Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel

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The complete amino-acid sequence of the cyclic GMP-gated channel from bovine retinal rod photoreceptors, deduced by cloning and sequencing its complementary DNA, shows that the protein contains several putative transmembrane segments, followed by a region that is similar to the cyclic GMP-binding domains of cyclic GMP-dependent protein kinase. Expression of the complementary DNA produces cyclic GMP-gated channel activity in *Xenopus* oocytes.

VERTEBRATE photoreceptors respond to light by a transient hyperpolarization due to the closure of a cation channel in the plasma membrane. The channel is activated directly[1] by guanosine 3′,5′-cyclic monophosphate (cGMP), the internal messenger of visual transduction (for reviews, see refs 2–4). The channel does not discriminate very well between alkali cations[1,5–8] and is reversibly blocked by divalent cations[9–11] and *l-cis*-diltiazem[9]. The cGMP-gated channel from bovine retina consists of a single type of polypeptide of relative molecular mass (M_r) 63,000 (63K)[12]. The purified bovine cGMP-gated channel is functional when reconstituted into phospholipid vesicles[13] or artificial planar bilayers[14]. Channel activation occurs by the cooperative binding of at least three[10,11], but probably more[3,12,13], cGMP molecules, which suggests[12] that the functional channel is composed either of several 63K polypeptides each harbouring one or two cGMP-binding sites, or of only a single polypeptide containing three or more cGMP-binding sites. It has also been proposed that the cGMP-gated channel is composed of polypeptides of M_r 39K[15] or M_r 250K[16] or of rhodopsin[17] itself, which raises the possibility[18] that the channel is composed of different polypeptides, that the 63K polypeptide is a dimer of the 39K polypeptide or that the 39 K polypeptide results from proteolytic modification of the 63K polypeptide. Alternatively, these polypeptides may represent different cGMP-gated channels.

We have now cloned DNA that is complementary to bovine retinal messenger RNA coding for the 63K polypeptide and from the nucleotide sequence analysis of this cDNA we are able to predict the complete amino-acid sequence of the polypeptide. The cDNA has been functionally expressed in *Xenopus* oocytes, which suggests that this polypeptide alone is sufficient to form a functional cGMP-gated channel.

Cloning of cDNA

The initial approach to isolating cDNA for the cGMP-gated channel was to screen a cDNA library by hybridization with oligodeoxyribonucleotide probes synthesized on the basis of partial amino-acid sequence data. The cGMP-gated channel polypeptide of M_r 63K was solubilized from bovine rod outer segments and purified as described previously[12]. After SDS-PAGE of the purified preparation, the 63K polypeptide was electroeluted from the gel and digested with trypsin. The resulting peptides were fractionated by reverse-phase HPLC (Fig. 1). Fifteen fractions (I–XV) were collected and analysed for amino-

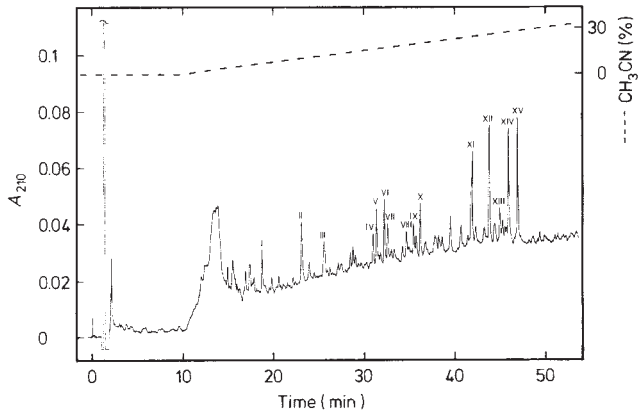

FIG. 1 Reverse-phase HPLC of tryptic peptides derived from the cGMP-gated channel. Solid line and left-hand axis, absorbance at 210 nm (A_{210}); broken line and right-hand axis, acetonitrile concentration. The amino-acid sequences determined for the fractions (I–XV) were identical to those of the following amino-acid residues deduced from the cDNA sequence (for amino-acid numbers, see Fig. 2): I, 639–641; II, 406–411; III, 591–596; IV, 454–460; V, 417–422; VI, 642–652; VII, 240–245; VIII, 513–518; IX, 668–677; X, 628–637; XI, 261–269; XII, 279–284; XIII, 435–443; XIV, 663–676; XV, 435–443. Identical amino-acid sequences were determined from fractions XIII and XV, and the sequence for fraction XIV overlapped that for fraction IX. METHODS. The 63K protein was purified as described previously[12]. After SDS-PAGE of the purified preparation, a piece of the gel carrying the 63K polypeptide was excised and the protein was electroeluted and dialysed[38]. The peptides resulting from tryptic digestion of the dialysed material (330 pmoles) were fractionated by reverse-phase HPLC and sequenced; the procedures used were essentially identical with those described previously[39].

