Gene, 169 (1996) 133–134

GENE 09506

Identification of calmodulin-binding peptide consensus sequences from a phage-displayed random peptide library *

(Combinatorial peptides; bacteriophage M13; affinity selection; calmodulin; sequence motif)

Nils B. Adey** and Brian K. Kay

Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA

Received by M.J. Benedik: 30 June 1995; Revised/Accepted: 18 October 1995; Received at publishers: 6 November 1995

SUMMARY

The calcium-binding protein, calmodulin (CaM), was used to screen a phage library displaying random peptides 26 amino acids (aa) in length. Twenty CaM-binding peptides were identified, 17 of which contained one of three consensus sequence motifs: +W−OλR, WRAAV or WRXXAAAL, where +, −, O, λ and X are positively charged, negatively charged, hydrophobic, leucine or valine, and any residue, respectively. The Trp residue in these motifs is located within 14 aa of the N-terminus of the displayed peptide. Previous studies [Dedman et al., J. Biol. Chem. 268 (1993) 23025–23030] using a library displaying random peptides 15 aa in length identified CaM-binding peptides which contained a Trp-Pro dipeptide motif. These results suggest that the type of CaM-binding motif identified can vary between different types of combinatorial peptides.

Calmodulin (CaM) is a ubiquitous, eukaryotic Ca^{2+}-binding protein that interacts with a variety of other proteins to regulate their activity (Means et al., 1991). To ascertain its protein binding specificity, previous studies (Dedman et al., 1993) screened CaM with a phage library displaying random 15-aa peptides (Devlin et al., 1990). These studies identified a number of Ca^{2+}-dependent CaM-binding peptides, all of which contained a Trp usually followed by a Pro residue. However, this WP dipeptide motif is not found in known CaM-binding regions from natural proteins (Dedman et al., 1993). To determine if isolation of this motif is a result of the type of random peptide library used, we decided to screen another combinatorial peptide library.

The library was constructed by annealing and extending two long degenerate oligos with a complementary region at their 3′ termini (Kay et al., 1993). The 6-nt complementarity corresponded to the *Sac*II recognition sequence and encoded the tripeptide:

(A/S/P/T)A(A/D/E/G/V)

This design, which fixed Ala as the central aa, permits the subdividing of the long peptides for the analysis of binding residues by *Sac*II digestion of the DNA insert. The library consisted of 2×10^8 recombinants, each expressing the peptide sequence:

SRX_{12}(A/S/P/T)A(A/D/E/G/V)$X_{12}SR$

at the mature N-terminus of pIII. Even though the central aa of the peptides displayed by the R26 library were not entirely random, we felt that the library would still contain potential CaM-binding peptides since these central aa often occur in the CaM-binding domains of natural proteins (O'Neil and DeGrado, 1990).

Correspondence to: Dr. B.K. Kay, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA. Tel. (1-919) 962-2273; Fax (1-919) 962-1625; e-mail: kay.fordham@mhs.unc.edu

* On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note.

** Present address: Myriad Genetics, Inc., 390 Wakara Way, Salt Lake City, UT 84108 USA. Tel. (1-801) 582-3400.

Abbreviations: aa, amino acid(s); BSA, bovine serum albumin; CaM, calmodulin; ELISA, enzyme-linked immunosorbent assay; oligo, oligodeoxyribonucleotide; X (x), any aa.

SSDI 0378-1119(95)00804-7

Through affinity selection, twenty different CaM-binding phage were isolated from this library, and all were Ca^{2+}-dependent in their binding. As seen in Fig. 1, 17 of the 20 CaM-binding peptides contained one of three sequence motifs: +W−OλR, WRAAV or WRXXAAAL, where +, −, O and λ are positively charged, negatively charged, hydrophobic, and Leu/Val, respectively. The Trp residue in these motifs was located within 14 aa of the N-terminus of mature pIII. Furthermore, λ residues were often present in the +W−OλR containing peptides (Fig. 1), three and eight residues C-terminal to the Trp residue.

```
Øb-2              S SWDVLR EAFTSRHPADLVHQADSQLSRASR
Øa-1              S SWDTVR ERLLKSYTADHSKTPPNRTAISSR
Øc-3             SS RWEIVR TGLLTRPAGITNASPPTITESR
Øc-4***          SS KWDLLR GVFWEGDRGDALSGTDTHGRTSR
Øa-2             SS GWERVR SWAASSSAARNTSVSVTPSDQSR
Øc-5            SSS HWDVLR GAVTLPAADSNAGRSWRTSTSR
Øb-1           SSRD HWSMLR GCFSSAGCSYWPDSRSHINSR
Øc-2          SSYAL RWDALR DCIAAGCHRTDHYVRSVDSR
Øb-5        SRCEADL HWALDR WSAAVKAGGTMPGSACSR
Øb-4       SSGDARGS HWGFLR SAVNSSQLINTRSLTSR
Ød-5       SSTSNRTP GWERLR AAVNNGMKSLNDLGPSR
Ød-4     SSATGGSTAS RWAAAR LRSFSPPSVIQSR
                    +W-OλR

Øb-3    SSTSSVGRAFDV WRAAV LYSTHAVPPEQSSR
Øc-1    SSLGLNSGDRTG WRAAV DQLLRLNKNKFDSR
Ød-3  SSAVTDPATRSTK WAAAV ADIIRSKNMQKSR
                    WRAAV

Ød-1      SSSELSSSSR WRGLAAAL MGYDTSSQQGPSR
Øb-6      SSERDSIGRM WRQSAAAL RSSFAHSQTNDSR
                    WRxxAAAL

Øb-8    SSADHALGEARRANTADKSSWPSVKRVLHSR
Ød-2    SSTPNSELGVYKQYSAANIFRSWASRAASR
Øc-7    SRGNGERELWWKAFSAVTEGKIKKAPGHTSR
```

