Cardin, A. D., & Behnke, W. K. (1978) *Biochim. Biophys. Acta 537*, 436–445.
Christie, D. J., Alter, G. M., & Magnuson, J. A. (1978) *Biochemistry 17*, 4425–4430.
Christie, D. J., Munske, G. R., & Magnuson, J. A. (1979) *Biochemistry 18*, 4638–4644.
Grimaldi, J. J., & Sykes, B. D. (1975) *J. Biol. Chem. 250*, 1618–1624.
Hallenga, K., & Koenig, S. H. (1976) *Biochemistry 15*, 4255–4264.
Harrington, P. C., & Wilkins, R. G. (1978) *Biochemistry 17*, 4245–4250.
Harrington, P. C., Moreno, R., & Wilkins, R. G. (1981) *Isr. J. Chem. 21*, 48–51.
Kalb, A. J., & Levitzki, A. (1968) *Biochem. J. 109*, 669–672.
Koenig, S. H., & Schillinger, W. S. (1969) *J. Biol. Chem. 244*, 4256–4262.
Koenig, S. H., Brown, R. D., & Brewer, C. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 475–479.
Koenig, S. H., Brewer, C. F., & Brown, R. D., III (1978) *Biochemistry 17*, 4251–4260.
Koenig, S. H., Brown, R. D., III, Brewer, C. F., & Sherry, A. D. (1982) *Biochem. Biophys. Res. Commun. 109*, 1047–1053.
Meirovitch, E., Luz, Z., & Kalb (Gilboa), A. J. (1974a) *J. Am. Chem. Soc. 96*, 7538–7542.
Meirovitch, E., Luz, Z., & Kalb (Gilboa), A. J. (1974b) *J. Am. Chem. Soc. 96*, 7542–7546.
Palmer, A. R., Bailey, O. B., Behnke, W. D., Cardin, A. D., Yang, P. B., & Ellis, P. D. (1980) *Biochemistry 19*, 5063–5070.
Pandolfino, E. R., Appel, D. M., Christie, D. J., & Magnuson, J. A. (1980) *Biochem. Biophys. Res. Commun. 96*, 1248–1252.
Sherry, A. D., Buck, A. E., & Peterson, C. A. (1978) *Biochemistry 17*, 2169–2173.
Sherry, A. D., Lindorfer, M. A., Adam-Stemler, P., & Milanes, O. A. (1981) *Biochemistry 20*, 3492–3499.
Strazza, S., & Sherry, A. D. (1982) *Biochem. Biophys. Res. Commun. 106*, 1291–1297.
Yariv, J., Kalb, A. J., & Levitzki, A. (1968) *Biochim. Biophys. Acta 165*, 303–305.

# Amino Acid Sequence of the Catalytic Subunit of Bovine Type II Adenosine Cyclic 3′,5′-Phosphate Dependent Protein Kinase†

Shozo Shoji,‡ Lowell H. Ericsson, Kenneth A. Walsh, Edmond H. Fischer, and Koiti Titani*

ABSTRACT: The 350-residue amino acid sequence of the catalytic subunit of bovine cardiac muscle adenosine cyclic 3′,5′-phosphate dependent protein kinase is described. The protein has a molecular weight of 40 862, which includes an *N*-tetradecanoyl (myristyl) group blocking the $NH_2$ terminus and phosphate groups at threonine-197 and serine-338. Seven methionyl bonds in the S-carboxymethylated protein were cleaved with cyanogen bromide to yield eight primary peptides. These fragments, and subpeptides generated by cleavage with trypsin, pepsin, chymotrypsin, thermolysin, and Myxobacter AL-1 protease II, were purified and analyzed to yield the majority of the sequence. The primary peptides were aligned by analyses of overlapping peptides, particularly of methione-containing tryptic peptides generated after in vitro [$^{14}$C]methyl exchange labeling of methionyl residues in the intact protein.

The varied physiological actions of adenosine cyclic 3′,5′-phosphate (cAMP) in eukaryotic organisms are mediated primarily by the specific phosphorylation of cellular proteins by cAMP-activated protein kinases (ATP:protein phosphotransferase, EC 2.7.1.37). They were widely distributed and well recognized to play a major general role in the regulation of energy metabolism and other physiological functions (Krebs, 1972; Rubin & Rosen, 1975; Rosen et al., 1977; Cohen, 1978, 1982; Krebs & Beavo, 1979). The enzyme is known to be composed of two regulatory (R) and two catalytic (C) subunits that together constitute an inactive holoenzyme ($R_2C_2$). The addition of cAMP activates protein kinase according to the following scheme:

$$R_2C_2(\text{inactive}) + 4\text{cAMP} \rightleftharpoons R_2(\text{cAMP})_4 + 2C(\text{active})$$

The enzyme is present in mammalian tissues in two forms, type I, the predominant form in skeletal muscle, and type II, the predominant form in bovine cardiac muscle (Reimann et al., 1971; Rubin et al., 1972). The differences between the two types are attributed to differences in the structure of the R subunit; the C subunits are thought to be identical (Hofmann et al., 1975). Recently, the roles, function, and location of critical sulfhydryl groups and of the ATP–Mg binding site of the C subunit of the enzyme were described (Zoller et al., 1981; Nelson & Taylor, 1981; Jimenez et al., 1982; Kupfer et al., 1982). In order to relate its molecular structure to the mechanism of action of the enzyme and the manner in which it is regulated, we have determined the amino acid sequence of the protein. The location of two sites that are phosphorylated and the occurrence of the unusual myristyl blocking group have already been described (Shoji et al., 1979; Carr

† From the Department of Biochemistry and the Howard Hughes Medical Institute Laboratory, University of Washington, Seattle, Washington 98195. *Received November 16, 1982; revised manuscript received April 13, 1983.* This work was supported by grants from the National Institutes of Health (GM 15731 and AM 7902).

* Address correspondence to this author at the Department of Biochemistry.

‡ Present address: Department of Biochemistry, Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-Honmachi, Kumamoto 862, Japan.

0006-2960/83/0422-3702$01.50/0 

et al., 1982), and a preliminary report of the sequence has been presented (Shoji et al., 1981).

This paper describes the details of proof of sequence of the C subunit of the bovine cardiac muscle enzyme (type II). Together with our recent description of the sequence of the regulatory subunit of the same enzyme (Takio et al., 1982a), this work accounts for the 750 amino acid residue sequence of the whole protein (1500 residues per tetramer).

## Materials and Methods

The catalytic subunit of bovine cardiac muscle type II cAMP-dependent protein kinase was purified to homogeneity according to Peters et al. (1977). Myxobactor AL-1 protease II (Wingard et al., 1972) was a gift from Dr. H. Wood (Case Western Reserve University), and carboxypeptidase Y was a gift from Dr. M. Ottesen (Carlsberg Laboratory). Thermolysin was purchased from Calbiochem, and pepsin, $\alpha$-chymotrypsin, carboxypeptidases A and B, and Tos-Phe-$CH_2Cl$-trypsin were Worthington products. Citraconic anhydride, dithioerythritol, cyanogen bromide, and fluorescamine were products of Pierce Chemical Co. Iodoacetic acid and guanidine hydrochloride were purchased from Sigma and Heico, respectively. Iodo[$^{14}$C]acetic acid and [$^{14}$C]methyl iodide were purchased from New England Nuclear. Sequenal-grade chemicals for sequencing were obtained from Beckman, Pierce, and Burdick & Jackson. Various grades of Sephadex, SP-Sephadex C-25, and DEAE-Sephacel were products of Pharmacia, and the ion-exchange resin AG 50W-X2 was a product of Bio-Rad.

