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Complete amino acid sequence of γ-subunit of the GTP-binding protein from cattle retina

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The complete amino acid sequence of the γ-subunit of the GTP-binding protein from cattle retina has been established. The polypeptide chain of the γ-subunit consists of 69 amino acid residues and contains the unusual sequence Cys35-Cys36. The M_r of the γ-subunit is 8008.7.

GTP-binding protein *γ-Subunit* *Amino acid sequence* *Rhodopsin*

1. INTRODUCTION

The GTP-binding protein or transducin is a peripheral protein located in retinal rod outer segments. Transducin is an amplifier and one of the transducers of a visual impulse, which performs the coupling between rhodopsin and cGMP-phosphodiesterase [1]. The α-subunit of transducin (~39 kDa) contains the guanine nucleotide binding site and serves to activate phosphodiesterase; the β(~36 kDa)- and γ(8 kDa)-subunits are required for the GTPase activity and for replacement of GDP by GTP catalyzed by photoexcited rhodopsin [2].

This study is part of complex investigations on the primary structures and mechanism of the function of the proteins involved in visual signal transduction. It is devoted to the establishment of the amino acid sequence of the γ-subunit of transducin from cattle retina. (The results were reported by Yu.A. Ovchinnikov in his plenary lecture at the 16th FEBS Meeting, Moscow, 1984, and at the Symposium 'Recent Advances in Biophysical Chemistry', Boston, 1984 (see also [3,4].)

2. MATERIALS AND METHODS

Transducin was isolated as in [5]. The α- and γ-subunits were obtained by high-performance liquid chromatography (HPLC) on a reversed-phase column. Transducin (2 mg) in 2 ml of 0.1 M Tris–HCl, 0.3 M NaCl, 0.005 M dithiothreitol, 0.0005 M EDTA (pH 7.5) was applied to a 10 μ Silasorb Phenyl (Lachema) column. Use was made of an acetonitrile gradient (0–90%) in 0.1% trifluoroacetic acid (TFA), the γ(180 μg)- and α(430 μg)-subunits were eluted in 54% and 74% acetonitrile, respectively. The β-subunit was irreversibly adsorbed to the column support. Homogeneity of the isolated subunits was detected by sodium dodecyl sulphate electrophoresis in gradient (4–30%) polyacrylamide gel [6].

To elucidate the primary structure of the transducin γ-subunit the following procedures were applied:

(i) Hydrolysis with *Staphylococcus aureus* protease (V8, Miles) in 0.1 M ammonium bicarbonate (pH 8.0) for 6 h at 37°C, enzyme:substrate ratio is 1:20.
(ii) Hydrolysis with chymotrypsin (Worthington) in 0.2 M ammonium bicarbonate (pH 8.4) for 2.5 h at 37°C, enzyme-substrate ratio, 1:50.
(iii) Hydrolysis with clostripain (Boehringer Mannheim) in 0.1 M sodium phosphate, 0.001 M calcium chloride, 0.005 M dithiothreitol (pH 7.8) for 1 h at 20°C, enzyme:substrate ratio, 1:100.
(iv) Cyanogen bromide cleavage in 70% formic acid at a 200-fold mole reagent excess.

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Both the intact γ-subunit and radioactively labeled (with iodo[^{14}C]acetamide) samples were hydrolysed with *S. aureus* protease. For radioactive labeling, 20 nmol of the γ-subunit in 200 μl of 0.1 M Tris–HCl, 8 M guanidine–HCl (pH 8.0) were reduced with a 3-fold mole excess of mercaptoethanol (9 μl) for 4 h at 20°C and then carboxymethylated with a 10-fold excess of moniodo[^{14}C]acetamide (50 μCi, Amersham). In 30 min mercaptoethanol excess was added. On treatment of the γ-subunit with iodoacetic acid under the same conditions cysteine residues were not carboxymethylated.

Peptides obtained on the hydrolysis of the γ-subunit with *S. aureus* protease, chymotrypsin, and cyanogen bromide were separated by HPLC on a reversed-phase Nucleosil C_{18} column (Macherey-Nagel) with an acetonitrile gradient (0–50%) in 0.1% TFA. Clostripain peptides were separated from salts and the non-split γ-subunit by gel filtration on a Spherogel 2000SW column (0.75 × 60 cm, Altex).

