Volume 196, number 1 FEBS 3334 February 1986

Sequence analysis of bovine retinal S-antigen

Relationships with α-transducin and G-proteins

Graeme J. Wistow, Albine Katial, Cheryl Craft and Toshimichi Shinohara

Laboratory of Molecular and Developmental Biology, NEI Bldg 6, Room 204, National Institutes of Health, Bethesda, MD 20892, USA

Received 25 November 1985

S-Antigen is a major soluble protein of the retina and pineal. It is capable of inducing experimental autoimmune uveitis (EAU) in laboratory animals and also seems to play an important role in the visual cycle. The results of partial cDNA sequence analysis reveal interesting homologies with α-transducin, a GTP-binding protein of retina and other purine nucleotide-binding proteins. In particular S-antigen shows over 50% identity to the proposed pertussis toxin ADP-ribosylation site of α-transducin. It also contains the Gly-X-X-X-X-Gly-Lys pattern common to phosphoryl binding sites. A possible relationship between S-antigen and purine nucleotide-binding proteins is discussed. There is also evidence for a repetitious β-structure in the C-terminal half of S-antigen, with a monoclonal antibody epitope in a helical region at the C-terminus.

(Bovine retina) S-Antigen Uveitis cDNA sequence α-Transducin ADP-ribosylation

1. INTRODUCTION

S-Antigen is a soluble protein of the retina and pineal [1]. Its molecular mass by laser-light scattering and ultracentrifugation is 42–44 kDa [2] (although SDS-PAGE gives an estimate of 50 kDa). It seems to exist in immunochemically conserved forms from molluscs to mammals [3]. Recently, it has been shown that S-antigen binds photoexcited-phosphorylated rhodopsin [4] and may therefore play an important role in the visual cycle. In light-adapted bovine retinas, S-antigen and rhodopsin have been detected in quantities approaching a 1:1 molar ratio [5].

The protein is also highly antigenic and anti-S-antigen activities have been detected in some patients suffering from the inflammatory condition uveitis [6]. Furthermore, purified S-antigen is able to induce similar symptoms in various laboratory animals in the condition known as experimental autoimmune uveitis (EAU) [1,7,8]. In spite of the interest in this molecule no sequence data have yet been presented. Here we describe the determination of the sequence of the C-terminal 239 residues of bovine S-antigen by cDNA sequencing. Examination of the predicted amino-acid sequence suggests that S-antigen has an internally repetitious structure. It has also allowed the position of an anti-S-antigen monoclonal antibody epitope to be predicted. Sequence comparisons have revealed some interesting regions of homology with α-transducin and other purine nucleotide-binding proteins, the functional family which includes G-proteins, elongation factors and ras proteins among others.

2. MATERIALS AND METHODS

2.1. *Isolation of cDNA clones*

cDNA clones were isolated from two bovine retina libraries as described elsewhere (Craft et al., submitted). Three overlapping cDNAs of approx. 420, 220 and 150 bp were isolated by immunoreactivity from the λgt11 [9] library of Dr Oprian (MIT). The 420 bp cDNA was used to screen a

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00145793/86/$3.50

larger, 880 bp cDNA from the λgt10 library of Dr Nathans (Stanford) [10] by hybridization.

2.2. *M13 subcloning*

The *Eco*RI fragment cDNA inserts were subcloned into the *Eco*RI site of M13 mp19 [11] using reagents from Bethesda Research Laboratories (Gaithersburg, MD). The inserts were also further digested using *Sst*I, *Bam*HI and *Bgl*II. These fragments were subcloned into M13 mp18 and mp19 [11] in order to obtain both strands of each fragment in the sequencing vector. The recombinant phages were then transfected into *E. coli* JM101 [12].