Automated sequence analyses were performed with a Beckman sequencer (Model 890C) according to the method of Edman & Begg (1967) with either the protein program described by Hermodson et al. (1972) or the Quadrol program of Brauer et al. (1975), each of which gave consistently repetitive yields of 93–96%. Small peptides were sequenced in the presence of Polybrene (Tarr et al., 1978) or analyzed with a Sequemat by the method of Laursen (1971) after attachment to the solid phase (triethylenetetramine resin or diisothiocyanate–polyamino glass) via carboxyl-terminal homoseryl or lysyl residues (Horn & Laursen, 1973; Laursen et al., 1972). Phenylthiohydantoin derivatives of the amino acids were identified by two complementary systems of reversed-phase high-performance liquid chromatography (Ericsson et al., 1977; Hermann et al., 1978). Amino acid analyses were performed with a Dionex amino acid analyzer (Model D-500). Methionyl residues of the *S*-carboxymethyl protein were labeled with [$^{14}$C]methyl iodide in vitro by the method of Link & Stark (1967).

Large fragments generated by cyanogen bromide cleavage were separated by gel filtration and SP-Sephadex chromatography in the presence and absence of either 7 M urea or 6 M guanidine hydrochloride. The molecular weights of large fragments were determined by $NaDodSO_4$[1]–polyacrylamide gel electrophoresis (Weber & Osborn, 1969). The conditions for protein carboxymethylation with iodo[$^{14}$C]acetic acid, cyanogen bromide cleavage, succinylation, tryptic digestion, chymotryptic digestion, cleavage at tryptophanyl bonds, gel filtration on Sephadex, chromatography on SP-Sephadex C-25 or Bio-Rad AG 50W-X2, and paper electrophoresis were described by Koide et al. (1978) and were used with the following minor modifications. Trypsin digestions generally were applied to the $\epsilon$-N-citraconylated (Atassia & Habeeb, 1972) *S*-carboxymethyl protein. Citraconyl groups were removed in 9% formic acid (3 h at 37 °C).

*Peptide Nomenclature.* The following prefixes indicate the primary source of the peptide: CB, cleavage by cyanogen bromide; R, tryptic cleavage at arginine of N-acylated protein; MTc, tryptic digest of [$^{14}$C]methionine-labeled, citraconylated protein. Hyphenated suffixes identify the products of subdigestion by trypsin (T), chymotrypsin (C), pepsin (P), thermolysin (Th), or Myxobacter protease (Mx) and products of chemical cleavage at tryptophan (W).

## Results

*General Strategy.* Cleavage at the seven methionyl residues yielded four large fragments (>45 residues) and four small ones (<10 residues). Edman degradation of these fragments and of their subdigestion products (primarily after tryptic cleavage at arginyl residues) yielded their complete sequences. These eight primary fragments were aligned by using tryptic peptides derived from the whole protein and selected for their methionyl residues, which had been radiolabeled by [$^{14}$C]-methyl exchange. Details of this strategy are summarized in Figure 1 in which large boxes enclose information derived from each of the four large fragments (CB1, CB4, CB7, and CB8) to distinguish them from both the intervening small peptides (CB2, CB3, CB5, and CB6) and the methionine-containing overlapping MTc peptides. In presenting these results, we show the separation and compositional data only for the primary CB and MTc fragments. Analogous data for subdigestion products are available in the supplementary material as indicated by the suffix S in figures or tables (see paragraph at end of paper regarding supplementary material).

*Isolation of CB Peptides.* Treatment of the *S*-carboxy-[$^{14}$C]methyl protein with cyanogen bromide yielded a mixture that was finally resolved into eight fragments by a combination of gel filtration and SP-Sephadex chromatography (Figure 2). The mixture was initially divided into large (fraction I, Figure 2A) and small fragments by Sephadex G-25 gel filtration. The pool of large fragments was further resolved by Sephadex G-75 gel filtration (Figure 2B) into three fractions, the second of which was homogeneous by $NaDodSO_4$ gels and by sequenator analysis. It contained no homoserine, indicating that it represented the carboxyl terminus of the protein (CB8).

The first fraction (I-1 in Figure 2B) was resolved into two fragments by rechromatography in a denaturing solvent (Figure 2C). Edman degradation of each gave the same $NH_2$-terminal sequence (Phe-Ser-His-), but $NaDodSO_4$ gels (Figure 1-S) showed that a fragment in the first pooled fraction (CB7–8) had a molecular weight equal to the sum of that in the second fraction (CB7) and that labeled CB8 in Figure 2B. The compositions of these three fractions (Table I) confirm that fraction CB7–8 contains a product of incomplete cleavage between CB7 and the carboxyl-terminal fragment CB8.

The third fraction in Figure 2B (I-3) yielded a single band on $NaDodSO_4$ gels and a single $NH_2$-terminal sequence on Edman degradation but proved to be a mixture of an $NH_2$ terminal blocked fragment CB1 and another peptide, CB4, of approximately the same mass. These were resolved on SP-Sephadex (Figure 2D).

Four small fragments (CB2, CB3, CB5, and CB6) were purified by paper electrophoresis of the fractions indicated in Figure 2A. Fraction II in Figure 2A appeared to contain a peptide overlapping CB2 and CB3, but it was not obtained in pure enough form to use as proof of sequence.

*Identification of the $NH_2$-Terminal Structure.* It proved to be impossible to obtain Edman degradation products from the intact subunit, CB1, R1 (a blocked tryptic peptide obtained by tryptic digestion of citraconyl CB1), CB1-T1 (a tryptic

[1] Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Myr, myristyl.

