

Product Information

Calmodulin from bovine testes

Catalog Number **P1431**

Storage Temperature –20 °C

CAS RN 77107-46-1

Synonyms: calcium dependent regulator protein (CDR), phosphodiesterase 3':5' cyclic nucleotide activator, CaM

Product Description

The protein calmodulin (CaM) is said to have roles in intracellular Ca²⁺ homeostasis, cell proliferation, smooth muscle contraction, microtubular function, exocytotic secretion of cellular products, and cell motility. Several review articles on CaM have been published.^{1,2}

CaM contains many acidic amino acids and lacks cysteine, hydroxyproline, and tryptophan. The abundance of acidic carboxyl groups allows for reversible binding of Ca²⁺. The absence of cysteine and hydroxyproline allows for a very flexible tertiary structure for interaction with various calmodulin-regulated proteins. CaM also has a high ratio of phenylalanine (8 residues) to tyrosine (2 residues), and has a distinctive UV spectrum with five peaks at 252 nm, 259 nm, 265 nm, 269 nm, and 277 nm, with a shoulder at 282 nm.¹

X-ray crystallography of CaM, in the presence of Ca²⁺, indicates a long dumbbell-shaped structure 65 Å long. Each globular end contains two Ca²⁺ binding domains. These domains are common among many Ca²⁺ binding proteins and are described as helix-loop-helix “EF-Hand” regions. The Ca²⁺ binding regions are connected by an extended 40 Å, 28-amino acid α-helical region.³⁻⁵ Upon Ca²⁺ binding, CaM undergoes a conformational change in which the hydrophobic regions become exposed. These hydrophobic regions are said to be involved with enzyme binding.^{6,7} Phosphorylation of CaM *in vivo* has been reported when cells are stimulated with insulin⁸⁻¹⁰ and *in vitro* by various protein kinases.¹¹⁻¹⁶

The four Ca²⁺ binding sites of CaM are designated I, II, III, and IV, starting from the site closest to the N-terminus. The order of Ca²⁺ binding to CaM is believed to be III, IV, I, and II. Sites III and IV have affinity for Ca²⁺ 10–20 times higher than sites I and II.^{17,19}

CaM has been found to activate such enzymes as ATPase,²⁰⁻²⁵ calmodulin kinases I, II, and III,²⁶⁻³⁰ phosphorylase kinase,³¹⁻³⁴ cyclic nucleotide phosphodiesterase,³⁵⁻³⁸ adenylate cyclase,³⁹ NADPH oxidase,⁴⁰ and myosin light chain kinase.⁴¹

Molecular Mass:

- 16.79 kDa (amino acid sequence)⁴²
- 18.7 kDa (sedimentation equilibrium)⁴³
- 19 kDa (SDS in presence of EGTA)

Note: The migration rate in SDS is faster when Ca²⁺ is present and slower when EGTA removes the Ca²⁺.⁴⁴

Stokes Radius: 20.9 Å (calculated)⁴³

E₂₇₆^{1%} = 1.8 (0.1 M Imidazole-HCl, pH 7.0 with 1 mM EDTA)²¹

Note: Addition of 0.5 mM CaCl₂ results in an 8% decrease in absorption.⁴⁵

Isoelectric point (pl):^{46,47} 3.9–4.3

Purity: ≥98% (SDS-PAGE)

This product is purified from bovine testes by a modification of a published procedure.⁴⁸ It is supplied as an essentially salt-free lyophilized powder. The calcium content of a representative lot was found to be <0.05%.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at –20 °C. Stored properly, as supplied in the powder form, calmodulin should remain active for a minimum of two to three years.

Preparation Instructions

Aqueous solutions at ≥1 mg/mL in such solvents as saline or 0.1 M Tris-HCl, pH 7.5, remain active at 2–8 °C for 1–2 days, or frozen for ~1 week. If there are difficulties with solubility, small μL aliquots of concentrated base (e.g. 1 M NaOH) may be used to help to dissolve the material.