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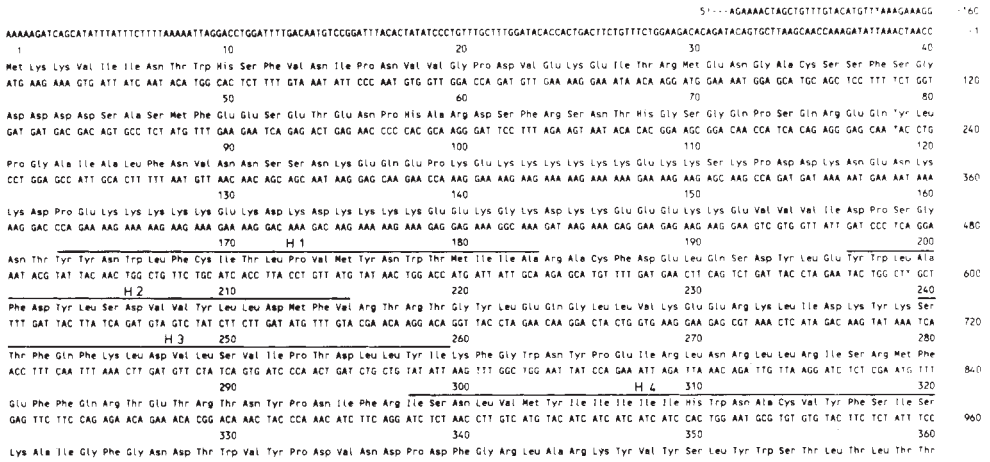

FIG. 2 Nucleotide sequence of cloned cDNA encoding the cGMP-gated channel from bovine retinal rod photoreceptors and the deduced amino-acid sequence. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG initiation triplet and the preceding residues are indicated by negative numbers; numbers of the nucleotide residues at the right-hand end of the individual lines are given. Nucleotide 2,488 is followed by a poly(dA) tract connected with the vector DNA sequence[40]. Amino-acid residues are numbered beginning with the initiating methionine. The hydrophobic segments with predicted secondary structure (H1–H6) are overlined; the termini of each segment are tentatively assigned on the basis of the hydropathicity profile and the amino-acid sequence. METHODS. The Okayama-Berg cDNA library[40] used, derived from bovine retinal poly(A)$^+$ RNA, was the same as that described previously[41]. It was screened with the 5′-end-labelled probes 5′-C$^{GA}_{TG}$AA$^{A}_{G}$TGCAT$^{A}_{G}$TA$^{T}_{C}$TG-3′ and 5′-TG$^{A}_{G}$AA$^{A}_{C}$AA$^{T}_{C}$TC$^{A}_{G}$AACAT-3′, synthesized on the basis of partial amino-acid sequences of tryptic peptides V and XII, respectively, to yield clone pCG24 (carrying nucleotides 112-2,488 and the poly(dA) tract) which hybridized with both probes. Clone pCG101 (carrying nucleotides −194 to 279) was selected from the cDNAs that were prepared[31] with the synthetic primer 5′-GCTGCTGTTGTTAACAT-3′ (complementary to nucleotides 263–279), tailed with poly(dC) and cloned into poly(dG)-tailed pBR322 (ref. 42). The probe used was the 5′-end-labelled *Hph*I(131)/*Bst*NI(243) fragment excised from pCG24; restriction endonuclease sites are identified by numbers (in parentheses) indicating the 5′-terminal nucleotide generated by cleavage. DNA sequencing[43,44] was carried out on both strands.

acid sequence (see Fig. 1 legend). Two synthetic oligodeoxyribonucleotide probes, prepared on the basis of partial amino-acid sequences of tryptic peptides V and XII, were used to screen a cDNA library derived from bovine retinal poly(A)$^+$ RNA, thus allowing a cDNA clone (pCG24) that hybridized with both probes to be isolated. Using a restriction fragment derived from the upstream region of pCG24 as a hybridization probe, we selected a collection of cDNA clones resulting from elongation of a synthetic primer. Clone pCG101 thus isolated carried a cDNA sequence extending beyond the translational initiation site. Details of the cloning procedure are described in Fig. 2 legend.

