Volume 221, number 1, 73-76 FEB 05052 August 1987

α-Actinin and spectrin have common structural domains

Veli-Matti Wasenius[+], Outi Närvänen, Veli-Pekka Lehto and Matti Saraste*

Departments of Pathology, [+]Oncology and **Medical Chemistry, University of Helsinki, 00290 Helsinki, Finland*

Received 3 July 1987

The α- and β-subunits of spectrin are made of repeated homologous units of 106 residues. In the recently reported partial sequence of the chicken non-muscle α-actinin, a repetitive sequence homologous to the internal repeat in spectrin occurs several times. Both spectrin and α-actinin are components of the cytoskeletal network, the integrity of which is based on multiple and complex interactions. We suggest that the shared domain structure indicates common structural principles or interactions of spectrin and α-actinin and reflects their common evolution.

Spectrin; α-Actinin; Sequence homology; Actin binding

1. INTRODUCTION

Spectrin is the major component of the so-called membrane skeleton and is present in practically all types of animal cells [1]. Spectrin forms dimers containing 240-kDa α- and 220-kDa β-subunits. Two heterodimers form tetramers which in erythroid cells are capable of forming larger oligomers [2]. α-Actinin is a dimer of identical 110-kDa monomers [3] and can be found in muscle and non-muscle cells [3]. Both of these proteins are rod-like molecules that bind F-actin [4].

2. EXPERIMENTAL

We are currently sequencing the cDNA of chicken brain spectrin. In the course of this study we compared the predicted amino acid sequence of the brain α-spectrin with the recently published sequence of chicken and *Dictyostelium discoideum* α-actinins [5,6]. This was carried out by using a computer program DIAGON where scoring is based on a mutation data matrix [7,8]. The span used in calculation was 21 and the dots shown have a score 245.

Correspondence address: V.-M. Wasenius, Department of Pathology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland

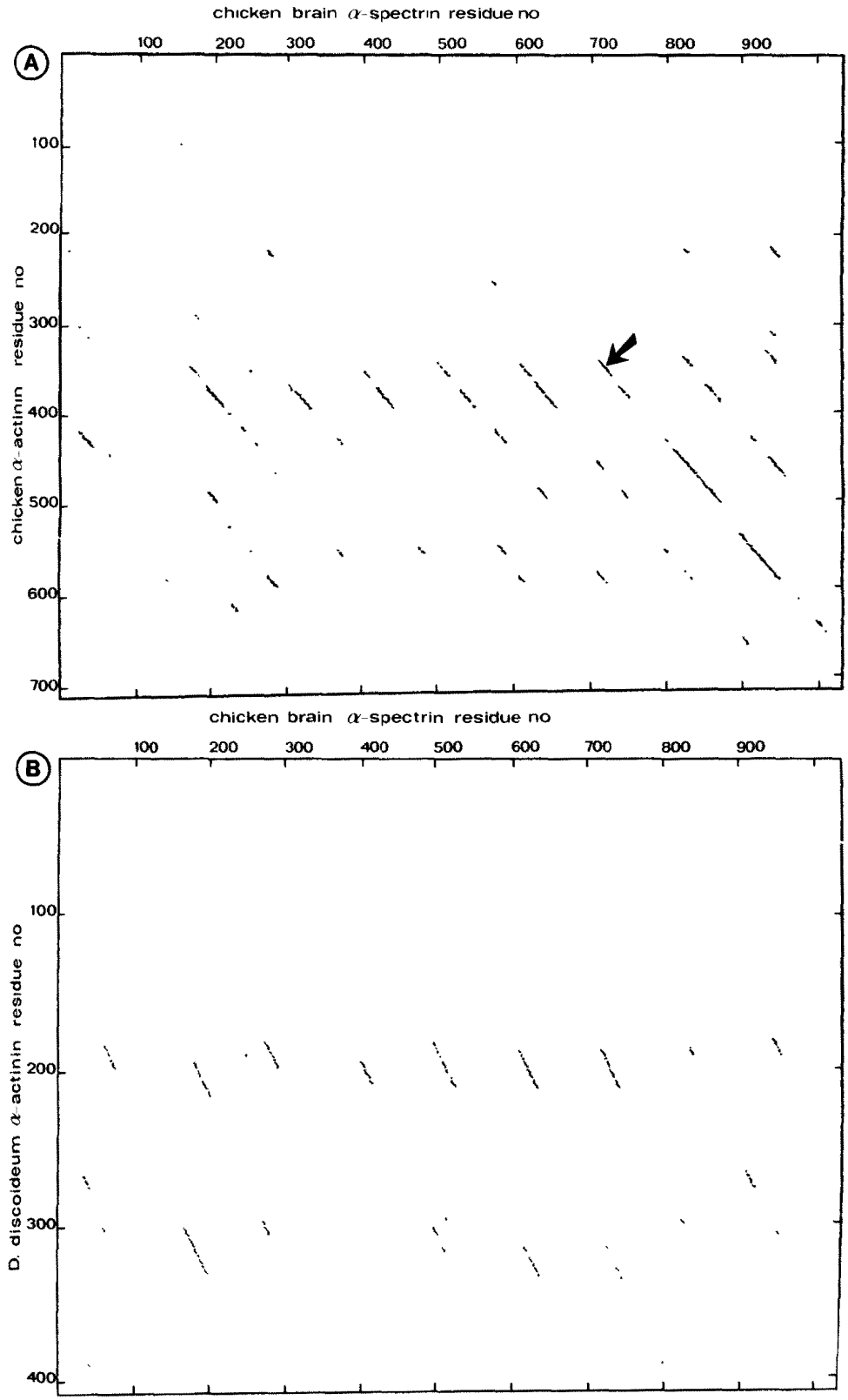
3. RESULTS

Fragments of the deduced amino acid sequences of α- and β-spectrins [9] and *D. discoideum* [6] and chicken fibroblast α-actinin [5] have been published. In this study we also utilized about 3.1 kb of our unpublished sequence data on chicken brain α-spectrin. This sequence extends in both the 5′- and 3′-direction of our previously published data [9].

Comparison of the segment containing approximately nine 106-amino-acid-long units of the chicken brain α-spectrin with the recently reported primary structure of the chicken α-actinin (comprising 86% of the full-length sequence [5]) reveals striking homology between the sequences (fig.1A); a part of each spectrin unit finds homologous sequences in the C-terminal half of the α-actinin sequence. A similar, but weaker homology was seen when the comparison was made between spectrin and *D. discoideum* α-actinin (comprising about ⅓ of the full-length sequence [6]) (see fig.1B).

In fig.2, the homologous sequences of chicken brain spectrin and chicken α-actinin are aligned. A

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50


chicken brain α-spectrin residue no
100
200
300
400
500
600
700
800
900
A
chicken α-actinin residue no
100
200
300
400
500
600
700
chicken brain α-spectrin residue no
100
200
300
400
500
600
700
800
900
B
D. discoideum α-actinin residue no
100
200
300
400

```
698               715                   735                   755                   775        785
QFHWKADVVE SWIGEKENSL KTDDYGRDLS SVQTLLTKQE TFDAGLQAFQ QEGIANITAL KDQLLAAKHI QSKAIEVRHA SLMKRWNQLL
 *| ** | * **   **  *    **      *   |* *          *   *| | |* *|    *  |     *    |*    |   * |*
KFRQKASIHE SWTDGKEAML QQKDYETATL SEIKALLKKH EAFESDLAAH QDRVEQIAAI AQELNELDYY DSPSVNARCQ KICDQWDNLG
340               357                   377                   397                   417        427

788               805                   825                   845                   865   870
ANSAARKKKL LEAQEHFRKV EDLFLTFAKK ASAFNSWFEN AEEDLTDPVR CNSLEEIKAL REAHDAFRSS LSSAQADFNQ LAELD-----
* |  *|  *          |   * * |**| *  ** * *  * *** *       |  *  *   **| *| | *  *  | |  || |
ALTQKRREAL ERTEKLLETI DQLYLEYAKR AAPFNNWMEG AMEDLQDTPI VHTIEEIQGL TTAHEQFKAT LPDADKERQA ILGIHNEVSK
430               447                   467                   487                   507        517

871               888                   908                   928                   948        958
--RQIKSFRV ASNPYTWFTM EALEETWRNL QKIIKERELE LQKEQRRQEE NDKLRQEFAQ HANAFHQWIQ ETRTYLLDGS CMVEESGTLE
     |   |  |****  *    |   *  |   ||  *|   *  *  **   *||**       **|   ***      |   *      |
IVQTYHVNMA GTNPYTTITP QEINGKWEHV RQLVPRRDQA LMEEHARQQQ NERLRKQFGA QANVIGPWIQ TKMEEIGRIS IEMHGTLEDQ
520               537                   557                   577                   597        617

961               978                   998                  1017
SQLEATKRKH QEIRAMRSQL KKIEDLGAAM EEALILDNKY TEHSTVGWRS SGTSLTSWE
      |      *   |  |   |*        **** ***  *  |    *      * |
LNH--LRQYE KSIVNYKPKI DQLEGDHQQI QEALIFDNKH TNYTMEHIRV GWEQLLTTI
610               625                   645                   664
```

