View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Volume 300, number 2, 123–126 FEBS 10859 March 1992
© 1992 Federation of European Biochemical Societies 00145793/92/$5.00

The vegetalizing factor

A member of the evolutionarily highly conserved activin family

Heinz Tiedemann[a], Friedrich Lottspeich[b], Michael Davids[a], Sigrun Knöchel[c], Peter Hoppe[a] and Hildegard Tiedemann[a]

[a]*Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33, Germany,* [b]*Max-Planck-Institut für Biochemie, Am Klopferspitz 18A, D-8033 Martinsried, Germany* and [c]*Abteilung Biochemie, Universität Ulm, Oberer Eselsberg, D-7900 Ulm, Germany*

Received 9 December 1991; revised version received 11 February 1992

The mesoderm and endoderm inducing vegetalizing factor was partially sequenced after BrCN cleavage. A sequence which is highly conserved in activin A near the C-terminal end was identified. This shows that the factor belongs to the activin family. The activins are not confined to embryos and gonads, but widely distributed in other tissues like calf kidney and calf liver. Functional aspects are discussed.

Embryonic induction; Vegetalizing factor; Activin A; Chicken embryo; Calf kidney; Calf liver

1. INTRODUCTION

Chemical factors are involved in the determination of organ pattern in vertebrate embryos, which takes place during the very early stages of embryonic development. The factors are protein in nature. An electrophoretically homogeneous factor, which was isolated from chicken embryos, induces endodermal and mesodermal tissues [1–3]. The factor has been called vegetalizing factor, because these tissues are formed in the vegetal half of the embryo. The factor has an estimated molecular mass of 26 kDa, an isoelectric point at about pH 8.0 and is dissociated into two subunits of 13 kDa after the reduction of disulfide bonds. Reduction completely inactivates the factor. It has recently been shown that the transforming growth factors β1 and β2 (TGF β's), which are related to the vegetalizing factor in their chemical properties, also induce mesodermal organs [4,5]. This suggested that the vegetalizing factor could be related to the TGF-β superfamily of proteins, but the inducing activity of the TGF's is much lower. In 1989, Asashima showed that activin A has a mesoderm inducing activity similar to the vegetalizing factor [6]. Activin A, a member of the TGF-β superfamily of proteins, was originally known as a gonadal protein which stimulates the release of pituitary follicle-stimulating hormone (FSH [7,8]). It is identical with the erythroid differentiation factor (EDF), which is capable of inducing Friend erythroleukemia cells into more mature hemoglobin producing erythroid cells [9]. Further experiments have shown that the vegetalizing factor has the same erythroid differentiation activity as recombinant EDF [3] and that the factor is, like activin A (EDF), inhibited by follistatin [10], a protein which possesses activin-binding activity [11]. In this communication we describe a partial sequence of the chicken vegetalizing factor. Similar factors have been isolated from other sources. Their chemical properties are compared.

Correspondence address: H. Tiedemann, Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33, Germany.

2. MATERIALS AND METHODS

2.1. *Isolation and biological tests of the inducing factors*

The vegetalizing factor was isolated from 1000 g batches of 11-day-old chicken embryo trunks as described previously [2,3]. Calf kidney and calf liver were immediately frozen in dry ice. After the extraction of these tissues 800 g batches were homogenized with 4000 ml 96% ethanol, 80 ml 92% HCl and 1200 ml distilled water. Columns with Eurosil Bioselect 300-C18-5 μm (Knauer, Berlin) were used for the first RP-HPLC. SDS-polyacrylamide gel electrophoresis was carried out as described [12].

The inducing activity was tested by the implantation method on early gastulae of *Triturus alpestis* [13–15] or on isolated ectoderm from middle to late blastula stages of *Xenopus laevis* [16]. After inspection under the dissection microscope, the explants and embryos were fixed in Bouin solution and the histological sections examined.

2.2. *Amino acid sequence analysis of the vegetalizing factor*

1 μg of pure vegetalizing factor with 5 μg dithioerythritol was incubated with 200 μl 10% cyanogen bromide (w/v) in 70% formic acid for 5 h at room temperature in the dark. After dilution with destilled water and evaporation to about 50 μl the cleavage mixture was applied onto reversed phase HPLC. A Supersphere Select B column (2 × 125 mm, Merck, Darmstadt) was used and a gradient from 0 to 90% acetonitril in 0.1% trifluoroacetic acid was applied at a flow rate of

0.3 ml/ min. Detection was performed at 206 nm. Amino acid sequence analysis was done using a gas phase sequencer 477A equipped with an on-line 120A PTH amino acid analyser (both Applied Biosystems, Weiterstadt) according to the instructions of the manufacturer.