Protein structure

Figure 2 shows the 2,682-nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the cGMP-gated channel from bovine retinal rod photoreceptors. There is an open reading frame that encodes 690 amino acids, including all the partial amino-acid sequences determined (see Fig. 1 legend). The translational initiation site was assigned to the first ATG triplet which appears downstream of nonsense codons found in frame. The nucleotide sequence surrounding the initiation codon agrees reasonably well with the consensus sequence[19]. The polyadenylation signal AATAAA (residues 2,469-2,474) is found 15 nucleotides upstream of the poly(dA) tract. The size of the cGMP-gated channel mRNA was estimated to be ~3,200 nucleotides by blot hybridization analysis[20] of bovine retinal poly(A)$^+$ RNA with a cDNA probe (*Bst*NI(243)/*Bst*NI(1,213) fragment). The calculated M_r (including the initiating methionine) of this protein is 79,601. The difference between this value

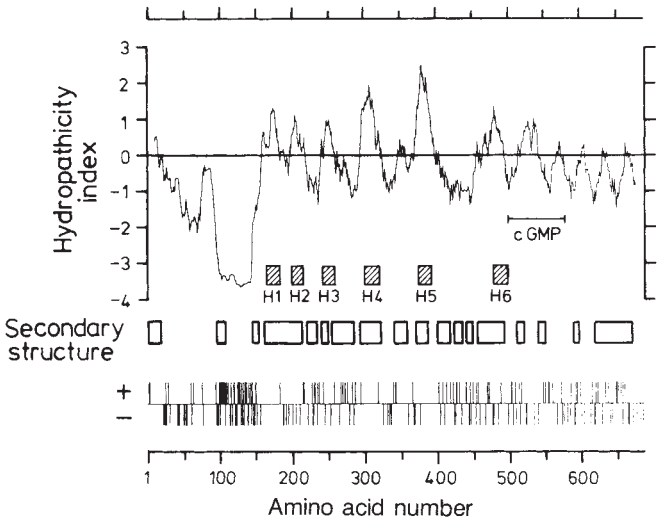

FIG. 3 Hydropathicity profile and predicted secondary structure of the cGMP-gated channel. The averaged hydropathicity index[45] of a nonadecapeptide composed of amino-acid residues $i-9$ to $i+9$ is plotted against i, the amino-acid number. The positions of the predicted structures of α-helix and/or β-sheet[46] that have a length of 10 or more residues are shown by open boxes below. The positions of the positively charged residues (Lys and Arg) and negatively charged residues (Asp and Glu) are indicated by upward (+) and downward (−) vertical lines, respectively. Segments H1–H6 are indicated by hatched boxes, and the putative cGMP-binding region by a horizontal bar.

FIG. 4 Amino-acid sequence similarity between a region of the cGMP-gated channel and the cGMP-binding domains of cGMP-dependent protein kinase (cGK). Amino-acid residues 498–577 (in one-letter code) of the cGMP-gated channel are aligned with the sequences of the two cGMP-binding domains of bovine lung cGK[23] (referred to as cGK (1) and cGK (2)); numbers of the amino-acid residues at both ends are given. Identities between the cGMP-gated channel and cGK (1) or cGK (2) are indicated by solid boxes, and conservative substitutions[47] by dotted boxes. Between the cGMP-gated channel and cGK (1), 32% of the aligned positions are occupied by identical residues, and 57% by identical or conserved residues; the probability that the sequence similarity occurs by chance[22] is 1.2×10^{-11}. The corresponding degrees of similarity between the cGMP-gated channel and cGK (2) are 30% and 51% (probability, 5.2×10^{-11}). For evaluating sequence similarity, a continuous stretch of gaps (–) has been counted as one substitution regardless of its length. Filled arrowheads indicate identities, and open arrowheads conservative substitutions between the cGMP-gated channel and the cyclic AMP-binding domain of the *E. coli* CAP; the comparison is based on the sequence alignment of CAP with cGK (2) (ref. 23).

and the M_r estimated by SDS-PAGE[12] (63K) may be attributed to the inaccuracy inherent in the measurement of M_r of membrane proteins by the electrophoretic method[21] and/or to proteolytic modification *in vivo* or during preparation of the channel.

Similarity matrix analysis[22] of the cGMP-gated channel detects no extensive amino-acid sequence similarity to other ionic channels, membrane transporters and G-protein-coupled receptors of known sequence. The deduced amino-acid sequence of the cGMP-gated channel was analysed for local hydropathicity and predicted secondary structure (Fig. 3). There are six hydrophobic segments with predicted secondary structure (referred to as H1–H6). These segments comprise ~20 amino-acid residues. Segments H4 and H5 probably represent transmembrane α-helices. Some or all of the four remaining segments may also span or interact with the membrane (see below). Segments H2, H3, H5 and H6 contain one to four charged (mostly negative) residues and segment H4, one histidine residue. In segments H2 and H5, the charged and polar side chains are clustered mainly on one side of the α-helix.