Fig.2. Alignment of chicken brain α-spectrin and chicken α-actinin sequences in the region showing the highest degree of homology (match marked by an arrow in the diagonal plot in fig.1A). The standard single letter code is used. Asterisks denote identical and vertical lines homological residues. The homology arises from using structurally related amino acids of the following families: A, I, L, V; Q, N; F, Y, W; P; E, D; H, R, K; S, T; G; C; M. Arrows indicate the positions of the tryptophans which are highly conserved in spectrin repeats thus far sequenced [9–12].

gap is introduced into the spectrin sequence to obtain optimal alignment. The consensus sequence seems to start just prior to the tryptophan residue that is shown to be invariant in the 106-amino-acid repeats of both erythroid and non-erythroid spectrins [9–12]. The similarity of deduced amino acid sequences, when optimal alignment is used, is 25%. When conservative changes are counted, the degree of homology is 40%. Especially significant homology is found in the regions where the invariant tryptophan residues of spectrin occur [9].

Fig.3 shows schematically the structural relationship between spectrin and chicken and *D. discoideum* α-actinins. Amino terminal halves of α-actinins, harboring the actin-binding site [13], are almost identical except for a short stretch in *D. discoideum* α-actinin with a spectrin-like sequence. The homology abruptly ends at positions 320 (chicken α-actinin) and 280 (*D. dictyostelium* α-actinin) after which there is a 20-amino-acid-long

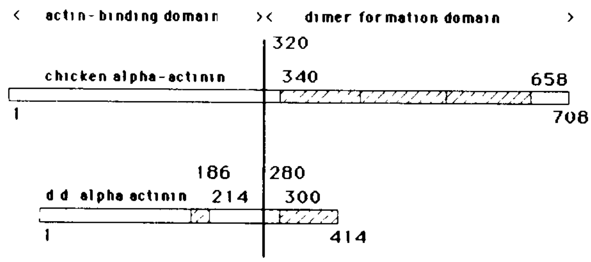


Fig.3. Schematic representation of the spectrin-like sequences in chicken and *D. discoideum* α-actinins. The numbering is based on the sequences published in [5] and [6]. Spectrin-like sequences are marked by hatched boxes. The thick cross bar shows the position at which the N-terminal identity of the two α-actinins ends.

stretch in which the sequences diverge. In these stretches no spectrin-like sequences can be found. From amino acid 340 onwards in chicken α-actinin there are three repeats which show a high degree of

Fig.1. Diagonal plot of amino acid matches between chicken brain α-spectrin and chicken α-actinin (A) and between brain α-spectrin and *D. discoideum* α-actinin (B). Arrow marks the match which is shown in sequence alignment in fig.2.

homology with the 106-amino-acid repeats of spectrin. This homology ends at about 50 amino acids from the carboxy-terminus of α-actinin. In *D. discoideum* α-actinin the homology with spectrin is lower.