2.3. *cDNA sequencing*

Sequences were obtained by the dideoxy chain termination method of Sanger et al. [13], modified for ^{35}S-ATP (Amersham, Arlington Heights, IL) essentially as described in the BRL M13 cloning/dideoxy sequencing manual. Enzymes were obtained from BRL and International Biotechnologies (New Haven, CT). Nucleotides, both deoxy- and dideoxy- were obtained from Pharmacia-PL Biochemicals (Piscataway, NY). Sequences were run on 8% bisacrylamide urea gels which were dried for autoradiography by the procedure of Vasil [14]. Most sequences were generated by using the universal M13 15-mer primer, sequencing across internal restriction enzyme sites or across the *Eco*RI sites resulting from the use of linkers in the cloning procedure. Some confirmatory overlapping sequences (fig.1) were later obtained by the use of synthetic 15-mer primers designed from the determined sequence and synthesized by OCS Labs (Denton, TX).

3. RESULTS AND DISCUSSION

The strategy for the determination of the nucleotide sequence of the complete 880 bp fragment, together with the overlapping 420, 220 and 150 bp fragments is shown in fig.1. Each arrow represents at least two and up to six different M13 subclones, except for that reading in from the extreme 3′-end of the 880 bp fragment. Only one M13 clone for this strand was obtained. It is possible that some part of the cDNA insert in that sense compromises the viability of the M13 vector. Similarly, a 10-fold excess of M13 clones for one strand of the 420 bp *Eco*RI-*Eco*RI fragment was observed compared to the reverse strand. To confirm certain parts of the sequence, specific, 15-mer oligonucleotide primers were designed to allow sequence initiation close to the desired region. Sequences were read by compiling data from successively longer gel runs of the same sequencing reaction.

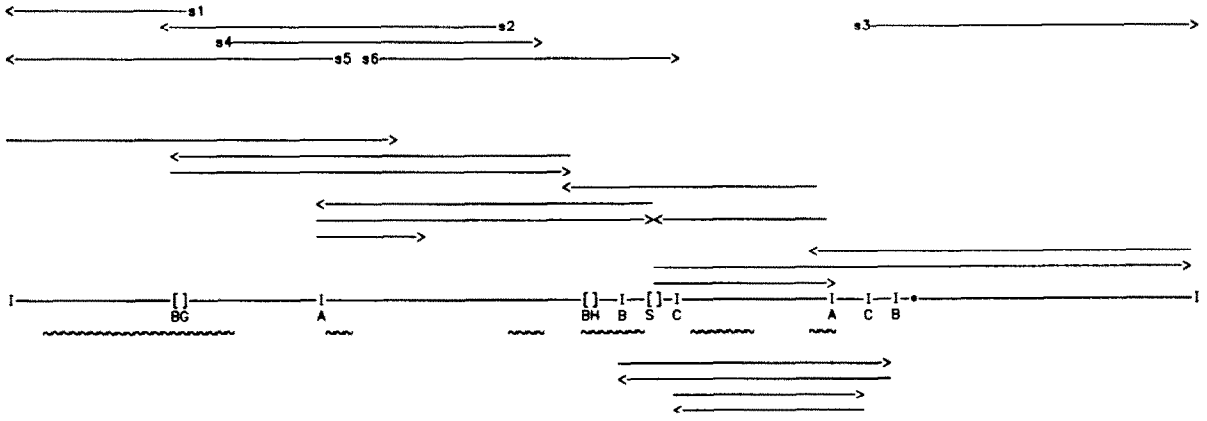

Fig.1. Determination of the nucleotide sequence of the 880 bp and smaller cDNAs for bovine S-antigen. Restriction sites are shown boxed: BH, *Bam*HI; BG, *Bgl*II; S, *Sst*I. A, B, C indicate the 5′- and 3′-ends of the 420, 220 and 150 bp fragments, respectively. Arrows show the origin and extents of sequences from different classes of M13 subclones. S1–S6 mark the positions of synthetic 15-mer primers used for sequence confirmation. (~~) Equivalent positions of peptide sequences determined by amino-acid sequencing (Dietzschold et al., in preparation).