References

1. Cheung, W., *Science*, **207(4426)**, 19-27 (1980).
2. Hinrichsen, R., *Biochim. Biophys. Acta*, **1155(3)**, 277-293 (1993).
3. Babu, S. et al., *Nature*, **315(6014)**, 37-40 (1985).
4. Babu, S. et al., *J. Mol. Biol.*, **204(1)**, 191-204 (1988).
5. Kretsinger, R., in *Calcium Transport in Contraction and Secretion* (Carafoli, E. et al., eds.). North-Holland Pub. Co., pp. 469-478 (1975).
6. Newton, D. et al., *J. Biol. Chem.*, **259(7)**, 4419-4426 (1984).
7. Newton, D. et al., *Biochim. Biophys. Acta*, **845(3)**, 533-539 (1985).
8. Sacks, D. et al., *Biochem. J.*, **286(Pt 1)**, 211-216 (1992).
9. Fukami, Y. et al., *Proc. Natl. Acad. Sci. USA*, **83(12)**, 4190-4193 (1986).
10. Colca, J. et al., *J. Biol. Chem.*, **262(24)**, 11399-11402 (1987).
11. Graves, C. et al., *J. Biol. Chem.*, **261(22)**, 10429-10438 (1986).
12. Sacks, D., and McDonald, J., *J. Biol. Chem.*, **263(5)**, 2377-2383 (1988).
13. Sacks, D. et al., *Biochem. J.*, **262(Pt 1)**, 803-812 (1989).
14. Meggio, F. et al., *FEBS Lett.*, **215(2)**, 241-246 (1987).
15. Sacks, D. et al., *Biochem. J.*, **283 (Pt 1)**, 21-24 (1992).
16. Sacks, D. et al., *Biochem. Biophys. Res. Comm.*, **188(2)**, 754-759 (1992).
17. Olwin, B. et al., *J. Biol. Chem.*, **259(17)**, 10949-10955 (1984).
18. Haiech, J. et al., *J. Biol. Chem.*, **266(6)**, 3427-3431 (1991).
19. Starovasnik, M. et al., *Protein Science*, **1(2)**, 245-253 (1992).
20. Watterson, D.M. et al., *J. Biol. Chem.*, **251(15)**, 4501-4513 (1976).
21. Blum, J. et al., *J. Cell Biol.*, **87(2 Pt 1)**, 386-397 (1980).
22. Carafoli, E. et al., *Ann. N.Y. Acad. Sci.*, **402**, 304-328 (1982).
23. Caroni, P., and Carafoli, E., *J. Biol. Chem.*, **256(7)**, 3263-3270 (1981).
24. McConnell, E.J. et al., *Circ. Res.*, **86(2)**, 191-197 (2000).
25. Yingst, D.R. et al., *Arch. Biochem. Biophys.*, **295(1)**, 49-54 (1992).
26. Laird, A., and Greengard, P., *J. Biol. Chem.*, **262(15)**, 7273-7281 (1987).
27. Colbran, R., and Soderling, T., *Curr. Top. Cell. Regul.*, **31**, 181-221 (1990).
28. Laird, A., and Palfrey, H., *J. Biol. Chem.*, **262(36)**, 17299-17303 (1987).
29. Laird, A. et al., *Proc. Natl. Acad. Sci. USA*, **82(23)**, 7939-7943 (1985).
30. Krasel, C.J. et al., *J. Biol. Chem.*, **276(3)**, 1911-1915 (2001).
31. Newsholme, P. et al., *J. Biol. Chem.*, **267(2)**, 810-818 (1992).
32. Chan, K.-F.J., and Graves, D.J., *J. Biol. Chem.*, **257(10)**, 5948-5955 (1982).
33. Cohen, P., in *Molecular Aspects of Cellular Regulation*, Volume 5: Calmodulin (P. Cohen and C.B. Klee, eds.). Elsevier (Amsterdam), pp. 123-144 (1988).
34. Juminaga, S. et al., *J. Biol. Chem.*, **269(3)**, 1660-1667 (1994).
35. Beavo, J. et al., *Mol. Cell. Endocrinol.*, **28(3)**, 387-410 (1982).
36. Dedman, J. et al., *J. Biol. Chem.*, **252(23)**, 8415-8422 (1977).
37. Cheung, W., *Biochem. Biophys. Res. Commun.*, **38(3)**, 533-538 (1970).
38. Rossi, P. et al., *J. Biol. Chem.*, **263(30)**, 15521-15527 (1988).
39. Brostrom, C.O. et al., *Proc. Natl. Acad. Sci. USA*, **72(1)**, 64-68 (1975).
40. Jones, H. et al., *Biochim. Biophys. Acta*, **714(1)**, 152-156 (1982).
41. Stull, J.T., in *Molecular Aspects of Cellular Regulation*, Volume 5: Calmodulin (P. Cohen and C.B. Klee, eds.). Elsevier (Amsterdam), pp. 91-122 (1988).
42. Watterson, D.M., *J. Biol. Chem.*, **255(3)**, 962-975 (1980).
43. Klee, C.B. et al., *Ann. Rev. Biochem.*, **49**, 489-525 (1980).
44. Klee, C.B. et al., *Proc. Natl. Acad. Sci. USA*, **76(12)**, 6270-6273 (1979).
45. Klee, C.B., *Biochemistry*, **16(5)**, 1017 (1977).
46. Lin, Y.M. et al., *J. Biol. Chem.*, **249(15)**, 4943-4954 (1974).
47. Crouch, T.H. et al., *Biochemistry*, **19(16)**, 3692-3698 (1980).
48. Gopalakrishna, R., and Anderson, W., *Biochem. Biophys. Res. Commun.*, **104(2)**, 830-836 (1982).

RC,GCY,PHC,MAM 07/19-1