The region comprising 80 amino-acid residues located on the C-terminal side of segment H6 shows significant amino-acid-sequence similarity to the two tandem cGMP-binding domains of cGMP-dependent protein kinase[23] (Fig. 4), segments of which are similar in sequence to the regulatory subunits of cAMP-dependent protein kinase and the *Escherichia coli* catabolite gene activator protein (CAP)[23,24]. The dimeric crystal structure of CAP with two bound molecules of AMP has been determined[25]. By analogy with CAP, the side chain of Arg 559 of the cGMP-gated channel may interact with the phosphate group, and the side chain of Glu 544 may interact with the 2′-OH group of the ribose ring of cGMP. Moreover, glycines 508, 520, 539 and 543 are conserved in cGMP-dependent protein kinase as well as in CAP, and each of the corresponding glycines in CAP is located at the end of a β-strand or in a bend between two β-strands. Thus, it is suggested that these amino acids are important for the correct formation of the cGMP-binding pocket. The putative cGMP-binding region of the cGMP-gated channel is partly hydrophobic, which may indicate that a portion of the binding pocket is buried in the membrane. The region preceding segment H1 contains the consensus sequence Asn-Lys-X-Asp (residues 119–122), where X can be any amino acid, for binding the guanine ring of GTP/GDP (ref. 26), although these amino-acid residues are part of a long sequence composed of mostly Lys, Glu and Asp residues. It is possible that these residues may also be involved in the cGMP-binding site.

The relatively low hydrophobicity of segments H1, H2, H3 and H6 makes it difficult to predict the number of transmembrane segments and thus the transmembrane topography of the cGMP-gated channel. It is reasonable to assign the putative cGMP-binding region, located on the C-terminal side of segment H6, to the cytoplasmic side of the membrane. The cGMP-gated channel does not possess a hydrophobic N-terminal sequence indicative of a typical signal sequence. On the assumption that the N terminus is located on the cytoplasmic side, there should be an even number of transmembrane segments. By analogy with other channels of known sequence, we favour the view that the cGMP-gated channel has either six (like the potassium channel[27] or one repeat of the sodium channel[28] and the calcium channel[29,30]) or four transmembrane segments (like the ryanodine receptor[31] or subunits of neurotransmitter-gated ionic channels[28,32]), as schematically shown in Fig. 5*a*, *b*. The possibility that there are only two transmembrane segments (probably H4 and H5) cannot be excluded. The proposed models are consistent with one of the five potential *N*-glycosylation sites (Asn 423 for the model in Fig. 5*a* or Asn 327 for the model in Fig. 5*b*) being located on the extracellular side of the membrane; the remaining potential sites are asparagines 90, 91 and 177.

Functional expression

To examine whether the cloned cDNA actually encodes a functional cGMP-gated channel, we performed expression studies. The cDNA, including the poly(dA) tract, was linked with the bacteriophage SP6 promoter and transcribed *in vitro* with SP6 polymerase. The resulting mRNA was injected into *Xenopus* oocytes, which were incubated for 2-3 days before being tested.

Inside-out patches of large diameter[33] excised from injected *Xenopus* oocytes showed a large cGMP-activated outward current (membrane voltage V of +50 mV; Fig. 6*a*). The cGMP-activated current was reversible and its amplitude remained

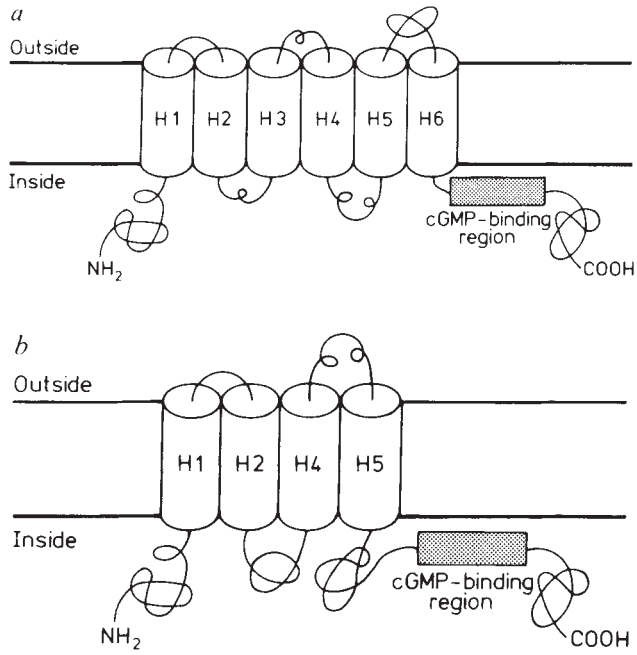

FIG. 5 Proposed transmembrane topography of the cGMP-gated channel. The presence of six (*a*) or four (*b*) putative transmembrane segments (indicated by cylinders) is assumed, which are displayed linearly; in *b*, it is possible that H3 and/or H6, instead of H1 and/or H2, span the membrane. The putative cGMP-binding region is also shown.

constant for several seconds, suggesting that the channel does not desensitize or inactivate in the presence of the agonist cGMP. All the 16 oocytes tested (5 injections, 20 patches) except one showed cGMP-activated currents, maximal amplitudes at saturating cGMP concentrations (≥200 μM) being 2–5 nA. No such response to cGMP was detected in three non-injected oocytes (5 patches) and in two oocytes (7 patches) that had been injected with mRNA specific for a potassium channel[34]. The cGMP-activated current was reversibly blocked to one half by ~40 μM *l-cis*-diltiazem (data not shown).