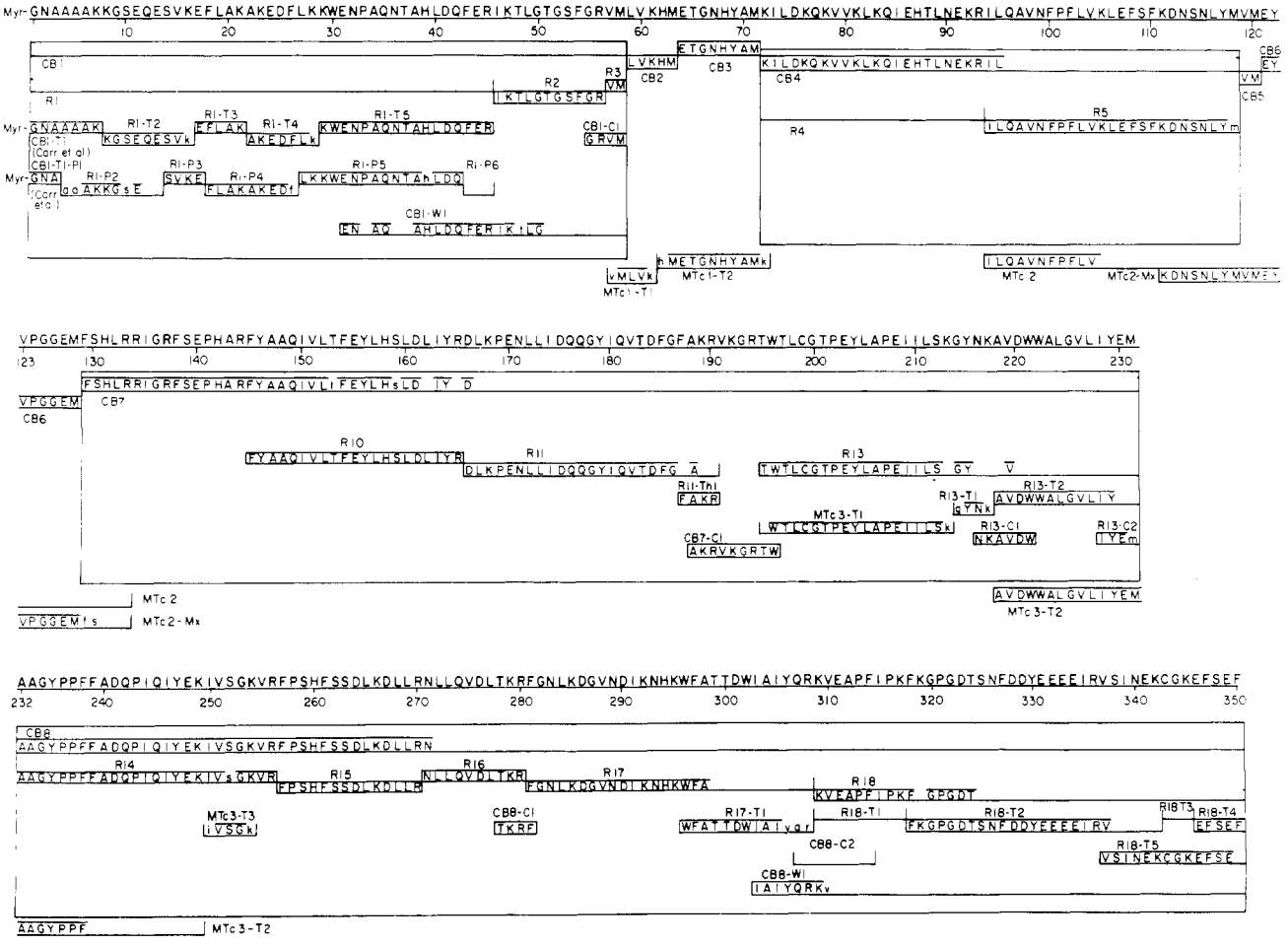

FIGURE 1: Summary proof of amino acid sequence of catalytic subunit of type II cAMP-dependent protein kinase from bovine cardiac muscle. The large boxes enclose data from the large CB peptides generated with CNBr. Smaller bars enclose summary data from the various fragments analyzed. The one letter code (Table I where D, N, E, and Q signify Asp, Asn, Glu, and Gln, respectively) within a bar designates amino acid residues in that peptide; large capital letters indicate residues positively identified by Edman degradation, and small letters indicate residues that were more tentatively placed by composition, cleavage specificity, or marginal identification of the phenylthiohydantoin derivative. The length of each bar indicates the length of the peptide; enclosure of the top of the bar indicates a proven portion of sequence; gaps in the upper enclosure indicate portions of sequence that are either tentative or lacking. The deduced sequence is summarized above the residue number line. Analyses of the two blocked amino-terminal peptides, CB1-T1 and CB1-T1-P1, were reported by Carr et al. (1982). The abbreviation Myr signifies a myristyl group.

peptide of decitraconylated R1), or CB1-T1-P1 (a peptic peptide derived from CB1-T1). A collaborative study with Dr. K. Biemann (Carr et al., 1982) of CB1-T1 and CB1-T1-P1 utilized mass spectrometry to establish that the blocking group is a myristyl moiety forming an amide linkage to the $NH_2$-terminal glycine in the sequence Myr-Gly-Asn-Ala-Ala-Ala-Ala-Lys. The peptide portion was previously incorrectly described by us (Shoji et al., 1981) with three rather than four alanine residues.

*Sequence Analysis of CB1 and CB4.* Five pure peptides (R1–R5) were obtained from tryptic digests of citraconylated CB1 and CB4 (Figures 2-S and 3-S). The composition of R4 corresponded to 22 residues of the $NH_2$-terminal sequence of CB4, and the sequence of R5 (Figure 1) overlapped and completed that sequence. The remaining three peptides are derived from CB1 where blocked R1 must be $NH_2$ terminal and the homoserine-containing R3 is carboxyl terminal. Two overlapping sets of peptides were obtained from R1 (Table I-S, Figure 1) by tryptic or peptic digestion of dicitraconylated R1. Together with analysis of a chymotryptic peptide derived from CB1 by the method of Horn & Lauresen (1973) and of a peptide purified by gel filtration after cleavage at tryptophan-30 by the method of Omenn et al. (1970), the complete sequence of CB1 was derived (Figure 1).

*Sequence Analysis of CB7.* Citraconylated CB7 was cleaved with trypsin and resolved into three fractions by gel filtration on Sephadex G-50 superfine in 0.1 M $NH_4HCO_3$ (Figure 4-S). The first fraction contained a pure peptide (R10 in Figure 1); the second fraction contained two peptides, R11 and R13, which were resolved on DEAE-Sephacel (Figure 4-S); the third comprised small peptides that were not further purified. $NH_2$-Terminal analysis of CB7 (Figure 1) established the sequence and contiguity of four putative small fragments (which, if they had been isolated from the third fraction, would have been named R6 through R9), provided a complete overlap through R10, and identified aspartic acid as the $NH_2$ terminus of the subsequent tryptic peptide.

Sequenator analyses of R11 and R13 were incomplete. The carboxyl terminus of R11 was provided by analysis of a thermolytic peptide isolated from R11 by paper electrophoresis. A chymotryptic peptide from CB7 (CB7-C1) overlapped the carboxyl terminus of R11 with the $NH_2$ terminus of R13 and provided an intervening peptide Val-Lys-Gly-Arg that must have also been in the third fraction of the tryptic digest of CB7.

Peptide R13 contained homoserine and, hence, must represent the carboxyl-terminal peptide of CB7. Subdigestion with chymotrypsin or with trypsin (after decitraconylation) yielded four overlapping peptides that provided the carbox-

Table I: Amino Acid Composition of Cyanogen Bromide Fragments[a]

