Proline- and alanine-rich N-terminal extension of the basic bovine β-crystallin B_1 chains

G.A.M. Berbers, W.A. Hoekman, H. Bloemendal, W.W. de Jong, T. Kleinschmidt* and G. Braunitzer*

Laboratorium voor Biochemie, Universiteit van Nijmegen, Geert Grooteplein Noord 21, 6525 EZ Nijmegen, The Netherlands and **Max-Planck-Institut für Biochemie, Abteilung Proteinchemie, D-8033 Martinsried bei München, FRG*

Received 22 June 1983

The amino acid sequence of the N-terminal region of the two basic bovine β-crystallin B_1 chains has been analyzed. The results reveal that βB_{1b} is derived in vivo from the primary gene product βB_{1a} by removal of a short N-terminal sequence. It appears that the βB_1 chains have the same domain structure as observed in other β- and γ-crystallin chains. They have, however, a very long N-terminal extension in comparison with other β-chains. This extension is mainly composed of a remarkable Pro- and Ala-rich sequence, which suggests an interaction of these structural proteins with the cytoskeleton and/or the plasma membranes of the lens cells.

Protein sequence *Bovine β-crystallin* *N-terminal extension* *Proline- and alanine-rich Domain structure*

1. INTRODUCTION

The crystallins are evolutionary highly conserved structural eye lens proteins, which can be divided into 4 classes: α-, β-, γ- and δ-crystallin [1]. The β- and γ-crystallins show considerable sequence homology [2–6]. The 3-dimensional structure of the bovine γ-crystallin II chain has been determined [7], and a similar tertiary fold has been predicted for two β-crystallin chains [8,9]. The β- and γ-crystallins are built up of two domains, which show considerable sequence similarity, suggesting an intragenic duplication in the ancestral gene of these proteins. The oligomeric β-crystallins differ mainly from the monomeric γ-crystallins by the presence of N- and C-terminal extensions [8,9].

Bovine β-crystallins comprise 6 or more primary gene products of M_r between 23000 and 33000, which can associate to oligomers, varying from dimers and trimers (β_{low}) to octamers (β_{high}) [10]. Two highly basic chains, βB_{1a} and βB_{1b} with M_r 33000 and 31000, respectively, are characteristic for β_{high} [11]. βB_{1a} is a primary gene product, from which βB_{1b} arises by post-translational modification [10–12], most probably a proteolytic step [13]. βB_{1a} is also one of the few crystallins besides α-crystallin A_2, which strongly associates with the lens plasma membranes [13].

Here we present the characteristic amino acid sequence of the N-terminal region of these two basic β-crystallin subunits.

2. MATERIALS AND METHODS

β-Crystallin was isolated from calf lens cortices, and the polypeptides β_{1a} and βB_{1b} were purified as in [10,14].

2.1. *Limited proteolysis with trypsin*

Limited digestion of βB_{1a} and βB_{1b} was carried out at 37°C in 0.1 M ammonium bicarbonate (pH 8.0) at a protein concentration of 1 mg/ml, using

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

0.1% (w/w) trypsin (Worthington, TR TPCK). Trypsin inhibitor (Sigma) was used to stop the digestion. Aliquots were taken at different times and analyzed by slab gel electrophoresis (13% polyacrylamide, 0.35% methylene-bisacrylamide and 0.1% SDS) [15].

2.2. *Characterization of the βB_1-chains*

Protein chemical methods were used as in [2]. The chains were subjected to end-group analysis with dansylchloride. After *S*-β-aminoethylation of βB_1 and cleavage with CNBr, the resulting fragments were separated on a column of Sephadex G-75 sf in 20% HAc at a flow rate of 0.1 ml/min. The N-terminal CNBr-fragments were digested with trypsin and chymotrypsin (Calbiochem A grade). Peptides were isolated and analyzed after high-voltage paper electrophoresis at pH 6.5, followed in the second dimension by descending chromatography. In some cases the neutral zone was submitted to reelectrophoresis at pH 3.8.

2.3. *Liquid phase sequencing*

Sequence analyses were obtained by automatic Edman-degradation with a Beckman Model 890 C Sequencer (Palo Alto CA). The βB_{1b}-chain and the Pro- and Ala-rich peptide, which was modified with Reagent IV [16] at lysine, were sequenced with a 0.25 M Quadrol program as in [17]. In each cycle a second cleavage step was performed because of the high proline content. The conversion was automatic, in an auto-convertor Sequemat P-6 (Kontrol Technics, GmbH, Eching). PTH-amino acids were identified by thin-layer chromatography in 3 consecutive systems [18,19], and by high pressure liquid chromatography (HPLC, 1084 B, Hewlett Packard, Böblingen).