Figure 6*b* shows dose-response relations for the cGMP-activated macroscopic current from five different patches. The solid curve was calculated according to:

$$I/I_{max} = C^n/(C^n + K_{1/2}^n)$$

where I/I_{max} is the normalized current amplitude, C the cGMP concentration, $K_{1/2}$ the concentration of cGMP at which the current amplitude is half-maximal, and n the Hill coefficient ($K_{1/2} = 52.3$ μM; range 43–71 μM and $n = 1.75$; range 1.5–2.1). This result indicates that the channel is cooperatively activated

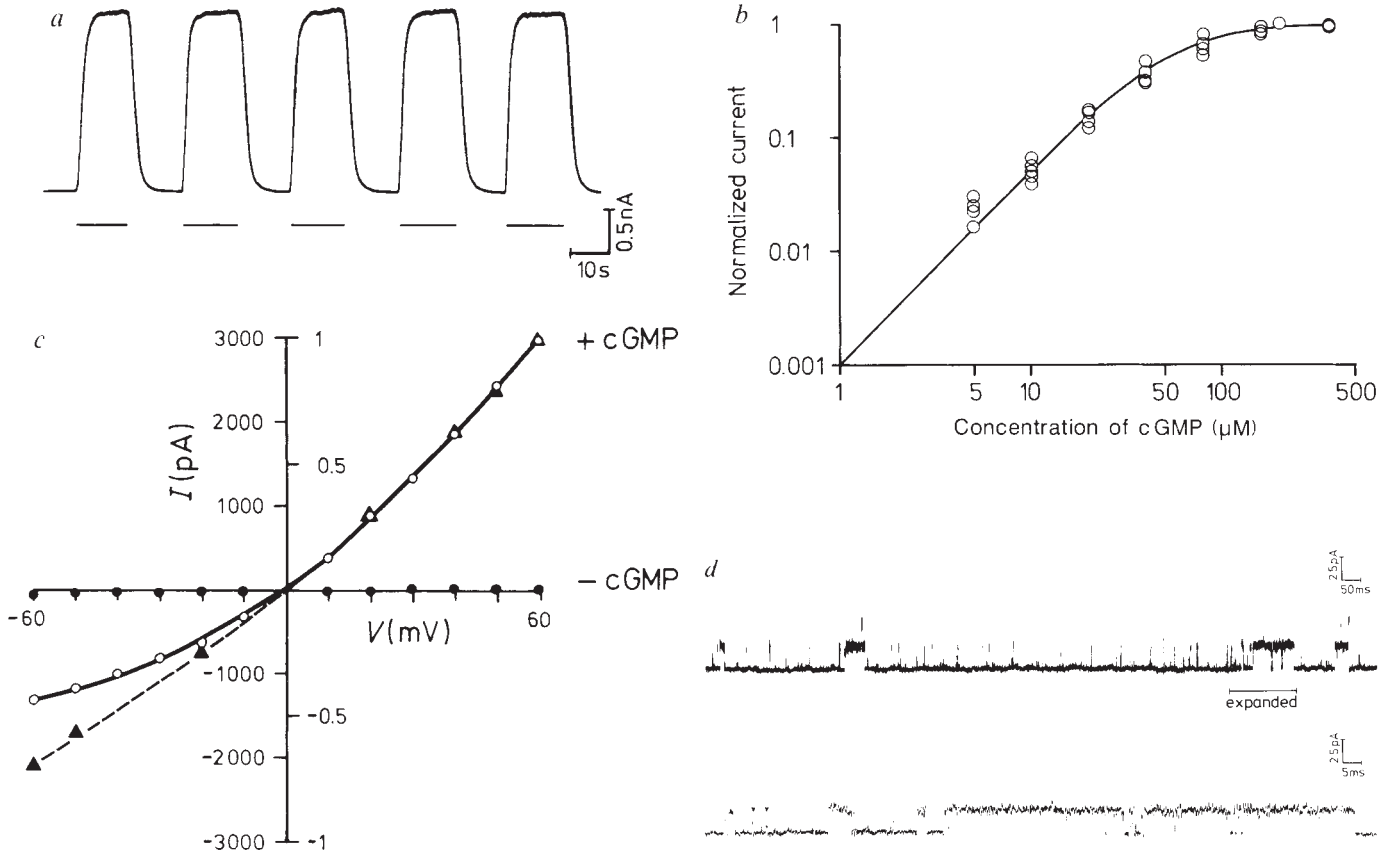

FIG. 6 Properties of the cGMP-gated channel expressed in *Xenopus* oocytes injected with mRNA derived from the cloned cDNA. *a*, Macroscopic currents activated by 200 μM cGMP. Inside-out patch. Membrane voltage (V) = +50 mV. The duration of perfusion with cGMP is indicated by bars. *b*, cGMP-dependence of the current amplitudes normalized from five experiments in double logarithmic coordinates. The solid line has been drawn according to the equation in the text with $K_{1/2} = 52.3$ μM and $n = 1.75$ ($V = +50$ mV in four experiments and $V = -40$ mV in one experiment). *c*, Current-voltage relation of the cGMP-gated channel. Macroscopic current in the presence (open circles) and absence (filled circles) of 200 μM cGMP; single-channel current in the presence of 5 μM cGMP (filled triangles). Numbers on the left-hand side of the ordinate refer to macroscopic currents, and numbers on the right-hand side to single-channel currents. *d*, Traces of single-channel currents activated by 5 μM cGMP at $V = +120$ mV. Inside-out patch. Outward current upwards. Low-pass filtering at 2 kHz (−3 dB). Mean amplitudes of single-channel currents from two experiments were 2.38 ± 0.21 pA and 2.48 ± 0.22 pA (mean ± s.d.; from ~800 and ~1,200 single-channel events, respectively). The lower trace represents an expanded form of the current trace indicated by a bar. At least two classes of elementary currents are observed; smaller current events are indicated by arrowheads.

METHODS. The pSP65 recombinant (pRCG1) carrying the complete protein-coding sequence for the cGMP-gated channel was constructed as follows. The 457-base-pair (bp) *Nde*I(2,332)/*Nde*I (vector) fragment derived from clone pCG24 was cleaved by *Pvu*II, and the resulting 227-bp *Nde*I(2,332)/*Pvu*II(vector) fragment was ligated with the *Hpa*I(268)/*Nde*I(2,332) fragment from