| | CB1 | CB2 | CB3 | CB4 | CB5 | CB6 | CB7 | CB8 | CB7-8 | Sum of CB1 to CB8 | Whole Subunit[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Residues | 1-58 | 59-63 | 64-71 | 72-118 | 119-120 | 121-128 | 129-231 | 232-350 | 129-350 | | |
| Fig. No. | 2D | 2A | 2A | 2D | 2A | 2A | 2C | 2B | 2C | | |
| Sephadex Fraction | | III | IV | | VI | V | | | | | |
| Subsequent Purification | | HVE[b] | HVE | | HVE | HVE | | | | | |
| Cm Cys, C | | | | | | | 0.7 (1) | 0.5 (1) | 1.8 (2) | 1.4 (2) | 1.5 (2) |
| Asx, D & N | 5.6 (5) | | 1.1 (1) | 6.2 (6) | | | 6.8 (7) | 14.0(16) | 22.8(23) | 35.2(35) | 34.8(35) |
| Thr, T | 2.7 (3) | | 0.9 (1) | 1.4 (1) | | | 4.3 (5) | 3.9 (4) | 8.9 (9) | 13.2(14) | 14.3(14) |
| Ser, S | 2.7 (3) | | | 1.8 (2) | | | 4.1 (4) | 5.5 (7) | 10.9(11) | 15.4(16) | 16.0(16) |
| Glx, E & Q | 8.1 (9) | | 1.2 (1) | 6.1 (6) | | 1.8 (2) | 10.2(10) | 12.4(13) | 22.4(23) | 39.8(41) | 40.8(41) |
| Pro, P | 1.2 (1) | | | 1.2 (1) | | 0.9 (1) | 4.0 (4) | 6.1 (7) | 10.8(11) | 13.9(14) | 14.3(14) |
| Gly, G | 4.6 (5) | | 1.3 (1) | | | 2.1 (2) | 7.6 (7) | 6.8 (7) | 14.0(14) | 23.2(22) | 22.3(22) |
| Ala, A | 6.8 (8) | | 1.0 (1) | 0.9 (1) | | | 7.0 (7) | 6.0 (6) | 13.2(13) | 21.7(23) | 22.2(23) |
| Val, V | 2.4 (2) | 1.1 (1) | | 3.6 (4) | 1.0 (1) | 1.0 (1) | 5.0 (5) | 5.8 (6) | 11.0(11) | 19.8(20) | 20.0(20) |
| Met, M[d] | 0.6 (1) | 1.1 (1) | 1.3 (1) | 0.5 (1) | 1.1 (1) | 1.1 (1) | 0.3 (1) | | 1.0 (1) | 6.0 (7) | 6.5 (7) |
| Ile, I | 1.2 (1) | | | 2.8 (3) | | | 6.9 (8) | 7.7 (9) | 16.8(17) | 19.2(21) | 20.8(21) |
| Leu, L | 4.6 (4) | 1.0 (1) | | 7.0 (7) | | | 12.6(13) | 7.4 (7) | 20.0(20) | 32.8(32) | 32.4(32) |
| Tyr, Y | | | 0.9 (1) | 1.1 (1) | | 0.9 (1) | 6.9 (7) | 3.9 (4) | 10.8(11) | 13.8(14) | 14.0(14) |
| Phe, F | 4.0 (4) | | | 3.8 (4) | | | 6.0 (6) | 9.6(11) | 15.8(17) | 23.4(25) | 23.9(25) |
| His, H | 1.0 (1) | 1.0 (1) | 1.2 (1) | 1.2 (1) | | | 3.0 (3) | 2.0 (2) | 5.0 (5) | 9.4 (9) | 8.7 (9) |
| Lys, K | 8.0 (8) | 1.0 (1) | | 8.0 (8) | | | 4.4 (5) | 10.6(12) | 16.9(17) | 32.6(34) | 33.6(34) |
| Arg, R | 2.0 (2) | | | 1.4 (1) | | | 6.9 (7) | 5.1 (5) | 12.0(12) | 15.4(15) | 14.9(15) |
| Trp, W | (1) | | | | | | (3) | (2) | (5) | (6) | 7.5[e](6) |
| No. of Residue | 58 | 5 | 8 | 47 | 2 | 8 | 103 | 119 | 222 | 350 | 350 |
| Yield (%) | 56 | 85 | 88 | 51 | 70 | 82 | 96 | 98 | 7 | | |
| Cpm[f] | | | | | | | 253 | 237 | 455 | | 538 |

[a] Residues/molecule by amino acid analysis or (in parentheses) from the sequence (Figure 1).

[b] HVE, high voltage paper electrophoresis at pH 3.6 and 2.5 KV for 1 h.

[c] Values for serine and threonine extrapolated to zero time after 24, 48, 72, and 96 h hydrolysis; values for valine and isoleucine are after 96 h.

[d] Determined as homoserine and its lactone.

[e] After hydrolysis with mercaptoethanesulfonic acid by Oemaille et al (1977), adjusted to 23 alanine/mol.

[f] Specific radioactivity of [$^{14}$C]-carboxymethyl cysteine (cpm/nmol of peptide).

yl-terminal 17 residues, including the homoseryl residue. The partial sequence Ser-X-Gly-Tyr-X-X-X-Val, which was derived toward the end of the $NH_2$-terminal analysis of R13, was consistent with the alignment of R13-T1 and R13-T2 in Figure 1. Other data (described later) provided lysine-213 (in MTc3-T1) and confirmed the alignment of R13-C1, R13-T2, and R13-C2 (in MTc3-T2).

*Sequence Analysis of CB8.* The $NH_2$-terminal 40 residues of this largest primary fragment of CNBr cleavage placed two of its five arginyl residues. Tryptic digests of succinylated (or citraconylated) CB8 were resolved into five major peptides (Figure 5-S, Table III-S), two of which (R14 and R15) confirmed the sequence of the $NH_2$-terminal region (Figure 1). Of the other three peptides, only R16 had an $NH_2$-terminal asparagine, placing it next to R15 by a minimal overlap. Another single residue overlap in CB8-C1 (a peptide isolated by paper electrophoresis from a chymotryptic digest of CB8) links R16 to R17. R17 and R18 were each decitraconylated and subdigested at lysyl residues by trypsin. The products provided all but three residues of R17 and extended the sequence of R18 through an uncleaved Arg–Val bond. On the assumption that cleavage of Arg–Val had been prevented by the acidic character of residues 328–334, calcium acetate (10 mM) was added to succinylated R18 and the mixture incubated with trypsin at 37 °C overnight. Sequenator analysis of the unfractionated digestion products (containing a succinylated $NH_2$ terminus) yielded the sequence R18-T5 (Figure 1), which began with valine-337 and overlapped R18-T4. R17 was completed, and an overlap to R18 was provided by peptide CB8-W1 after cleavage of CB8 at tryptophan by the method of Omenn et al. (1970).

*Exchange Radiolabeling of Methionyl Residues.* The *S*-carboxymethyl protein (4 mg) was dissolved in 1 mL of 6 M guanidine hydrochloride containing 0.1 M $KNO_3$, and the pH was adjusted to 5.0 with 1.0 N NaOH. This solution was added to $^{14}CH_3I$ (100 $\mu$Ci/47.9 $\mu$mol), and the mixture was stirred at room temperature in the dark overnight. The excess reagents were removed on a column (2.5 × 25 cm) of Sephadex G-25 superfine in 9% formic acid in the presence of 6 M guanidine hydrochloride. The protein fraction was then desalted by dialysis and lyophilized. This [$^{14}$C]methyl protein (3.5 mg) should be labeled only on its methionyl residues as their methyl sulfonium salt derivatives (Link & Stark, 1967). Labeling was measured to be 400 828 cpm/mg. Methionyl residues were then regenerated by the method of Christie et al. (1979) with 2-mercaptoethanol in 6 M guanidine hydrochloride at pH 8.6 for 22 h at 37 °C. The product (2 mg) was desalted as above and found to contain 215 500 cpm/mg, in accord with the expected random demethylation. No traces of 1-methylhistidine or 3-methylhistidine were detected in the acid hydrolysates (110 °C, 24 h) of the $^{14}$C-labeled protein by paper electrophoresis.