One long open reading frame (fig.2) terminating after 239 predicted residues was observed after computer translation of the nucleotide sequence. This open frame matched that predicted for the λgt11 clones which were detected as the result of fusion protein expression [9]. The size of the predicted polypeptide (26822 Da) corresponds to about two-thirds of that expected for a 42 kDa protein. No poly(A) tail or polyadenylation signal was observed in the nucleotide sequence.

The predicted amino-acid sequence was then aligned with the partial peptide sequences obtained independently by Dietzschold et al. (in preparation) (figs 1 and 2), confirming the identity of the cDNA clones. There were no matches between the peptide sequences and any translated reading frame of the nucleotide sequence 3′- to the first stop codon.

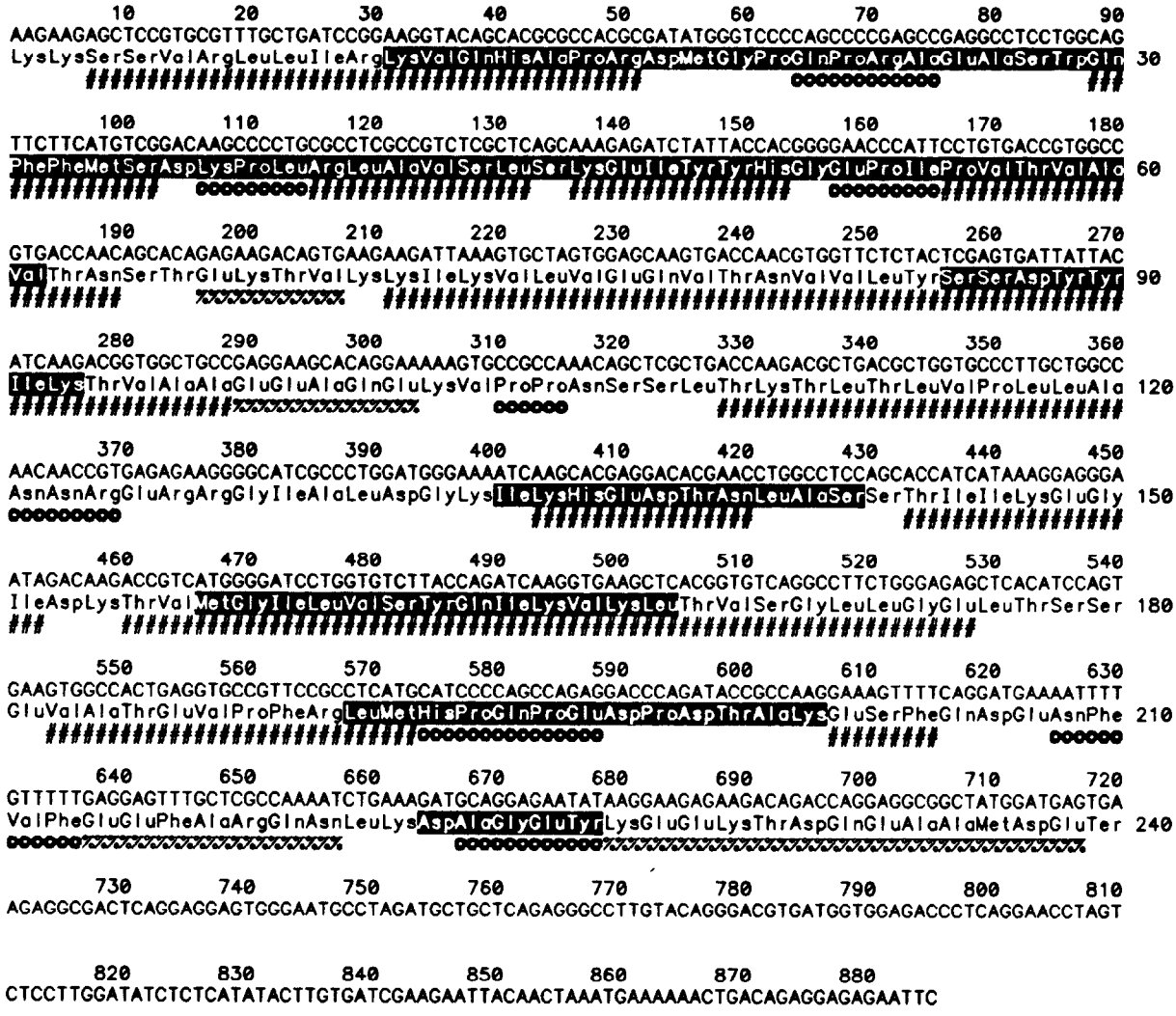

Fig.2. The sequence of the 880 bp cDNA clone for bovine S-antigen. The upper line shows the nucleotide sequence. The lower line shows the predicted amino-acid sequence with the positions of peptides determined by amino-acid sequencing (Dietzschold et al., in preparation) indicated by reverse tone. Regions of secondary structure predicted by the method of Garnier et al. [16] are shown: (#) extended (β) conformation, (●) coil or turn, (%) helical (α) conformation. Nucleotide numbers are given horizontally, predicted amino acids are numbered vertically.

3.1. *Secondary structure*

The algorithms of Chou and Fasman [15] and Garnier et al. [16], as implemented in the package IDEAS [17], were used to predict secondary structure for the predicted 239 C-terminal residues of S-antigen. For the method of Garnier et al., in the absence of circular