pCG24. The 2,292-bp ligation product was isolated by agarose (1%) gel electrophoresis and cloned into the *Hin*cII site of pSP65 in the same orientation as the SP6 promoter to yield pSP65CG. Finally the *Rsa*I (−176)/*Hpa*I(268) fragment from pCG101 was cloned into the *Hin*cII site of pSP65CG to yield pRCG1. mRNA specific for the cGMP-gated channel was synthesized *in vitro*[48], using *Hin*dIII-cleaved pRCG1 as template; transcription was primed[49] with the cap dinucleotide m^7G (5′)ppp(5′)G (0.5 mM). The size (2.8 kb) of the mRNA, estimated by agarose (1.2%) gel electrophoresis, agreed with that expected from the structure of the plasmid. The mRNA was injected into *Xenopus laevis* oocytes (mRNA concentration, 0.4 μg μl^{-1}; average volume injected per oocyte, ~50 nl). Macroscopic and single-channel current measurements on excised inside-out patches[33] were made after incubation[33] of injected oocytes for 2–3 days, followed by removal of the follicular cell layer and the vitelline membrane[50]. The solution in the pipette and the perfusion medium contained (in mM): 100 KCl, 10 EGTA-KOH and 10 HEPES-KOH (pH 7.2). For determination of relative ion permeabilities from the reversal voltage (V_{rev}) under biionic conditions, KCl in the pipette solution was replaced by NaCl, and KCl in the perfusion medium by the respective ion species. In both solutions, HEPES-KOH buffer was replaced by Tris-HCl buffer, and the EGTA concentration was lowered to 1 mM. Measurements *in situ* on excised patches of isolated bovine rod outer segments confirmed that replacement of HEPES-KOH by Tris-HCl did not affect the cGMP-stimulated membrane current (H. Lühring, unpublished observation). Junction potentials varied between +2.0 and −1.9 mV and were neglected. cGMP-containing solution was applied by pressure through a glass pipette in front of the patch pipette. Macroscopic currents were either acquired on-line using a PDP 11/73 computer or stored on magnetic tape for off-line analysis. Single-channel currents were filtered at 2 kHz (−3 dB), stored on magnetic tape and analysed off-line on an Atari personal computer using standard single-channel analysis programs (Instrutech, Mineola, New York).

by cGMP. The $K_{1/2}$ and n values are similar to those observed *in situ* in amphibian[4,10,11] and mammalian[8] rod photoreceptors and cAMP failed to activate the channel at 1 mM concentration.