The peptides were sequenced by the Edman procedure; amino acids were identified as 1-dimethylaminonaphthalene-5-sulphonyl derivatives or phenylthiohydantoins (PTG) [7]. Large peptides and the whole γ-subunit were determined by the automated degradation on a 890C sequencer (Beckman) by using the 122974 program. PTG amino acids were identified by HPLC on an Ultrasphere ODS column (Altex) and eluted with acetonitrile gradient in 0.03 M sodium phosphate (pH 5.4). Amino acid compositions of the protein and peptides were determined on a D-500 amino acid analyzer (Durrum).

3. RESULTS AND DISCUSSION

Determination of the primary structure of transducin was begun with the γ-subunit. Proline was established to be its N-terminal residue. The N-terminal amino acid sequence of the γ-subunit was studied by the Edman procedure and on a sequencer. As a result, the sequence of 34 amino acid residues was determined.

The complete structure of the γ-subunit was deciphered by the block method. Modification of SH-groups was not observed upon treatment of the γ-subunit with iodoacetic acid, therefore the intact protein sample was studied. Hydrolysis of the γ-subunit with *S. aureus* protease and chymotrypsin resulted in 9 and 12 peptides, respectively. The N- and C-terminal cyanogen bromide fragments were also isolated. Determination of amino acid sequences of these peptides made possible their localization in the polypeptide chain. The γ-subunit was found to contain 69 amino acid residues. However, we failed to identify the amino acid residues in positions 35 and 36. They were suggested to be cysteine residues which were not carboxymethylated with iodoacetic acid for one or another reason.

Amino acid analysis of the protein after its oxidation with performic acid confirmed that two cysteine residues were in the polypeptide chain of the γ-subunit. It also appeared that iodoacetamide, in contrast to iodoacetic acid, was capable of carboxymethylating the γ-subunit reduced with dithiothreitol. That is why the *S. aureus* protease hydrolysis of the γ-subunit radioactively labeled with iodo[^{14}C]acetamide was repeated. Two radioactive peptides were isolated by HPLC from the hydrolysate obtained. They were the analogues of the same peptide, Arg 29-Glu 38, but each of them was carboxymethylated at one of two neighboring non-identified residues, which were determined as cysteine residues.

New techniques for ionization of molecules (fast atom bombarding [FAB] and field desorbtion) introduced to mass-spectrometry sharply enhanced the efficiency of this method for the structural

Table 1

Mass numbers of molecular ions of clostripain peptides of γ-subunit

Peptide	*m/e* of ion $[M+H]^+$	*m/e* of ion $[M+2H]^{2+}$
Pro^1-Lys^{13}	1513	757
Pro^1-Lys^{15}	1754	
Leu^{14}-Arg^{29}	1958	979.5
Met^{16}-Arg^{29}	1717	
Met^{30}-Arg^{40}	1342	
Met^{30}-Arg^{40a}	1344	
Asp^{41}-Arg^{46}	810	
Ser^{47}-Lys^{54}	844	422.5
Ser^{47}-Lys^{67}	2339	
Gly^{55}-Lys^{67}	1514	757.5

