amino acid sequence determined by the analysis of CNBr peptides of η chain is underlined. The termination codon (TGA) at positions 904–906 and the polyadenylylation signal (AATAAA) at positions 1671–1676 are emphasized by the double lines.

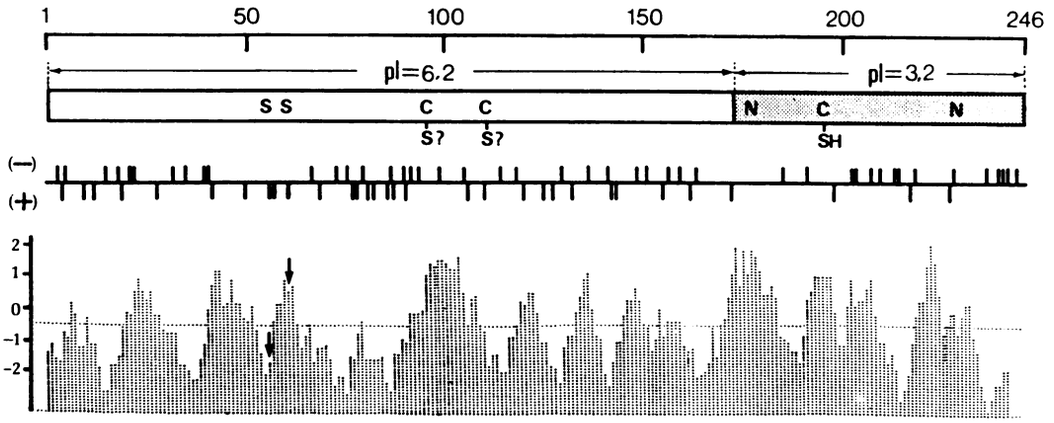

FIG. 6. Linear model of the primary structure of 14-3-3 η chain emphasizing the extremely acidic carboxyl-terminal region. Serine (S), cysteine (C), and asparagine (N) residues discussed in the text are indicated within the bar, and their positions in the sequence of 246 residues are shown to scale. The sulfhydryl (SH) of Cys-194 is indicated below the bar; it is not known whether Cys-97 and Cys-112 (S?) form an intrachain disulfide bond. Acidic (aspartic and glutamic) and basic (lysine and arginine) amino acid residues are shown below the sequence by upward (−) and downward (+) vertical lines, respectively. At the bottom is a plot of the hydropathy index generated by the algorithm of Kyte and Doolittle (16) with a window size of 6 residues. Arrows indicate the positions of serine residues that are potential sites of phosphorylation.

mechanism similar to that proposed for the anions described above. A previous study (20) indicates that the 14-3-3 protein binds to TyrOHase and TrpOHase only after the enzymes are phosphorylated, suggesting that the phosphorylated serine is involved in interaction with the 14-3-3 protein or that the phosphorylation results in a conformational transition that enables the 14-3-3 protein to bind the regulatory domain, thus causing a second transition leading to hydroxylase activation. However, whether this or some other mechanism is the basis of kinase II-dependent activation of TyrOHase and TrpOHase awaits further investigation.

To our knowledge, the sequence of 14-3-3 protein represents the first sequence of a protein whose activity is associated with the regulatory function of protein kinase. Knowledge of the amino acid sequence and availability of its cDNA should aid elucidation of its mechanism of action and permit a structure–function analysis of its potential biological function in monoamine biosynthesis. This study should also facilitate studies of the 14-3-3 gene and its possible relation with neuropsychiatric disorders involving monoamine biosynthesis, such as Parkinson disease, manic-depressive illness, and schizophrenia.

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