dichroism data or other estimates of secondary structure a range of decision constants DCH (75–158) and DCE (88–50) were used. The results, summarized in fig.1, all indicated a predominantly extended conformation (β-sheet conformation) interspersed with short regions of turn, containing little predicted helical conformation. In contrast, the C-terminal 27 residues were predicted to have helical structure with a central break of turn or coil.

It was then observed that several sections of the sequence exhibiting the pattern of extended conformation followed by turn or coil had some sequence similarity. The most striking resemblance is between residues 2–30, and residues 178–206 which have 11 identical residues (34% identity) allowing for gaps (fig.3a). These two segments have interesting positions in the S-antigen sequence. If a total length of about 380 residues is assumed for a 42 kDa protein and if the C-terminal helical region of about 30 residues is ignored, it is apparent that residues 2–30 lie at the C-terminal end of the first half of the molecule while residues 178–206 lie at the equivalent position in the second half. This would be consistent with a 2-fold repeat in the protein sequence.

With weaker agreement other segments (41–66, 85–105, 106–139, 142–163), can also be fitted to this general alignment of similar secondary structures with limited sequence homology (fig.3a). It is possible that S-antigen has an internally repetitious structure, at least at the level of a 2-fold repeat, of the kind that has been observed for other groups of proteins, such as β,γ-crystallins [18,19] and acid proteases [20] and is often thought to be the result of gene multiplication and fusion.