2.4. *Numbering of residues*

In order to facilitate comparison with other β- and γ-crystallins, we assigned position no. 1 to the βB_1 residue corresponding with the first residue of the γ-crystallin II chain, and indicated the N-terminal residues by negative numbers from this residue backward, as in [8].

3. RESULTS AND DISCUSSION

The effect of limited tryptic proteolysis on βB_{1a} and βB_{1b} is demonstrated in fig.1. A small fragment is almost immediately cleaved off from the βB_{1a}-chain (fig.1A). When the limited proteolysis was stopped after 5 min with trypsin inhibitor, and the resulting digest subsequently analyzed by peptide mapping and amino acid analysis, this fragment was found to contain the peptides T -57/-52 and T -51/-36 (fig.2). Continued proteolysis resulted in the cleavage of a second fragment (fig. 1A), which contained only peptide T -35/-8 (fig.2), although some complete digestion of the βB_1-chain also takes place at prolonged digestion. Under these conditions, peptide T -51/-36 is cleaved, yielded peptides T -51/-38 and T -37/-36, and reelectrophoresis of the neutral zone at pH 3.8 was necessary to separate the peptides T -51/-38 and T -57/-52.

When the βB_{1b}-chain was subjected to limited tryptic proteolysis for 30 min (fig.1B), the large peptide T -35/-8 could again be identified as well as the peptides T -46/-38 and T -37/-36 (fig.2). The remaining polypeptide chain has a M_r around 25000 (fig.1), which corresponds to that of the other β-crystallins.

End-group determination of the βB_{1b}-chain revealed proline as N-terminus, and no result was obtained in the case of βB_{1a}, indicating that βB_{1a} might be N-terminally blocked like βB_p [2]. Automatic sequence analysis was thus started with the βB_{1b}-chain. Unfortunately, two N-termini were found, Ala-46 and Pro-43 (fig.2) in a ratio of 1:2, which made the identification of the PTH-amino acids very difficult after about 20 cycles. Therefore, the peptide T -35/-8 was isolated from preparative peptide maps and submitted to automatic sequence analysis. This revealed the remarkable presence of an 8-fold repeat of the sequence Pro–Ala (fig.2).

CNBr-treatment of the two proteins, followed by SDS-gel electrophoresis, allowed the identification of the N-terminal CNBr-fragments, because they still possess the difference in length (M_r 14000 for βB_{1a}-CB1 and M_r 12000 for βB_{1b}-CB1), while all other fragments are similar in molecular mass. End-group determination of these isolated N-terminal fragments gave the same results as for the whole proteins: Pro for βB_{1b} and no free N-terminus for βB_{1a}. After tryptic digestion of the N-terminal CNBr-fragment of βB_{1a} the same peptides could be recovered as identified by limited tryptic