*Isolation of [$^{14}$C]Methionine-Containing Peptides (MTc Peptides).* One micromole (40 mg) of *S*-carboxymethyl

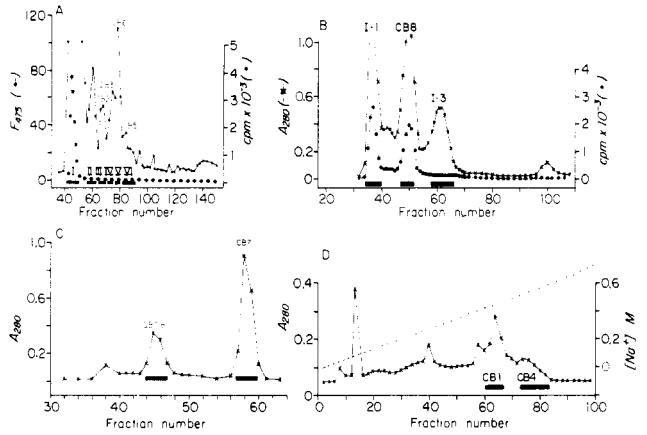

FIGURE 2: Separation of CB fragments of type II catalytic subunit (114 mg, 2.79 μmol) of cAMP-dependent protein kinase. Columns were monitored at 280 nm (×), by radioactivity (--), or with fluorescamine after hydrolysis of 2% of the effluent (—) and fractions pooled as indicated by horizontal bars. (A) Initial separation on a Sephadex G-25 superfine column (2.5 × 112 cm) eluted with 9% formic acid at 30 mL/h. Aliquots of 5 mL were collected at 30 mL/h. (B) Pooled fraction I of (A) was citraconylated and fractionated on a Sephadex G-75 superfine column (2.5 × 113 cm). Peptides were eluted at 20 mL/h with 0.1 M ammonium bicarbonate (pH 8.0). Aliquots of 5 mL were collected. (C) Fraction I-1 of (B) was further purified under the same conditions as in (B) but with the inclusion of 6 M guanidine hydrochloride. (D) Further fractionation of fraction I-3 (B) on a SP-Sephadex C-25 column (0.9 × 60 cm). Fragments were eluted at 12 mL/h by a linear gradient of 0.1 M sodium formate–7 M urea, pH 2.9 (100 mL), to 0.75 M sodium formate–7 M urea, pH 3.9 (100 mL). Fractions of 2 mL were collected. The first 50 fractions contained no peptides. Fractions eluting before CB1 and between CB1 and CB4 contained mixtures.

Table II: Amino Acid Compositions[a] of Tryptic Peptides from [$^{14}$C] Methionine-labelled Catalytic Subunit

| | MTc1-T1[b] | MTc1-T2[b] | MTc2 | MTc3-T1[c] | MTc3-T2 | MTc3-T3[d] |
|---|---|---|---|---|---|---|
| Residues | 57-61 | 62-72 | 94-133 | 195-213 | 218-249 | 250-254 |
| Fig. No. | 3C | 3C | 3B | 3C | 3C | 3C |
| Sephadex Fraction | II-3 | II-3 | | II-2 | | II-3 |
| Subsequent Purification[e] | HVE | HVE | | DEAE | | HVE |
| Cm Cys, C | | | | 0.8 (1) | | |
| Asx, D & N | | 1.0 (1) | 4.4 (4) | | 2.3 (2) | |
| Thr, T | | 1.0 (1) | | 2.7 (3) | | |
| Ser, S | | | 2.5 (3) | 1.2 (1) | | 1.0 (1) |
| Glx, E & Q | | 1.1 (1) | 4.6 (4) | 1.6 (2) | 4.4 (4) | |
| Pro, P | | | 2.0 (2) | 2.0 (2) | 2.9 (3) | |
| Gly, G | | 1.1 (1) | 2.5 (2) | 0.9 (1) | 1.9 (2) | 1.2 (1) |
| Ala, A | | 1.0 (1) | 1.3 (1) | 1.0 (1) | 5.0 (5) | |
| Val, V | 2.0 (2) | | 3.8 (4) | | 2.0 (2) | 1.0 (1) |
| Met, M | 0.9 (1) | 1.5 (2) | 1.7 (3) | | 1.4 (1) | |
| Ile, I | | | 1.5 (1) | 1.3(2) | 2.6 (3) | 0.7 (1) |
| Leu, L | 1.0 (1) | | 5.4 (5) | 2.6 (3) | 2.3 (2) | |
| Tyr, Y | | 0.8 (1) | 1.8 (2) | 0.9 (1) | 2.5 (3) | |
| Phe, F | | | 4.7 (5) | | 2.3 (2) | |
| His, H | | 1.6 (2) | 0.9 (1) | | | |
| Lys, K | 1.2 (1) | 1.4 (1) | 1.9 (2) | 0.8 (1) | 1.3 (1) | 1.4 (1) |
| Arg, R | | | 1.6 (1) | | | |
| Trp, W | | | | (1) | (2) | |
| No. of Residues | 5 | 11 | 40 | 19 | 32 | 5 |
| Yield (%) | 28 | 17 | 24 | 55 | 57 | 36 |
| Cpm[f] | 13080 | 26350 | 44000 | | 14790 | |

[a] Residues/molecule by amino acid analysis or (in parentheses) from the sequence (Figure 1).

[b] MTc1-T1 and MTc1-T2 were obtained from the further purification of fraction II-3 (Figure 3C) by paper electrophoresis.

[c] MTc3T1 is not a radioactive peptide, but it was useful to confirm the sequence from residues 195 to 213.

[d] MTc3-T3 is not a radioactive peptide, however, it was used to confirm the sequence of residues 250-254.

[e] HVE, high voltage paper electrophoresis at pH 6.5, 2.5 KV for 1 hr; DEAE, purified on DEAE-Sephacel (0.9 x 60 cm) with a linear gradient (150 ml) from 0.025M to 1.0 M $NH_4HCO_3$ (pH 8.0).

[f] Specific radioactivity (cpm/μmol of peptide)

protein, containing 13 600 cpm/μmol of methionine was citraconylated in 6 M guanidine hydrochloride, pH 8.8, and then

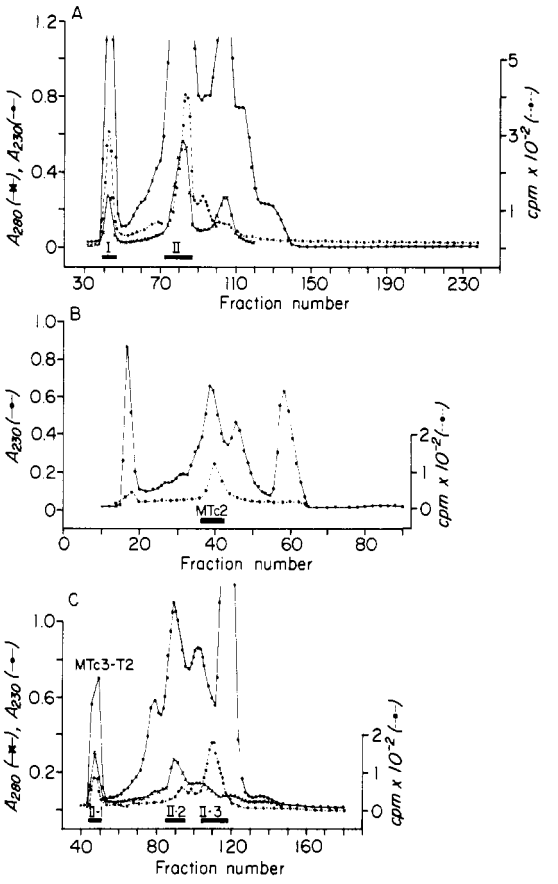

FIGURE 3: Separation of [$^{14}$C]methionine-containing tryptic peptides (MTc peptides) obtained from type II catalytic subunit (42 mg, 1.03 μmol) of cAMP-dependent protein kinase. Columns were monitored at 230 (—) and 280 nm (×) and for radioactivity in 4% of the aliquot (--). (A) Fragments generated by tryptic digestion of ε-*N*-citraconyl, *S*-carboxymethyl protein that had been *S*-[$^{14}$C]methyl-labeled at methionine. The mixture was separated on a column (2.5 × 113 cm) of Sephadex G-75 superfine with 0.1 M ammonium bicarbonate (pH 8.6). Fractions of 4 mL were collected at 20 mL/h, and the principal radioactive fractions were pooled as indicated by horizontal bars. (B) Fraction I was further separated under the same conditions but on a smaller column (1.5 × 85 cm) in the presence of 6 M guanidine hydrochloride. Aliquots of 2 mL were collected. (C) Fraction II was acidified to remove citraconyl groups, digested with trypsin, and fractionated on a column (1.5 × 200 cm) of Sephadex G-50 superfine at 10 mL/h with 0.1 M ammonium bicarbonate (pH 8.0). Fraction II-1 was a pure peptide; fraction II-2 was not radioactive but contained a peptide (MTc3-T1) that was useful in confirming a sequence within CB7; and fraction II-3 contained three useful peptides (Table II).