Fig. 1. Sequences displayed at the N-terminus of pIII by CaM-binding phage isolated from the R26 library. Three consensus motifs are explained in text. Trp residues (**W**) are indicated in bold, WP motifs are indicated by double underline. Representative phage from each motif class (Øb-1, b-2, c-1 and d-1) were verified to bind biotinylated CaM in solution as the phage-CaM complexes could be captured with immobilized streptavidin bridge. Binding of all phage was prevented by chelating Ca with 10 mM EGTA (not shown). Differences in binding affinity between phage could not be detected by ELISA. The libraries were constructed as described previously (Kay et al., 1993). By virtue of oligo design, the R26 library displays the following sequences at the N-terminus of pIII: NH_2-SRX_{12}(A/S/?/T)A(A/D/E/G/V)X_{12}SR (NNK codons). Fixed and semi-fixed aa (due to oligo design) are underlined. Asterisks (***) mark a clone that appears to have been derived from a frameshifted oligo. **Methods:** CaM (0.2 μg/well) was immobilized to 96-well polystyrene microtiter plates (Corning 8501, Corning, NY, USA; or Costar 3799, Cambridge, MA, USA) by non-specific absorption in 0.1 M $NaHCO_3$ (pH 8.5). Biotinylated CaM (0.2 μg/well) was immobilized using streptavidin (0.5 μg/well) absorbed as above. For libraries screening (Adey et al., 1995), 10^{11} phage particles (derived from 2×10^8 different clones) were incubated with immobilized CaM, non-binding phage washed away, binding phage recovered by acid elution and amplified in *E. coli*. After three rounds of screening, individual clones were amplified and the binding tested to CaM or BSA-coated wells by ELISA (Pharmacia, Piscataway, NJ, USA). The identity of the peptides displayed by CaM-binding clones were determined by sequencing both strands of the appropriate region of the viral genome.

Comparison of the CaM-binding peptides shown in Fig. 1 and those (15 aa) identified by Dedman et al. (1993) has revealed that both sets contain a Trp, which has been shown to insert into a hydrophobic pocket of CaM (Afshar et al., 1994). However, the aa which flank the Trp residue vary (i.e., +W−OλR, WRAAV or WRXXAAAL in Fig. 1 and WP in Dedman et al., 1993). Another distinction is that the Trp was located at or near the N-terminus of the phage-displayed random 15-aa, whereas its position varied more within the N-terminal half of the random 26-aa peptides. Thus different libraries may yield different binding sequences for a given target and that the choice of library may influence the results quite dramatically.

Presently identified CaM-binding motifs were used in a Swiss-Protein database search (Pearson and Lipman, 1988). A number of matches were obtained, but they were to non-relevant proteins. Thus, the presence of the +W−OλR, WRAAV and WRXXAAAL motifs in CaM-binding peptides appears to be biologically novel.

ACKNOWLEDGMENTS

The authors wish to thank Heather Hanson for technical advice. This work was supported by a grant from Cytogen Corp., Princeton, NJ.

REFERENCES

Adey, N.B., Mataragnon, A.H., Rider, J.E., Carter, J.M. and Kay, B.K.: Characterization of phage that bind plastic from phage-displayed random peptide libraries. Gene 156 (1995) 27–31.

Afshar, M., Caves, L., Guimard, L., Hubbard, R., Calass, B., Grassy, G. and Haiech, J.: Investigating the high affinity and low sequence specificity of calmodulin binding to its' targets. J. Mol. Biol. 244 (1994) 554–571.

Dedman, J.R., Kaetzel, M.A., Chan, H.C., Nelson, D.J. and Jamieson, J., G A: Selection of target biological modifiers from a bacteriophage library of random peptides: the identification of novel calmodulin regulatory peptides. J. Biol. Chem. 268 (1993) 23025–23030.

Devlin, J.J., Panganiban, L.C. and Devlin, P.E.: Random peptide libraries: a source of specific protein binding molecules. Science 249 (1990) 404–406.

Kay, B.K., Adey, N.B., He, Y.-S., Manfredi, J.P., Mataragnon, A.H. and Fowlkes, D.M.: An M13 library displaying 38-amino-acid peptides as a source of novel sequences with affinity to selected targets. Gene 128 (1993) 59–65.

Means, A., Van Berkum, M., Bagchi, I., Lu, K. and Rasmussen, C.: Regulatory functions of calmodulin. Pharmacol. Ther. 50 (1991) 255–270.

O'Neil, K.T. and DeGrado, W.F.: How calmodulin binds its targets: sequence independent recognition of amphiphilic α-helices. Trends Biochem. Sci. 15 (1990) 59–64.

Pearson, W. and Lipman, D.: Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA 85 (1988) 9481–9485.