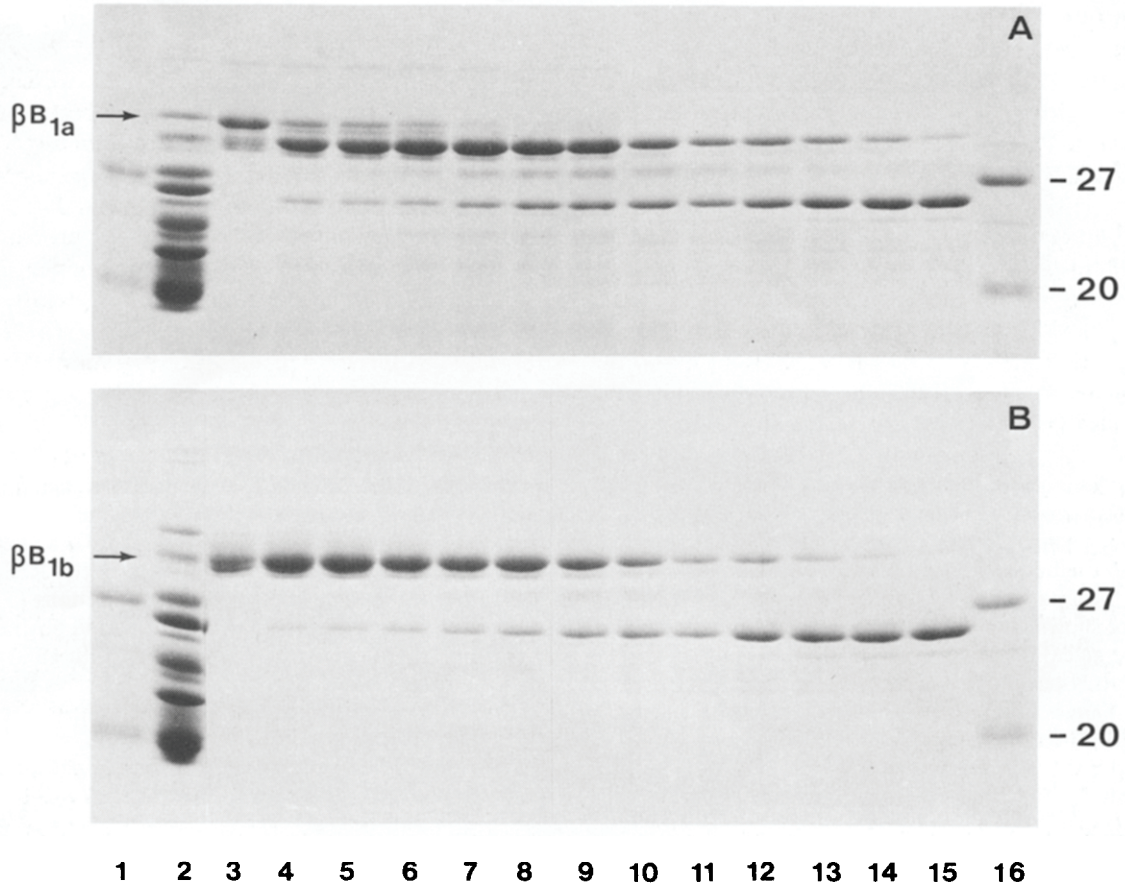

Fig.1. SDS gel electrophoresis of the limited tryptic digest of βB_{1a} (A) and βB_{1b} (B). Samples are: (1) marker proteins (α-chymotrypsinogen, M_r 27000; α-crystallin A, M_r 20000); (2) total soluble calf lens protein; (3) starting material; (4) after 1 min limited proteolysis; (5) 3 min; (6) 5 min; (7) 10 min; (8) 15 min; (9) 20 min; (10) 30 min; (11) 45 min; (12) 60 min; (13) 90 min; (14) 120 min; (15) 180 min; (16) marker proteins. The double band in the starting material of lane B3 is due to the N-terminal heterogeneity of βB_{1b} (cf. fig.2).

proteolysis,and in addition peptides which belong to the N-terminal domain, considering the homology with other β- and γ-crystallins (fig.2). The same tryptic peptides could be obtained from the N-terminal CNBr-fragment of βB_{1b}, obviously with the exception of the N-terminal peptides T -57/-52 and T -51/-38. The peptide T -43/-38 was found to be the N-terminus of the CNBr-fragment of βB_{1b}. Apparently, the Asn–Pro bond at position -44/-43 is easily broken by acid treatment, and the original N-terminus of βB_{1b} in vivo will probably be Ala-46.

Chymotryptic digestion of the N-terminal CNBr-fragment of βB_{1a} supplied all the necessary overlaps (fig.2). It is not sure whether the N-terminus of βB_{1a} is located at position -57. The difference in charge between βB_{1a} and βB_{1b}, as visualized on a 2-dimensional gel [10], cannot be accounted for by peptides T -57/-52 and -51/-47, because they are both neutral. We were, however, unable to detect any additional peptides, whether or not blocked, in the tryptic and chymotryptic digests of the N-terminal CNBr-fragment of βB_{1a}.

The sequence homology between the N-terminal domain of the βB_1-chains and those of bovine βB_p [2] and mouse $\beta 23$ [9] is about 50%, and compared to bovine γ-crystallin II [4] about 25%. This seems to warrant the presence of the same tertiary folds in these domains. In contrast, fig.2 shows that the N-terminal extension of βB_1 is totally different