3. RESULTS AND DISCUSSION

The vegetalizing factor was extracted from chicken embryos with acid–ethanol and purified by extraction with phenol, chromatography on Sephadex G100, isoelectric focusing and four consecutive steps of reversed phase (RP)-HPLC as previously described (see [2,3]). A direct N-terminal sequence analysis revealed that the factor is blocked at the amino terminal end. The factor was therefore subjected to cyanogen bromide cleavage and the peptides separated by RP-HPLC. At 30% acetonitrile a peptide was eluted which gave the amino acid sequence Leu-Tyr-Tyr-Asp-Asp-Gly-Gln-Asn-(Ile)-Ile-?-Lys. The sequence Met-Leu-Tyr-Tyr-Asp-Asp-Gly-Gln-Asn-Ile-Ile-Lys-Lys is part of the activin A (EDF) sequence near the C-terminal end [9]. It is conserved in activin A isolated from different species, whereas in activin B four amino acids of this sequence (Tyr⇌Phe; Gly⇌Glu; Gln⇌Tyr; Ile⇌Val) are replaced [17]. The closely related DPP-C protein, which contributes to the determination of dorsal structures in *Drosophila* embryos [18], and the bone morphogenetic proteins (BMP's) have a homologous region, which includes the first six amino acids, in common with the activin A partial sequence. In the homologous region two amino acids are replaced in the DPP-C protein (Tyr⇌Leu; Asp⇌Asn [19]) and in the BMP's (Tyr⇌Leu; Asp⇌Glu [20]). Transcripts of the BMP4 gene are present in amphibian oocytes and very early developmental stages [21]. The activin A partial sequence is not at all found in the TGF-βs [22]. This shows that the vegetalizing factor is either activin A or an activin A homologue.

By the same procedure as used for the purification of the vegetalizing factor from chicken embryos, mesoderm inducing factors were extracted from calf liver and calf kidney. These factors are eluted from Sephadex G100 columns in the same molecular weight range (24–28 kDa) as the chicken factor (Fig. 1). The inducing activity of these fractions is only slightly lower than the inducing activity of the similar fraction from chicken embryos (Table I). The fraction from liver induces (besides trunk and tail structures which contain muscle, notochord, nephric tubules and blood cells) hindheads

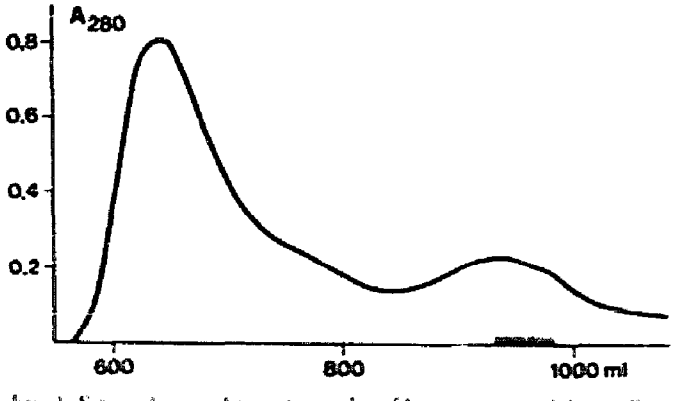

Fig. 1. Size exclusion chromatography of liver protein. Column, Sephadex G100, 100 × 5.4 cm; eluent, 1 M acetic acid/HCl/6 M urea (pH 2.5); flowrate, 50 ml/h at 4°C; bar, position of inducing activity.