dialyzed against 0.1 M $NH_4HCO_3$ at the same pH for 2 days. A subsequent tryptic digest (37 °C for 1 h) was resolved on Sephadex G-75 into two principal radioactive fractions (Figure 3A) with 31 897 cpm in fraction I and 76 805 cpm in fraction II. Rechromatography of fraction I, after decitraconylation, in the presence of 6 M guanidine hydrochloride (Figure 3B) yielded pure peptide MTc2 with its $NH_2$ terminus at residue 94 (Figure 1) and containing 44 000 cpm, indicating three methionyl residues in the peptide.

Fraction II was decitraconylated, redigested with trypsin, and resolved into two radioactive fractions (Figure 2C). The first contained pure peptide MTc3-T2 with 14 790 cpm; the second was resolved by paper electrophoresis into two radioactive peptides, MTc1-T1 and MTc1-T2, containing 13 080 and 26 350 cpm, respectively. Two unlabeled peptides, MTc3-T1 and MTc3-T3, isolated as byproducts of the above fractionation scheme (Table II), provided compositional evidence of lysine-213 and sequence data for serine-252, respectively.

*Alignment of CB Peptides with MTc Peptides.* The sequence of MTc3-T2 provided an overlap linking CB7 directly to the carboxyl-terminal peptide CB8. Since CB1 with its blocked $NH_2$ terminus must be the $NH_2$-terminal fragment, this leaves four small peptides, CB2, CB3, CB5, and CB6, to be placed around the larger fragment CB4.

Peptides MTc1-T1 and MTc1-T2 were sequenced by the method of Laursen (1971) after attachment through peptide amino groups. The nature of the method precluded identification of their $NH_2$-terminal residues and their carboxyl-terminal lysyl residues. However, the interior sequences of MTc1-T1 and MTc1-T2 clearly corresponded to CB2 and CB3, respectively. Since MTc1-T2 must have a carboxyl-terminal lysine, the composition (Table II) places histidine at its $NH_2$ terminus in the sequence His-Met-Glu-Thr, which links CB2 to CB3 (Figure 1). The carboxyl-terminal Ala-Met-Lys links CB3 to CB4, although by only a single residue overlap. Similarly, the composition of MTc1-T1 dictates an $NH_2$-terminal valine and the sequence Val-Met-Leu-Val-Lys. This is consistent with an overlap of CB1 and CB2. Although CB5 also consists of a Val-Met sequence, it must be preceded by methionine; furthermore, trypsin could not have generated MTc1-T1 from a CB5-CB2 alignment.

The largest tryptic peptide, MTc2, contained three residues of methionine that were not reached by sequenator analysis. Treatment of MTc2 with Myxobacter AL-1 protease II (Wingard et al., 1972) at 50 °C for 1 h at pH 8.0 generated a mixture of three peptides, which were examined together in the spinning cup. By a subtraction of the expected sequences of R5 and of residues 105–110, the sequence labeled MTc2-Mx in Figure 1 (residues 111–130) was deduced, including all three methionyl residues. This clearly linked CB4-CB5-CB6 in that order, excluded CB5 as an overlap candidate with CB2, and provided a marginal overlap into CB7. Thus, the seven cyanogen bromide fragments are aligned in the single 350-residue chain (Figure 1).

## Discussion

The catalytic subunit of bovine cardiac muscle, type II, cAMP-dependent protein kinase is composed of 350 amino acid residues in a single chain including two phosphorylation sites (Shoji et al., 1979) and an $NH_2$-terminal myristyl group (Carr et al., 1982), which together comprise a molecular weight of 40 862. Our strategy of sequence analysis was based upon only two primary cleavage procedures, with cyanogen bromide at methionine and with trypsin at arginine, followed, when needed, by secondary subdigestion of isolated fragments by appropriate proteases.

Six of the eight cyanogen bromide cleavage products were purified easily by traditional techniques, but the need for separation of the mixture of CB1 and CB4 was not at first recognized because CB1 was blocked at its $NH_2$ terminus and the mixture behaved like a single fragment during both Edman degradation and $NaDodSO_4$–polyacrylamide gel electrophoresis. Methionyl-containing tryptic peptides were selected during purification on the basis of [$^{14}$C]methyl label introduced by an exchange reaction before digestion.

Three large fragments (CB4, CB7, and CB8) required subdigestion to complement and complete analyses of their $NH_2$-terminal sequences. The $NH_2$-terminal fragment (CB1) was $\alpha$-N-myristylated (Carr et al., 1982), requiring subdigestion products throughout its analysis. Although compositional data and carboxypeptidase digestion had established the $NH_2$-terminal sequence X-Gly-Asn-Ala (Carr et al., 1982), our earlier report of the sequence misinterpreted the composition of the $NH_2$-terminal peptide X-Gly-Asn-Ala-Ala-Ala-Lys (CB1-T1, Figure 1) to include only three instead of four alanyl residues. Mass spectrometric analysis of CB1-T1 clearly established the existence of the fourth alanyl residue (Carr et al., 1982), as well as identifying the myristyl group.

In appraising the reliability of a sequence analysis, it is important to draw attention to the weakest aspects of the proof. In this case, the alignments of CB1/CB2 and of CB4/CB5/CB6/CB7 are not ideal. The first alignment is based on the composition of MTc-T1, which, together with solid-phase Edman degradation data, indicates an $NH_2$-terminal Val-Met that overlaps CB1 (Figure 1). The second alignment is based on analysis of a mixture of peptides, one of which is deduced to be that of MTc2-Mx with a marginal overlap from CB6 into CB7. A third weak point is the lack of a phenylthiohydantoin derivative from lysine-213, because MTc3-T1 was analyzed by the solid-phase technique. Other weaknesses in the proof are the single residue overlaps at glutamate-17, aspartate-166, asparagine-271, phenylalanine-281, lysine-309, and valine-337. Finally, confirmation of the carboxyl terminus was not possible by digestion of the whole subunit (or of CB8) with carboxypeptidase Y. A time course of amino acid release from R18-T4 (Glu-Phe-Ser-Glu-Phe) was difficult to evaluate because of the repeating Glu-Phe sequence. Nonetheless, the proposed carboxyl terminus is the only one possible because of the arginine-cleavage products only R18 lacked a carboxyl-terminal arginine, of the tryptic peptides only R18-T4 lacked lysine or arginine, and of the cyanogen bromide cleavage products only CB8 lacked homoserine. An identical octapeptide sequence was reported for the carboxyl terminus of the porcine catalytic subunit (Taylor et al., 1981).