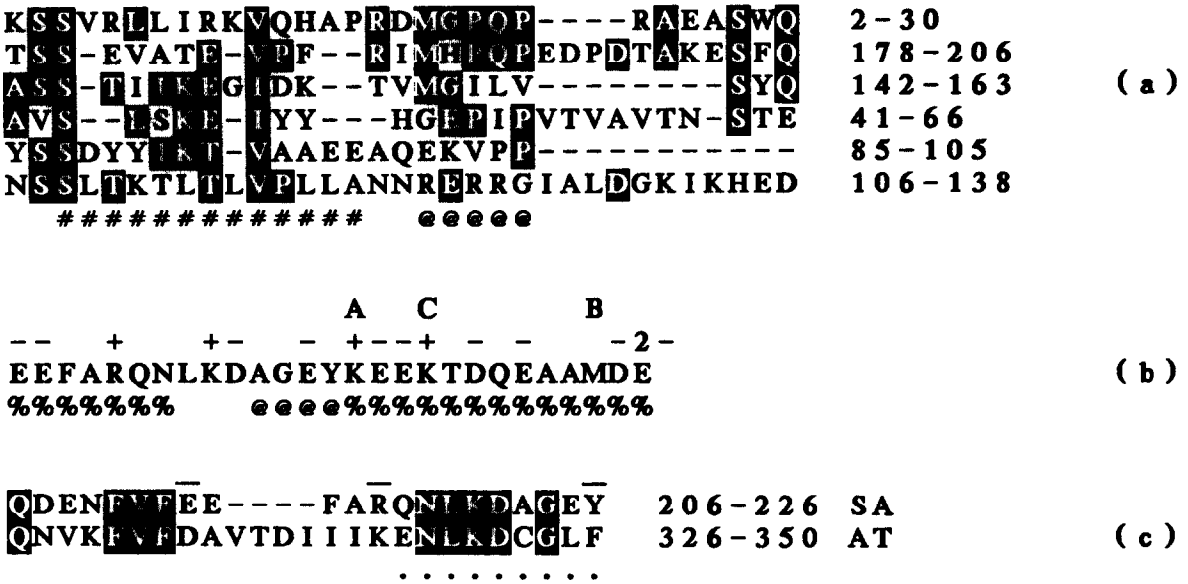

Fig.3. (a) Comparison of the amino-acid sequences of S-antigen peptides which have similar predicted secondary structures. Identical residues are boxed. (#) Predicted extended structure, (@) predicted coil or turn in the regions of approximate consensus in predicted conformation [15,16]. Single-letter amino-acid code is used. (b) C-terminal peptide of bovine S-antigen. Predicted secondary structures are shown: (%) predicted helix, (@) coil or turn. (+, −) Indicate the charge on each residue. A, B, C mark the C-termini of the peptide products of the 420, 220 and 150 bp cDNA fragments. (c) Comparison of residues 206–226 of the S-antigen (SA) sequence with residues 326–350 of α-transducin (AT) [26,27]. Identical residues are boxed. Closely conserved variations are indicated by −. The region identified with pertussis toxin ADP-ribosylation [26–28] is underlined with dots.

In contrast to the repetitious β-structure of the rest of the protein, the C-terminal polypeptide gives rise to a prediction for a predominately helical structure (fig.3b). It contains several charged groups (11 negative and 4 positive charges), is mainly hydrophilic in character and is predicted to be composed of two helices separated by a bend of coil or turn conformation. Interestingly, the 3 cDNA clones selected by immunoreactivity from the λgt11 expression library all terminate in this region. As described elsewhere (Craft et al., submitted), although all 3 reacted with the monoclonal antibody MAbA1-G5, the product of the 420 bp fragment gave a markedly weaker reaction. This suggests that the boundary between the C-terminal ends of the 420 bp and 150 bp fragment peptide products (fig.3b) is close to part of the antigenic site. Indeed, while the products of the 220 bp and 150 bp fragments are both predicted to contain the bend region and at least part of the final helix, the 420 bp product lacks all of the final helix. This implies that the epitope for the monoclonal antibody consists, at least in part, of the bend into the final helix.

3.2. *Inter-molecular comparisons*

Although the function of S-antigen in the visual system is not well understood, several pieces of evidence point to a possible relationship with purine nucleotide-binding proteins. Both S-antigen [4] and the GTP-binding protein transducin [21] interact with photoactivated rhodopsin. This leads respectively to the quenching or activation of the phosphodiesterase that mediates the light response in rod cells. Transducin has been shown to be closely related to the G-proteins of the adenylate cyclase system [22]. These proteins typically exhibit two different α-subunits, $G\alpha_s$ and $G\alpha_i$ (both of ~40 kDa), which have opposing stimulatory or inhibitory effects. The relationship between S-antigen and transducin in the activation/inactivation of phosphodiesterase has interesting parallels with this system.