In the absence of divalent cations the I-V relation of the macroscopic current was slightly outward rectifying (Fig. 6*c*, open circles) and closely matches the I-V relation observed in excised patches from amphibian[35] and mammalian[8] rod photoreceptors under similar conditions. The leak current recorded in the absence of cGMP was negligible (filled circles). The difference in voltage dependence between the macroscopic current (open circles) and the single-channel current (filled triangles) was small and became noticeable only at negative voltages. The small difference may be attributed to a weak voltage-dependence of gating and is in agreement with the observations *in situ*[10,11] and in the reconstituted system[14].

Figure 6*d* shows single-channel currents ($V = +120$ mV) recorded in the absence of divalent cations and at a low cGMP concentration. The mean current amplitude in 100 mM KCl was 2.4 pA corresponding to a single-channel conductance of 20 pS, but smaller conductance sublevels (8–10 pS) were also observed. The single-channel conductance observed *in situ*[10,11] is 25 pS and that in the reconstituted system[14] is 26 pS. At the membrane voltage used, periods with brief channel openings (mean open time $\tau_0 \sim 1$ ms) were interrupted by much longer openings ($\tau_0 \geq 10$ ms), indicating that the time distribution of opening is not uniform and may be described by at least two exponential time constants.

The ion selectivity of the expressed cGMP-gated channel was examined by measurement of the reversal voltage V_{rev} under symmetrical biionic conditions. The following values for V_{rev} were obtained (mean ± s.d.; n indicating the number of experiments): NH_4^+, $+27.3 \pm 1.7$ mV ($n = 3$); K^+, $+0.35 \pm 1.3$ mV ($n = 3$); Li^+, -11.6 ± 1.7 mV ($n = 5$); Rb^+, -14.6 ± 2.5 mV ($n = 5$); Cs^+, -25.6 ± 1.6 mV ($n = 4$). Permeability ratios P_i/P_{Na} calculated from V_{rev} according to the Goldman-Hodgkin-Katz equation yielded the following series of ion selectivity:

$$NH_4^+ > K^+ \sim Na^+ > Li^+ > Rb^+ > Cs^+$$
$$= 2.93 : 1.01 : 1 : 0.63 : 0.56 : 0.37$$

Similar relative ion permeabilities have been determined in excised patches from amphibian[1,5-7] and mammalian[8] rod photoreceptors.

Discussion

The primary structure of the rod photoreceptor cGMP-gated channel has been deduced by cloning and sequencing the cDNA. The predicted structure suggests the presence of multiple transmembrane segments, which may be involved in forming the channel portion. On the carboxyl side of the putative transmembrane region, there exists a segment of 80 amino acids which exhibits significant amino-acid sequence similarity to the cyclic nucleotide-binding regions of cAMP-binding proteins and in particular of cGMP-dependent protein kinase. This strongly suggests that the corresponding region of the cGMP-gated channel is also involved in binding of cGMP. The channel is cooperatively activated by at least three[10,11], probably more[3,12,13] cGMP molecules. Moreover, cGMP-dependent protein kinase which is cooperatively stimulated[36] by two cGMP molecules per monomer[37], contains two tandem homologous cGMP-binding regions, whereas the cGMP-gated channel contains only one such region. This indicates that the cGMP-gated channel is a homo-oligomeric complex, each constituent polypeptide having a single cGMP-binding site.

The expression of cGMP-gated channel activity by injection of *Xenopus* oocytes with specific mRNA derived from the cloned cDNA identifies the encoded polypeptide as the cGMP-gated channel protein of mammalian rod photoreceptors and suggests that this polypeptide alone is sufficient to form the functional channel with properties similar to those observed *in situ*. In particular the expressed channel is cooperatively activated by cGMP with a $K_{1/2}$ of several tens of micromolar; it represents a channel that does not discriminate very well between alkali cations and whose major single-channel conductance is 20 pS; the macroscopic cGMP-activated current exhibits a weak outward directed rectification, probably due to the weak voltage-dependence of gating and the almost linear I-V relation of the single channel; finally, the channel is blocked by the drug *l-cis*-diltiazem. □