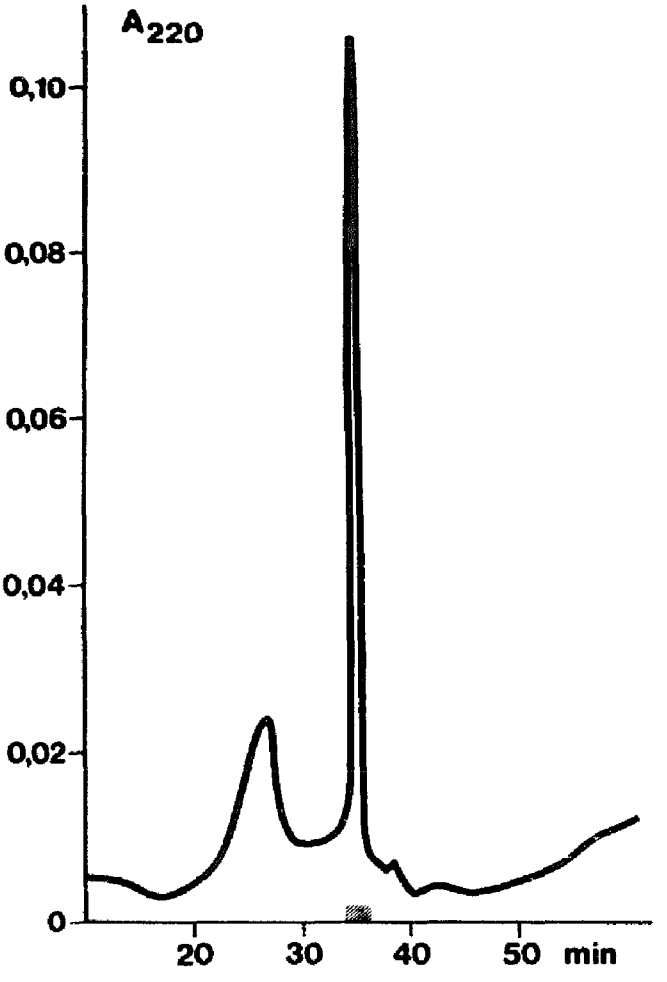

Fig. 2. Final RP-HPLC of calf kidney factor on a Nucleosil 300-CN-7 μm microbore column (60 × 2 mm). Solvent A, 0.1% TFA; solvent B, 0.08% TFA/100% acetonitril; gradient, 0–15% B/4 min, then 15–60% B/65 min; flow rate, 0.1 ml/min at 32°C; bar, position of inducing activity.

Table I

Inducing activity of Sephadex G100 fractions from calf liver, calf kidney and chicken embryos tested by the implantation method on *Triturus alpestris*

	Positive (%)	Size of induction (%)			Induced tissues (%)	
		Large	Med.	Small	Hind'h	Trunk/tail
Calf liver	94	54	13	27	13	94
Calf kidney	88	63	19	6	0	88
Chicken embryo	100	73	18	8	0	100

with rhombencephalon and ear vesicles (deuterencephalic structures) in a lower percentage. This is due to contamination with a neural inducing factor, which is present in calf liver with a higher activity than in calf kidney or chicken embryos, and at this step of the purification procedure is not yet completely separated from the vegetalizing factor. Liver from several vertebrate species has been shown to induce foreheads with telencephalon, diencephalon and eyes (archencephalic structures) as well as deuterencephalic structures besides mesodermal tissues [23]. Archencephalic structures are induced by a neuralizing factor, deuterencephalic structures by the combined action of mesodermalizing and neuralizing factors [24–26].

The factor from calf kidney was further purified to homogeneity by the same methods used for the chicken factor (Fig. 2). When tested on *Xenopus* ectoderm, 50% of the explants are induced at a concentration of 1.0–1.5 ng/ml. The molecular weight is about 26 kDa, the isoelectric point (in 6 M urea) at pH 8.0. The factor is completely inactivated after reduction with mercaptoethanol. The yield is about 0.7 μg/kg kidney. Similar factors which are activin or activin homologues are secreted from a *Xenopus* transformed fibroblast cell (XTC) line [27] and other cell lines [28,29]. They have been found in bovine amniotic fluid [30] and in human blood platelets [31]. Activin genes are transcribed in *Xenopus* embryos [32]. Mesoderm inducing activins have, however, not been found in neuroblastoma and retinoblastoma cell lines (Tiedemann et al., unpublished).

The activins are obviously evolutionarily highly conserved proteins which are not confined to functioning as embryonic inducing factors and gonadal hormones, but are synthesized in a number of organs of mesodermal and endodermal origin long after embryogenesis. The total amount of the inducing factors in calf kidney and liver is only slightly lower than in chicken embryos. The inducing activity depends on the concentration of the biologically active factor. It has been shown that the vegetalizing factor is in part bound in a latent form to protein complexes of higher molecular weight [33–36]. Whether follistatin, an activin binding protein which was isolated from ovaries [37], is present in these complexes is not yet known. The factor can be activated by different means like acidification or treatment with phenol, which lead to a dissociation of protein complexes.

Whether the activins have regulatory functions in the maintenance of the differentiated state or in the control of certain differentiated functions in later stages of development is not yet known. They could be needed to maintain the equilibrium between factors promoting or constraining cell division [38] and factors regulating differentiation. Which genes of the gene network are activated or inhibited by the activins remains to be shown.