In other work, a purine nucleotide (ATP)-binding activity has been proposed for an abundant retinal protein of similar size to S-antigen [23]. Furthermore, other workers have suggested that S-antigen is identical to one of the rhodopsin kinase activities of retina [24].

The predicted S-antigen amino-acid sequence was examined for homologies with the NBRF protein sequence database but no large-scale similarities were detected using IDEAS [17] or the search programs of Wilbur and Lipman [25]. However, a detailed comparison with purine nucleotide-binding proteins revealed some interesting similarities, particularly with bovine α-transducin [26–28]. Most interesting is the similarity between residues 206–226 of the S-antigen sequence and residues 326–350, the C-terminal 25 residues of α-transducin [26–28] (fig.3c). This part of α-transducin contains the proposed site for ADP-ribosylation by pertussis toxin [26–29] and this site in particular is more than 50% identical to the S-antigen sequence, with most non-identical residues conservatively varied (fig.3c). Since the modification of α-transducin by pertussis toxin [26–29] presumably occurs at a functionally important position [28,29], the similarity of the C-terminal region in S-antigen suggests the possibility of a functional relationship with α-transducin. It also raises the interesting possibility of a role for toxin-mediated modification of S-antigen in uveitis. Furthermore, the replacement of Cys 347 with Ala 223 (fig.3c) may question the hypothesis that Cys 347 is the functionally active residue in the α-transducin sequence and in equivalent positions in ras proteins [26–28].

Elsewhere, residues 144–149 of S-antigen (Ser-Thr-Ile-Ile-Lys-Glu) are similar to residues 43–48 of α-transducin (Ser-Thr-Ile-Val-Lys-Gln) which are part of a 22-residue sequence of unknown function conserved between α-transducin and the α-subunits of brain GTP-binding proteins [30].

Purine nucleotide-binding proteins in general have other conserved functional sequences [31,32]. The most characteristic of these is Gly-X-X-X-X-Gly-Lys at the phosphoryl binding site. Residues 127–133 of the predicted S-antigen sequence have the sequence Gly-Ile-Ala-Leu-Asp-Gly-Lys which fits this pattern. Another sequence, Asn-Lys-X-Asp is common to the guanine specificity regions of elongation factors, ras protein and α-transducin [32]. The closest matches to this sequence in S-antigen are Glu-Lys-Thr-Asp (229–232) and Asn-Leu-Lys-Asp (218–221). Another conserved sequence of the purine nucleotide-binding site, Asp-X-X-Gly, with a preference for Ala or Pro at the third position [31,32], is not observed in the part of S-antigen for which data exist.

These similarities are consistent with the possibility that there is a functional relationship between S-antigen and α-transducin and perhaps with other members of the large and important family of purine nucleotide-binding proteins, although the relationship is much more distant than that between α-transducin and ras proteins [26–28]. The phosphoryl binding site similarities may relate to the binding of phosphorylated rhodopsin rather than a purine nucleotide but in view of the presence of S-antigen in the mammalian pineal, where rhodopsin is absent [33], a more general function for S-antigen is possible.

ACKNOWLEDGEMENTS

We thank B. Dietzschold, J. Early and L. Donoso for information on the peptide sequences prior to publication, Dawn Chicchirichi for typing the manuscript and Dr J. Piatigorsky and C. Jaworski for helpful comments.