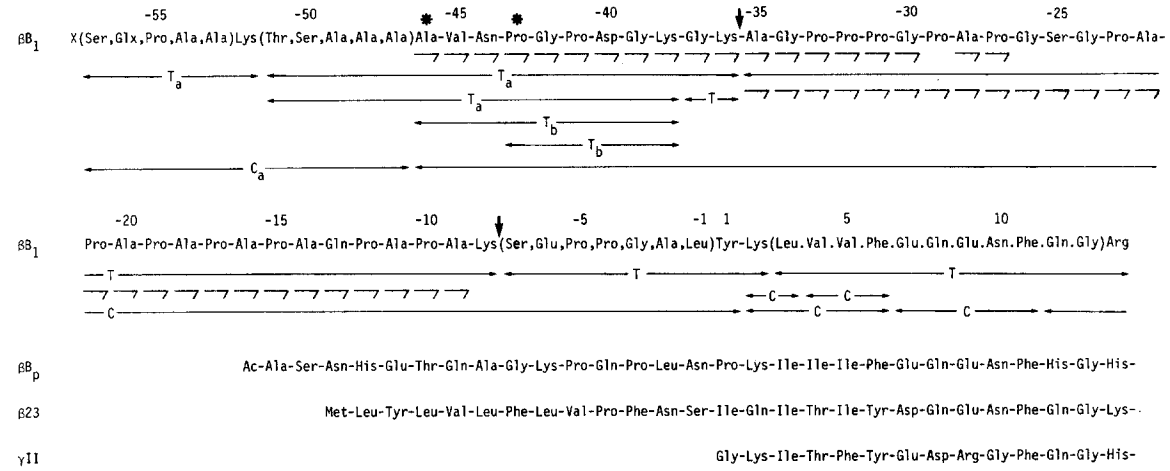

Fig.2. Amino acid sequence of the N-terminal region of the bovine β-crystallin B_1-chains. Sequences were determined by automatic liquid phase sequencing (→) of total βB_{1b}-chain and of T -35/-8, and on the basis of amino acid compositions of tryptic (T) and chymotryptic (C) peptides obtained from the N-terminal CNBr-fragments of βB_{1a} and βB_{1b}. Peptides indicated as T_a, C_a and T_b are only found in the digests of the βB_{1a}- and βB_{1b}-chains, respectively. Residues 1–14 form part of the N-terminal domain; the remaining residues of the N-terminal CNBr-fragments (positions 15–70) are not shown. For comparison the corresponding N-terminal regions of bovine βB_p [2], mouse $\beta 23$ [9] and bovine γII [4] are given. The two cleavage positions in the βB_{1a}-chain, found by limited proteolysis, are marked with an arrow. The two N-termini of the βB_{16}-chain, identified by sequence analysis, are marked with an asterisk. X indicates that the βB_{1a}-chain is blocked; it is however uncertain whether peptide T -57/-52 is the N-terminal one. The symbols (.) and (,) are used as in [27].

from that of the other two known β-chains. This may confer characteristically different functional properties upon the different β-chains. Interestingly, the βB_1 sequence of the rat, as determined by cDNA analysis, shows a very high homology (some 95%) in the N-terminal domain, while the N-terminal extension is again completely different from that of bovine βB_1 (J.T. den Dunnen, personal communication).

It should be noted that peptides T -35/-8 and C -46/+1 are readily soluble, despite their size and very high alanyl and prolyl content, and exhibit a remarkably hydrophilic behavior in chromatography. It may indeed be expected from studies on model peptides [20,21] that the repeated Ala–Pro sequences will induce extraordinary conformational properties. This might also explain the relatively great decrease in apparent M_r, which is observed on SDS-gel when the peptide T -35/-8 is cleaved off by limited tryptic proteolysis (fig.1). The Pro- and Ala-rich sequence is apparently not able to bind the normal amount of SDS molecules because of its conformation; therefore the βB_{1a}- and βB_{1b}-chains may be relatively retarded in SDS gel electrophoresis.

The putative hydrophobic N-terminal extension of mouse $\beta 23$ resembles a membrane anchor sequence, and might link this chain to the lens membranes or, alternatively, be involved in the association with other β-crystallin chains [9]. The N-terminal extension of the βB_1-chains, although not of such a hydrophobic nature, may have a similar role, as supported by the tight association of these chains with the lens membranes [13] and their oc-

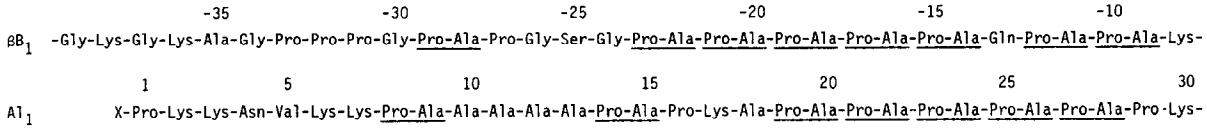

Fig.3. Comparison of the Pro- and Ala-rich sequences of the amino-terminal regions of the bovine β-crystallin B_1-chains and of the alkali light chain A_1 ($A1_1$) from rabbit myosin [22]. The underlining emphasizes the Pro–Ala repeats in both sequences.

currence in the oligomeric β_{high}-aggregates. In this respect the similarity with the N-terminal region of the alkali light chain A_1 from rabbit myosin [22] (fig.3) is suggestive of comparable properties. The N-terminal segment of $A1_1$ has been shown to interact with the C-terminal segment of actin [23–25]. Preliminary crosslinking experiments failed to reveal such an association between βB_1 and actin, which is present in the lens cytoskeleton [26]. We are currently searching for specific interactions between the N-terminal extension of the βB_1 chains and other membrane or cytoskeletal proteins.