A minor but novel aspect of our strategy was the technique to overcome the resistance of arginine-336 to tryptic cleavage in the sequence: Asp-Asp-Tyr-Glu-Glu-Glu-Glu-Ile-Arg-Val. It was assumed that the refractory nature of this bond was related to the neighboring acidic residues, as in the Asp-Asp-Asp-Asp-Lys-Ile sequence of trypsinogen where calcium ions are needed to overcome the negative character of the aspartyl residues and permit tryptic cleavage of the Lys–Ile bond (Abita et al., 1969; Radhakrishnan et al., 1969). Indeed, 10 mM calcium acetate overcame the resistance of R18 to tryptic attack, facilitating the completion of the sequence of R18.

The amino acid composition of the 350-residue sequence of the catalytic subunit (Figure 4) is compared in Table I with that determined in hydrolysates. Agreement is generally within experimental error except for an anomalously high estimate of tryptophan by Demaille et al. (1977), who also reported a high value (three) for cysteine. Our quantification of cysteine is based both upon estimates of *S*-(carboxymethyl)cysteine in hydrolysates and upon the quantitative recovery of carboxy-[$^{14}$C]methyl groups from protein labeled with iodo[$^{14}$C]acetate (Shoji et al., 1981). Nelson & Taylor (1981) also isolated only two cysteine-containing peptides from the *porcine* enzyme, one of which was identical with our residues 343–350 and the other clearly homologous to residues 195–213. However, their observation of a non-cysteinyl component labeled by iodo-[$^{14}$C]acetate was not duplicated in our experiments with the bovine enzyme. We have summarized previously the variability among published estimates of cysteine content (Shoji et al., 1981).

MgATP protects the porcine enzyme from affinity labeling by an analogue of ATP (Zoller & Taylor, 1979). Specifically, *p*-(fluorosulfonyl)benzoyladenosine sulfonylates a single lysyl residue that is clearly the porcine counterpart of lysine-72 in the bovine enzyme (Zoller et al., 1981). Of the 27 residues

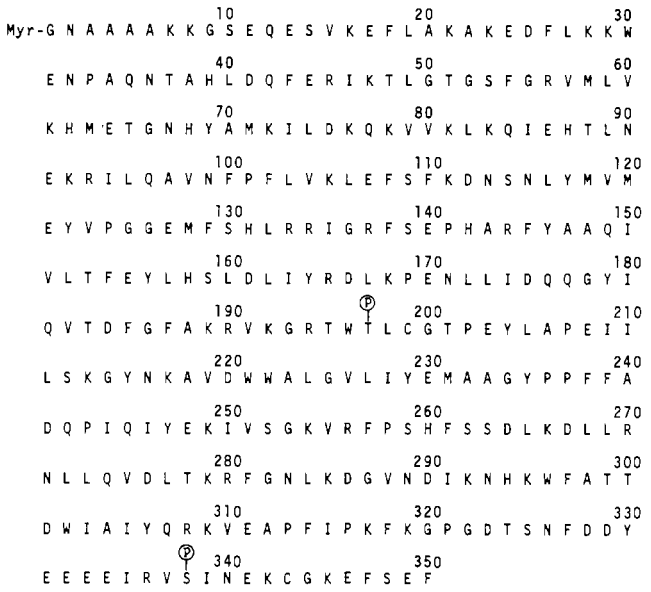


FIGURE 4: Amino acid sequence of catalytic subunit of type II cAMP-dependent protein kinase from bovine cardiac muscle. The one letter code abbreviations are shown in Table I. Myr signifies the $\alpha$-*N*-myristyl group identified by Carr et al. (1982). Each circled P signifies a phosphoryl group placed by Shoji et al. (1979). Lysyl residue 72 has been identified as a component of the ATP-binding site (see text).

surrounding the labeled lysyl residue in the porcine enzyme, 25 are identical in the bovine enzyme (residues 59–85), leaving little doubt that lysine-72 is located at the ATP-binding site of the bovine catalytic subunit. More recently, it has been shown that the same affinity label reacts with lysine in an identical Ala-Met-Lys-Ile-Leu sequence in cGMP-dependent protein kinase from bovine lung (Hashimoto et al., 1982) and that an extended sequence surrounding that pentapeptide is homologous with residues 56–92 of the catalytic subunit of the cAMP-dependent enzyme.

These data are part of a growing body of evidence that the sequence of the catalytic subunit of bovine cAMP-dependent protein kinase can be considered as a prototype of a family of homologous enzymes including not only the cGMP-dependent enzyme (Takio et al., 1982b) but also tyrosine-specific protein kinases of viral origin (Barker & Dayhoff, 1982). Currently, studies are underway to see whether calcium-dependent protein kinases belong to the same family. In this connection, it is interesting that a tyrosine-specific protein kinase also has an amino-terminal fatty acid (Henderson et al., 1983). At present, little can be said of a functional role of the myristyl group or, for that matter, for the two phosphoryl groups. It seems likely that each serves a yet unidentified control function in this enzyme.

## Acknowledgments

We are grateful for the sustaining interest and advice of Dr. Hans Neurath and for the help of Santosh Kumar with certain peptide isolations, of Roger D. Wade with solid-phase sequence analyses, and of Richard R. Granberg with amino acid analyses.

## Supplementary Material Available

Three tables (I-S–III-S) and five figures (1-S–5-S) giving descriptions of the purification and amino acid compositions of subpeptides derived from CB1, CB4, CB7, and CB8 (11 pages). Ordering information is given on any current masthead page.

**Registry No.** Protein kinase, 9026-43-1; protein kinase (bovine heart cAMP-dependent catalytic subunit protein moiety reduced), 84012-73-7.

## References

Abita, J. P., Delaage, M., Lazdunski, M., & Savrda, J. (1969) *Eur. J. Biochem. 8*, 314.

Atassi, M. Z., & Habeeb, A. F. S. A. (1972) *Methods Enzymol. 25*, 546.

Barker, W. C., & Dayhoff, M. O. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2836.

Brauer, A. W., Margolies, M. N., & Haber, E. (1975) *Biochemistry 14*, 3029.

Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C., & Titani, K. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6128.

Christie, D. L., Hancock, W. S., & Barling, P. M. (1979) *Endocrinology (Philadelphia) 105*, 1020.

Cohen, P. (1978) *Curr. Top. Cell. Regul. 14*, 118.

Cohen, P. (1982) *Nature (London) 296*, 613.

Demaille, J. G., Peters, K. A., & Fischer, E. H. (1977) *Biochemistry 16*, 3080.

Edman, P., & Begg, G. (1967) *Eur. J. Biochem. 1*, 80.

Ericsson, L. H., Wade, R. D., Gagnon, J., McDonald, R. M., Granberg, R. R., & Walsh, K. A. (1977) in *Solid Phase Methods in Protein Sequence Analysis* (Previero, A., & Colletti-Previero, M. A., Eds.) p 137, Elsevier/North-Holland, Amsterdam.

Hashimoto, E., Takio, K., & Krebs, E. G. (1982) *J. Biol. Chem. 257*, 727.

Henderson, L. E., Krutzsch, H. C., & Oroszlan, S. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 339.

Hermann, J., Titani, K., Ericsson, L. H., Wade, R. D., Neurath, H., & Walsh, K. A. (1978) *Biochemistry 17*, 5672.

Hermodson, M. A., Ericsson, L. H., Titani, K., Neurath, H., & Walsh, K. A. (1972) *Biochemistry 11*, 4493.