[a] Reduced peptide form

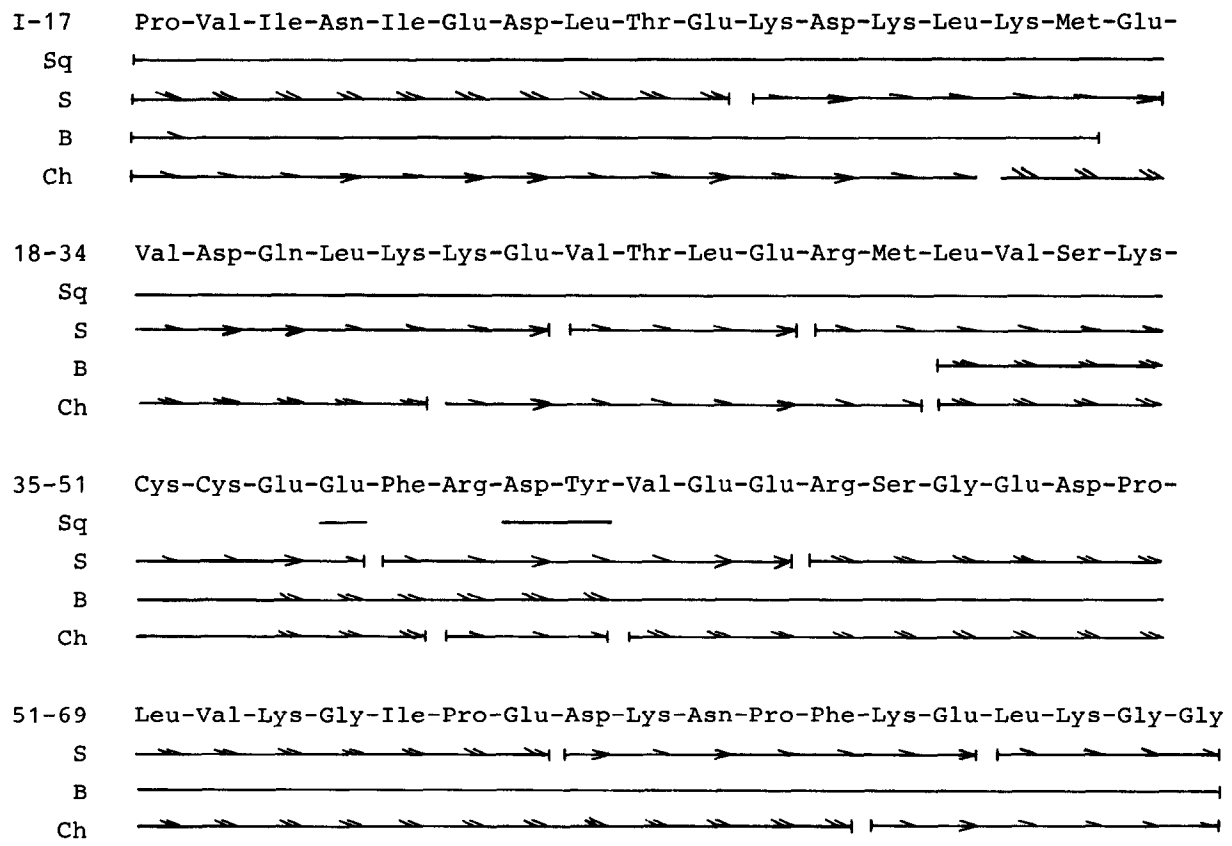

Fig.1. Primary structure of γ-subunit of the GTP-binding protein. Sequence data on individual peptides are indicated as follows: (⟶) sequenced by Beckman sequencer; (⟶) sequenced by the Edman method; S, B, Ch, peptides derived from cleavage with *S. aureus* protease, cyanogen bromide and chymotrypsin, respectively. Sq, sequence of γ-subunit determined by sequencer.

analysis of proteins and peptides. The clostripain hydrolysate of the γ-subunit was analyzed on a ZAB (VG) mass spectrometer by using ionization with FAB. The mass spectrum obtained was mainly composed of molecular ions of clostripain peptides of the γ-subunit. The complete coincidence of molecular masses of the peptides with the expected ones (table 1) supported the established structure.

The complete amino acid sequence of the γ-subunit is shown in fig.1. It consists of 69 amino acid residues (M_r 8008.7) and has the following composition: Asp-6, Asn-2, Thr-2, Ser-2, Glu-12, Gln-1, Pro-4, Gly-4, Met-2, Val-6, Cys-2, Ile-3, Leu-7, Tyr-1, Phe-2, Lys-10, Arg-3.

Noteworthy were the data on the presence of a disulfide bond between two neighboring cysteine residues (35 and 36) in the polypeptide chain of the γ-subunit. Firstly, an intense peak at *m/e* 1342 corresponding to peptide Met 30-Arg 40 with the disulfide bonds as well as a peak at *m/e* 1358 formed as a result of oxidation of the methionine residue in this peptide were present. Secondly, the γ-subunit was not bound to the active thiol-Sepharose 4B which formed the covalent bond to free sulfhydryl groups (the α- and β-subunits are tightly bound to thiol-Sepharose).

An unusual disulfide bond between the neighboring cysteines in the γ-subunit of transducin probably plays a substantial role in supporting its specific conformation. Interestingly, one of the sites of the rhodopsin polypeptide chain exposed into the cytoplasm also contains the sequence Cys-Cys [8]. According to [9], this sequence is accessible to modification after its ir-

radiation; from other data it is thought to form the disulfide bond. Both sequences are proposed to be functionally significant for light signal transfer.

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