REFERENCES

[1] Wacker, W.B., Donoso, L.A., Kalsow, C.M., Yankeelov, J.A. and Organisciak, D.T. (1977) J. Immunol. 14, 1949–1958.
[2] Broekhuyse, R.M. and Bessems, H.J.H. (1985) Exp. Eye Res. 40, 763–766.
[3] Mirshahi, M., Boucheix, C., Collenot, G., Thillaye, B. and Faure, J.-P. (1985) Invest. Ophthal. Vis. Sci. 26, 1016–1021.
[4] Pfister, C., Chabre, M., Plouet, J., Tuyen, V.V., DeKozak, Y., Faure, J.-P. and Kuhn, H. (1985) Science 228, 891–893.
[5] Broekhuyse, R.M., Tolhuizen, E.F.J., Janssen, A.P.M. and Winkens, H.J. (1985) Curr. Eye Res. 4, 613–618.
[6] Gregerson, D., Abrahams, I. and Thirkill, C. (1981) Invest. Ophthalmol. Vis. Sci. 21, 669–680.
[7] Faure, J.-P. (1980) in: Current Topics in Eye Research, vol.2, pp.215–302, Academic Press, New York.
[8] Forrester, J.V., Borthwick, G.M. and McMenamin, P.G. (1985) Invest. Ophthalmol. Vis. Sci. 26, 1281–1292.
[9] Young, A.R. and Davis, R.W. (1983) Proc. Natl. Acad. Sci. USA 80, 1194–1198.
[10] Nathans, J. and Hogness, D.S. (1984) Cell 34, 807–814.
[11] Norrander, J., Kempe, T. and Messing, J. (1983) Gene 26, 101–106.
[12] Hanahan, D. (1983) J. Mol. Biol. 166, 557–580.
[13] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[14] Vasil, M. (1984) Product Highlights, p.7, Boehringer Mannheim, Indianapolis, IN.
[15] Chou, P.Y. and Fasman, G.D. (1974) Biochemistry 13, 222–245.
[16] Garnier, J., Osguthorpe, D.J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.
[17] Kanehisa, M., Fickett, J.W. and Goad, W.B. (1984) Nucleic Acids Res. 12, 149–158.
[18] Blundell, T., Lindley, P., Miller, L., Moss, D., Slingsby, C., Tickle, I., Turnell, B. and Wistow, G. (1981) Nature 289, 771–777.
[19] Piatigorsky, J. (1984) Cell 38, 620–621.
[20] Tang, J., James, M.N.G., Hsu, I.N., Jenkins, J.A. and Blundell, T.L. (1978) Nature 271, 618–621.
[21] Stryer, L. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, 841–852.
[22] Gilman, A.G. (1984) Cell 36, 577–579.
[23] Zuckerman, R., Buzdygon, B. and Liebman, P. (1984) ARVO Abstr., Invest. Ophthalmol. Vis. Sci. 25, 112.
[24] Somers, R.L. and Klein, D.C. (1984) Science 226, 182–184.
[25] Wilbur, W.J. and Lipman, D.J. (1983) Proc. Natl. Acad. Sci. USA 80, 726–730.
[26] Medynski, D.C., Sullivan, K., Smith, D., Van Dorp, C., Chang, F.-H., Fung, B.K.-K., Seeburg, P.H. and Bourne, H.R. (1985) Proc. Natl. Acad. Sci. USA 82, 4311–4315.
[27] Yatsunami, K. and Khorana, G. (1985) Proc. Natl. Acad. Sci. USA 82, 4316–4320.
[28] Tanabe, T., Nukada, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H. and Numa, S. (1985) Nature 315, 242–245.
[29] Manning, D.R., Fraser, B.A., Kahn, R.A. and Gillman, A.G. (1984) J. Biol. Chem. 259, 749–756.
[30] Hurley, J.B., Simon, M.I., Teplow, D.B., Robishaw, J.D. and Gilman, A.G. (1984) Science 226, 860–862.
[31] La Cour, T.F.M., Nyborg, J., Thirup, S. and Clark, B.F.C. (1985) EMBO J. 4, 2385–2388.
[32] McCormick, F., Clark, B.F.C., La Cour, T.F.M., Kjeldgaard, M., Norskov-Lauritsen, L. and Nyborg, J. (1985) Science 230, 78–82.
[33] Converse, C.A. and Papermaster, D.S. (1975) Science 189, 469–472.