ACKNOWLEGEMENTS

We are grateful to Ms Marlies Versteeg for performing amino acid analyses, and to Dr Theo Cuypers for some preliminary data on βB_1. We wish to thank Mr Claus Krombach, Ms Barbara Schrank and Ms Lucia Gößl for their excellent sequence analyses. G.A.M. Berbers acknowledges a FEBS short-term fellowship. This investigation was carried out under the auspices of the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

[1] Bloemendal, H. (1983) in: Molecular and Cellular Biology of the Eye Lens (Bloemendal, H. ed) pp.1–241, Wiley, New York.
[2] Driessen, H.P.C., Herbrink, P., Bloemendal, H. and de Jong, W.W. (1981) Eur. J. Biochem. 121, 83–91.
[3] Inana, G., Shinohara, T., Maizel, J.V. jr and Piatigorsky, J. (1982) J. Biol. Chem. 257, 9064–9071.
[4] Croft, L.R. (1972) Biochem. J. 128, 461–470.
[5] Moormann, R.J.N., den Dunnen, J.T., Bloemendal, H. and Schoenmakers J.G.G. (1982) Proc. Natl. Acad. Sci. USA 79, 6876–6880.
[6] Tomarev, I.S., Krayev, A.S., Skryabin, K.G., Bayev, A.A. and Gause G.G. jr (1982) FEBS Lett. 146, 315–318.
[7] Blundell, T., Lindley, P., Miller, L., Moss, D., Slingsby, C., Tickle, I., Turnell, B. and Wistow, G. (1981) Nature 281, 771–777.
[8] Wistow, G., Slingsby, C., Blundell, T., Driessen, H.P.C., de Jong, W.W. and Bloemendal, H. (1981) FEBS Lett. 133, 9–16.
[9] Inana, G., Piatigorsky, J., Norman, B., Slingsby, C. and Blundell, T. (1983) Nature 302, 310–315.
[10] Berbers, G.A.M., Boerman, O.C., Bloemendal, H. and de Jong, W.W. (1982) Eur. J. Biochem. 128, 495–502.
[11] Vermorken, A.J.M., Herbrink, P. and Bloemendal, H. (1977) Eur. J. Biochem. 78, 617–622,
[12] Zigler, J.S., jr (1978) Exp. Eye Res. 26, 537–546.
[13] Bloemendal, H., Hermsen, T., Dunia, I. and Benedetti, E.L. (1982) Exp. Eye Res. 35, 61–67.
[14] Herbrink, P. and Bloemendal, H. (1974) Biochim. Biophys. Acta 336, 370–382.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Braunitzer, G., Schrank, B., Ruhfus, A., Petersen, S. and Petersen U. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1730–1732.
[17] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[18] Braunitzer, G., Schrank, B. and Ruhfus, A. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 1584–1590.
[19] Braunitzer, G., Schrank, B., Stangl, A. and Scheithauer, U. (1978) Hoppe-Seyler's Z. Phys. Chem. 359, 137–146.
[20] Isemura, T., Asakura, I., Shibata, S., Isemura, S., Saitoli, E. and Sanada, K. (1983) Int. J. Pept. Protein Res. 21, 281–287.
[21] Lisowski, M., Siemion, I.Z. and Sobczyk, K. (1983) Int. J. Pept. Protein Res. 21, 301–306.
[22] Frank, G. and Weeds, A.G. (1974) Eur. J. Biochem. 44, 317–334.
[23] Prince, H.P., Trayer, H.R., Henry, G.D., Trayer, I.P., Dalgarno, D.C., Levine, B.A., Cary, P.O. and Turner, C. (1981) Eur. J. Biochem. 121, 213–214.
[24] Winstanley, M.A. and Trayer, I.P. (1979) Biochem. Soc. Trans. 7, 703–704.
[25] Sutoh, K. (1982) Biochemistry 21, 3654–3661.
[26] Kibbelaar, M.A., Ramaekers, F.C.S., Ringens, P.J., Selten-Versteegen, A.M.E., Poels, L.G., Jap, P.H.K., van Rossum, A.L., Feltkamp, T.E.W. and Bloemendal, H. (1980) Nature 285, 506–508.
[27] Dayhoff, M.O. (1978) Atlas of Protein Sequence and Structure, vol.5, suppl. 3, National Biomedical Research Foundation, Silver Springs OH.