Hofmann, F., Beavo, J. A., Bechtel, P. J., & Krebs, E. G. (1975) *J. Biol. Chem. 250*, 7795.

Horn, M. J., & Laursen, R. A. (1973) *FEBS Lett. 36*, 285.

Jimenez, J. S., Kupfer, A., Gani, V., & Shaltiel, S. (1982) *Biochemistry 21*, 1623.

Koide, A., Titani, K., Ericsson, L. H., Kumar, S., Neurath, H., & Walsh, K. A. (1978) *Biochemistry 17*, 5657.

Krebs, E. G. (1972) *Curr. Top. Cell. Regul. 5*, 99.

Krebs, E. G., & Beavo, J. A. (1979) *Annu. Rev. Biochem. 48*, 923.

Kupfer, A., Jimenez, J. S., Gottlieb, P., & Shaltiel, S. (1982) *Biochemistry 21*, 1631.

Laursen, R. A. (1971) *Eur. J. Biochem. 20*, 89.

Laursen, R. A., Horn, M. J., & Bonner, A. G. (1972) *FEBS Lett. 21*, 67.

Link, T. P., & Stark, G. R. (1967) *J. Biol. Chem. 243*, 1082.

Nelson, N. C., & Taylor, S. S. (1981) *J. Biol. Chem. 256*, 3743.

Omenn, G. S., Fontana, A., & Anfinsen, C. B. (1970) *J. Biol. Chem. 245*, 1895.

Peters, K. A., Demaille, J. G., & Fischer, E. H. (1977) *Biochemistry 16*, 5691.

Radhakrishnan, T. M., Walsh, K. A., & Neurath, H. (1969) *Biochemistry 8*, 4020.

Reimann, E. M., Walsh, D. A., & Krebs, E. G. (1971) *J. Biol. Chem. 246*, 1986.

Rosen, O. M., Rangel-Aldao, R., & Erlichmann, J. (1977) *Curr. Top. Cell. Regul. 12*, 39.

Rubin, C. S., & Rosen, O. M. (1975) *Annu. Rev. Biochem. 44*, 831.

Rubin, C. S., Erlichmann, J., & Rosen, O. M. (1972) *J. Biol. Chem. 247*, 36.
Shoji, S., Titani, K., Demaille, J. G., & Fischer, E. H. (1979) *J. Biol. Chem. 254*, 6211.
Shoji, S., Parmelee, D. C., Wade, R. D., Kumar, S., Ericsson, L. H., Walsh, K. A., Neurath, H., Long, G. L., Demaille, J. G., Fischer, E. H., & Titani, K. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 848.
Takio, K., Smith, S. B., Krebs, E. G., Walsh, K. A., & Titani, K. (1982a) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2544.
Takio, K., Smith, S. B., Walsh, K. A., Wade, R. D., Kumar, S., Krebs, E. G., & Titani, K. (1982b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41*, 662.
Tarr, G. E., Beecher, J. F., Bell, M., & McKean, D. J. (1978) *Anal. Biochem. 84*, 622.
Taylor, S. S., Kerlavage, A. R., Zoller, M. J., Nelson, N. C., & Potter, R. L. (1981) *Cold Spring Harbor Conf. Cell Proliferation 8*, 3.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.
Wingard, M., Matsueda, G., & Wolfe, R. S. (1972) *J. Bacteriol. 112*, 940.
Zoller, M. J., & Taylor, S. S. (1979) *J. Biol. Chem. 254*, 8363.
Zoller, M. J., Nelson, N. C., & Taylor, S. S. (1981) *J. Biol. Chem. 256*, 10837.

# Structure of Alamethicin in Solution: Nuclear Magnetic Resonance Relaxation Studies†

Utpal Banerjee and Sunney I. Chan*

ABSTRACT: An NMR relaxation study at 500 MHz of the icosapeptide antibiotic alamethicin is reported. This study lends further support to the partly helical, partly extended, amphiphilic, and dimeric structure recently proposed for this peptide in methanolic solutions [Banerjee, U., Tsui, F. P., Balasubramanian, T. N., Marshall, G. R., & Chan, S. I. (1983) *J. Mol. Biol. 165*, 757]. The *N*-acetyl methyl groups toward the N terminus of alamethicin in this solvent system were found to exhibit unusual NMR relaxation behavior. The decay of the transverse magnetization due to these protons was nonexponential, but the spin–lattice relaxation recovery of the longitudinal magnetization was exponential. In a solution saturated with urea, however, both decays were exponential. These observations are shown to be consistent with the proposed structure. Studies in water yielded qualitatively similar but more complex results. The transverse relaxation times suggest further aggregation in water and indicate that the larger aggregates in water may be made up of the smaller units observed in methanol.

In our previous paper of this series (Banerjee et al., 1983), we reported the 500-MHz NMR spectra of the icosapeptide alamethicin (Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol) in two solvent systems, viz., methanol and water. Two-dimensional NMR was used in combination with double-resonance experiments to obtain a complete assignment of the resonances to the protons in the molecule. Our work confirmed and extended the earlier NMR results of Martin & Williams (1976) and Davis & Gisin (1981). From the peptide amide coupling constants and two-dimensional nuclear Overhauser effect (NOE) results, we deduced the conformation of the molecule in methanol that was consistent with these data as well as a line of other experimental observations.

The proposed dimeric structure in methanol is partly extended and partly helical. In accordance with the values of the NMR coupling constants and two-dimensional NOE results, in our model (a) the amide protons of residues 15–20 are intermolecularly hydrogen bonded with the corresponding residues of the opposing molecule to create a rigid, extended parallel $\beta$-pleated structure for the C-terminal end of the molecule, (b) the proline at position 14 breaks the continuity of this structure, and amino acids 10–14 are forced into an open, non-hydrogen-bonded conformation, and (c) amino acids 3–9 are folded into an $\alpha$ helix, with the Gln-7 side chains from the two strands in the right juxtaposition to facilitate a hydrogen bond between them. The resultant structure is highly amphipathic: one face is completely hydrophobic with the aliphatic side chains exposed, whereas the other face is primarily hydrophilic with polar side chains and peptide groups lining the extended $\beta$-sheet region.

In the above structure, the two helices at the N-terminal end of the proposed dimer are held rigidly together and are energetically stabilized by a side-chain amide hydrogen bond between the two Gln-7's. Extensive hydrophobic interaction between the side groups in the helical region no doubt contributes to the stability of the structure as well. Since one helical strand must be necessarily twisted slightly with respect to the other in this structure, an interesting consequence is that the two acetyl methyl groups at the N termini of the dimer are inequivalent in terms of the angle subtended by them with respect to the long axis of the dimer. Since this is the principal rotor axis, it follows on the basis of the Woessner et al. (1969) treatment of the relaxation of symmetric top methyl groups attached to the side chain of an anisotropically tumbling ellipsoid that the two sets of *N*-acetyl methyl protons should exhibit different spin–spin relaxation times ($T_2$'s). The C termini of the dimer are also inequivalent, but since the individual units are related to one another by a 2-fold screw axis, the side groups in this part of the molecule are symmetrically displaced with respect to the principal rotor axis of the aggregate. Protons from the two aromatic rings on the individual strands, for example, subtend the same angle to the rotor axis and should therefore exhibit equal spin–spin relaxation times.

† From the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125. *Received February 11, 1983.* Contribution No. 6787. Supported by Grant GM-22432 from the National Institute of General Medical Sciences, U.S. Public Health Service.

0006-2960/83/0422-3709$01.50/0 