4. DISCUSSION

Our results show that chicken non-muscle α-actinin contains a repetitive, spectrin-like sequence. It maps to the C-terminus which in earlier studies has been shown to be responsible for the formation of α-actinin dimers [13]. In contrast, no homology was noticed between the spectrin sequence and the N-terminal, actin-binding end of α-actinin [13,14]. A similar but weaker homology was found between *D. discoideum* α-actinin and chicken spectrin. The degree of homology could not be reliably assessed, however, due to the paucity of sequence data on *D. discoideum* α-actinin. On the other hand, the actin-binding N-terminal halves in both α-actinins showed a virtual identity with each other (not shown).

There are no sequence data yet available for the N-terminus of brain α-spectrin. However, from the present sequence data on erythroid and non-erythroid spectrins and on the basis of the structural similarities of erythroid and non-erythroid spectrins [10–12], we can infer that there are no sequences present in spectrins that are homologous to the actin-binding domain of α-actinin. Hence, α-actinin must be considered a hybrid molecule composed of an actin-binding domain and an internally repeated structure which in spectrin is present as the major structural motif.

The similarities in the primary structure of α-actinin and α-spectrin have important ramifications to the functions of these two cytoskeletal proteins. In spectrin, the 106-amino-acid repeat has been shown to have a predominantly α-helical structure, and the repeated motif may be considered as the basis of its rod-like structure [9–12]. These helical repeats are also thought to facilitate formation of dimers from α- and β-spectrin [1,11]. Thus it is logical to consider that the homologous portion in α-actinin also serves a similar function. The spectrin-like repeats in α-actinin may also carry membrane-anchoring functions since both spectrin and α-actinin have been shown to be in close contact with the cytoplasmic face of plasma membrane [1,15,16].

ACKNOWLEDGEMENT

This work was supported by the Academy of Finland.

REFERENCES

[1] Marchesi, V.T. (1985) Annu. Rev. Cell Biol. 1, 531–561.
[2] Morrow, J.S., Speicher, D.W., Knowles, W.J., Hsu, C.J. and Marchesi, V.T. (1980) Proc. Natl. Acad. Sci. USA 77, 6592–6596.
[3] Pollard, T.D. and Cooper, J.A. (1986) Annu. Rev. Biochem. 55, 987–1035.
[4] Burridge, K. and Feramisco, J.R. (1981) Nature 294, 565–567.
[5] Baron, M.D., Davison, M.D., Jones, P., Patel, B. and Critchley, D.R. (1987) J. Biol. Chem. 262, 2558–2561.
[6] Witke, W., Schleicher, M., Lottspeich, F. and Noegel, A. (1986) J. Cell Biol. 103, 969–979.
[7] Staden, R. (1982) Nucleic Acid Res. 10, 2951–2961.
[8] McLachlan, A.D. (1971) J. Mol. Biol. 61, 409–424.
[9] Wasenius, V.-M., Saraste, M., Knowles, J., Virtanen, I. and Lehto, V.-P. (1985) EMBO J. 4, 1425–1430.
[10] Speicher, D.W., Davis, G. and Marchesi, V.T. (1983) J. Biol. Chem. 258, 14938–14947.
[11] Speicher, D.W. and Marchesi, V.T. (1984) Nature 311, 177–180.
[12] Birkenmeier, C.S., Bodine, D.M., Repasky, E.A., Helfman, D.M., Hughes, S.H. and Barker, J.E. (1985) Proc. Natl. Acad. Sci. USA 82, 5671–5678.
[13] Arakawa, N., Goll, D.E., Robson, R.M. and Kleese, W.C. (1985) J. Cell Biochem. suppl. 9 (part B), 7.
[14] Wallraff, E., Schleicher, M., Modersitzki, M., Rieger, D., Isenberg, G. and Gerisch, G. (1986) EMBO J. 5, 61–67.
[15] Lehto, V.-P. and Virtanen, I. (1983) J. Cell Biol. 96, 703–716.
[16] Lazarides, E. and Burridge, K. (1975) Cell 6, 289–298.