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1. Fesenko, E. E., Kolesnikov, S. S. & Lyubarsky, A. L. *Nature* **313,** 310–313 (1985).
2. Stryer, L. *A. Rev. Neurosci.* **9,** 87–119 (1986).
3. Kaupp, U. B., Hanke, W., Simmoteit, R. & Lühring, H. *Cold Spring Harbor Symp. quant. Biol.* **53,** 407–415 (1988).
4. Yau, K.-W. & Baylor, D. A. *A. Rev. Neurosci.* **12,** 289–327 (1989).
5. Nunn, B. *J. Physiol., Lond.* **394,** 17P (1987).
6. Menini, A. & Torre, V. *Biophys. J.* **55,** 61a (1989).
7. Furman, R. E. & Tanaka, J. C. *Biophys. J.* **55,** 455a (1989).
8. Lühring, H. & Kaupp, U. B. *Biophys. J.* **55,** 377a (1989).
9. Stern, J. H., Kaupp, U. B. & MacLeish, P. R. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1163–1167 (1986).
10. Haynes, L. W., Kay, A. R. & Yau, K.-W. *Nature* **321,** 66–70 (1986).
11. Zimmerman, A. L. & Baylor, D. A. *Nature* **321,** 70–72 (1986).
12. Cook, N. J., Hanke, W. & Kaupp, U. B. *Proc. natn. Acad. Sci. U.S.A.* **84,** 585–589 (1987).
13. Cook, N. J., Zeilinger, C., Koch, K.-W. & Kaupp, U. B. *J. biol. Chem.* **261,** 17033–17039 (1986).
14. Hanke, W., Cook, N. J. & Kaupp, U. B. *Proc. natn. Acad. Sci. U.S.A.* **85,** 94–98 (1988).
15. Matesic, D. & Liebman, P. A. *Nature* **326,** 600–603 (1987).
16. Shinozawa, T., Sokabe, M., Terada, S., Matsusaka, H. & Yoshizawa, T. *J. Biochem.* **102,** 281–290 (1987).
17. Clack, J. W. & Stein, P. J. *Proc. natn. Acad. Sci. U.S.A.* **85,** 9806–9810 (1988).
18. Applebury, M. L. *Nature* **326,** 546–547 (1987).
19. Kozak, M., *Nucleic Acids Res.* **12,** 857–872 (1984).
20. Mishina, M. *et al. Nature* **321,** 406–411 (1986).
21. Grefrath, S. P. & Reynolds, J. A. *Proc. natn. Acad. Sci. U.S.A.* **71,** 3913–3916 (1974).
22. Toh, H., Hayashida, H. & Miyata, T. *Nature* **305,** 827–829 (1983).
23. Takio, K. *et al. Biochemistry* **23,** 4207–4218 (1984).
24. Weber, I. T., Steitz, T. A., Bubis, J. & Taylor, S. S. *Biochemistry* **26,** 343–351 (1987).
25. McKay, D. B. & Steitz, T. A. *Nature* **290,** 744–749 (1981).
26. Dever, T. E., Glynias, M. J. & Merrick, W. C. *Proc. natn. Acad. Sci. U.S.A.* **84,** 1814–1818 (1987).
27. Jan, L. Y. & Jan, Y. N. *Cell* **56,** 13–25 (1989).
28. Numa, S. *Harvey Lect.* **83,** 121–165 (1989).
29. Tanabe, T. *et al. Nature* **328,** 313–318 (1987).
30. Mikami, A. *et al. Nature* **340,** 230–233 (1989).
31. Takeshima, H. *et al. Nature* **339,** 439–445 (1989).
32. Barnard, E. A., Darlison, M. G. & Seeburg, P. *Trends Neurosci.* **10,** 502–509 (1987).
33. Stühmer, W., Methfessel, C., Sakmann, B., Noda, M. & Numa, S. *Eur. Biophys. J.* **14,** 131–138 (1987).
34. Stühmer, W., Stocker, M., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A. & Pongs, O. *FEBS Lett.* **242,** 199–206 (1988).
35. Yau, K.-W., Haynes, L. W. & Nakatani, K. in *Membrane Control of Cellular Activity* (ed. Lüttgau, H. C.) 343–366 (Gustav-Fischer, Stuttgart, 1986).
36. McCure, R. W. & Gill, G. N. *J. biol. Chem.* **254,** 5083–5091 (1979).
37. Corbin, J. D. & Døskeland, S. O. *J. biol. Chem.* **258,** 11391–11397 (1983).
38. Kawakami, K. *et al. Nature* **316,** 733–736 (1985).
39. Kubo, T., *et al. Nature* **323,** 411–416 (1986).
40. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2,** 161–170 (1982).
41. Tanabe, T. *et al. Nature* **315,** 242–245 (1985).
42. Noda, M. *et al. Nature* **295,** 202–206 (1982).
43. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
44. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
45. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157,** 105–132 (1982).
46. Chou, P. Y. & Fasman, G. D. *A. Rev. Biochem.* **47,** 251–276 (1978).
47. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. in *Atlas of Protein Sequence and Structure* Vol. 5, suppl. 3 (ed. Dayhoff, M. O.) 345–352 (National Biomedical Research Foundation, Silver Spring, Maryland, 1978).
48. Melton, D. A. *et al. Nucleic Acids Res.* **12,** 7035–7056 (1984).
49. Konarska, M. M., Padgett, R. A. & Sharp, P. A. *Cell* **38,** 731–736 (1984).
50. Methfessel, C. *et al. Pflügers Arch.* **407,** 577–588 (1986).

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