Acknowledgements: We thank Edith Born, Waltraud Mertens, Yvona Cichocka and Iris Ziglowsky for skilful assistance. The investigation was supported by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

REFERENCES

[1] Geithe, H.-P., Asashima, M., Asahi, K.-I., Born, J., Tiedemann, H. and Tiedemann, H. (1981) Biochim. Biophys. Acta 676, 350–356.
[2] Plessow, S., Davids, M., Born, J., Hoppe, P., Loppnow-Blinde, B., Tiedemann, H. and Tiedemann, H. (1990) Cell Differ. Dev. 32, 27–38.
[3] Asashima, M., Uchiyama, H., Nakano, H., Eto, Y., Ejima, D., Sugino, H., Davids, M., Plessow, S., Born, S., Hoppe, P., Tiedemann, H. and Tiedemann, H. (1991) Mechn. Dev. 34, 135–141.
[4] Rosa, F., Roberts, A.B., Danielpour, D., Dart, L.L., Sporn, M.B. and Dawid, I.B. (1988) Science 239, 783–785.
[5] Knöchel, W., Born, J., Loppnow-Blinde, B., Tiedemann, H., Tiedemann, H., McKeehan, W.L. and Grunz, H. (1987) Naturwissenschaften 74, 604–606.
[6] Asashima, M., Nakano, H., Shimada, K., Kinoshita, K., Ishii, K., Shibai, H. and Ueno, N. (1990) Roux's Arch. Dev. Biol. 198, 330–335.
[7] Ling, L., Ying, S.Y., Ueno, H., Shimasaki, S., Esch, F., Hotta, M. and Guillemin, R. (1986) Nature 321, 779–782.
[8] Vale, W., Rivier, S., Vaughan, S., McClintock, R., Corrigan, A., Woo, W., Kaw, D. and Spiess, S. (1986) Nature 321, 776–779.
[9] Murata, M., Eto, Y., Shibai, H., Sakai, M. and Muramatsu, M. (1988) Proc. Natl. Acad. Sci. USA 85, 2434–2438.
[10] Asashima, M., Nakano, H., Uchiyama, H., Sugino, H., Nakamura, T., Eto, Y., Eyima, D., Davids, M., Plessow, S., Cichocka, I. and Kinoshita, K. (1991) Roux's Arch. Dev. Biol. 200, 4–7.
[11] Nakamura, T., Takio, K., Eto, Y., Shibai, H., Titani, K. and Sugino, H. (1990) Science 247, 836–838.
[12] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
[13] Mangold, O. (1923) Wilhelm Roux's Arch. Entwicklungsmech. Org. 100, 198–301.
[14] Tiedemann, H. (1986), in: Cellular Endocrinology: Hormonal Control of Embryonic and Cellular Differentiation in: (G. Serrero and J. Hayashi, Eds.) Alan Liss, New York, pp. 25–34.
[15] Asahi, K.-I., Born, J., Tiedemann, H. and Tiedemann, H. (1979) Roux's Arch. Dev. Biol. 187, 231–244.
[16] Grunz, H., McKeehan, W.L., Knöchel, W., Born, J., Tiedemann, H. and Tiedemann, H. (1988) Cell Differ. 22, 183–190.
[17] Mason, A.J., Berkemeier, L.M., Schmelzer, Ch.H. and Schw[illegible] R.W. (1989) Mol. Endocrinol. 3, 1352–12.
[18] Padgett, R.W., Johnston, R.D.St. and Gelbart, W.M. (1987) Nature 325, 81–84.
[19] Panganiban, G.E.F., Rashka, K.E., Neitzel, M.D. and Hoffmann, F.M. (1990) Mol. Cell. Biol. 10, 2669–2677.
[20] Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Kriz, R.W., Hewick, R.M. and Wang, E.A. (1988) Science 242, 1528–1534.
[21] Köster, M., Plessow, S., Clement, J., Lorenz, A., Tiedemann, H. and Knöchel, W. (1991) Mech. Dev. 33, 191–200.
[22] Roberts, A.B. and Sporn, M.B. (1988) Adv. Cancer Res. 51, 107–129.
[23] Saxén, L. and Toivonen, S. (1962) Primary embryonic induction, Logos Press-Academic Press, London.
[24] Saxén, L. and Toivonen, S. (1961) J. Embryol. Exp. Morph. 9, 514–533.
[25] Tiedemann, H. and Tiedemann, H. (1964) Rev. Suisse de Zool. 71, 117–137.
[26] Tiedemann, H. (1990) Zool. Sci. 7, 171–186.
[27] Smith, J.C., Price, B.M.J., Van Nimmen, K. and Huylebroeck, D. (1990) Nature 345, 729–731.

[28] Nakano, H., Kinoshita, K., Ishii, K., Shibai, H. and Asashima, M. (1990) Dev. Growth Differ. 32, 165–170.
[29] Van den Eijnden-Van Raaij, A.J.M., Van Zoelent, E.J.J., Van Nimmen, K., Koster, C.H., Snoek, G.T., Durston, A.J. and Huylebroeck, D. (1990) Nature 345, 732–734.
[30] Chertov, O.Y. u. Krasnosel'skii, Bogdanov, M.E. and Hoperskaya, O.A. (1990) Biomed. Sci. 1, 499–506.
[31] Dau, H., Wedlich, D., Born, J., Loppnow-Blinde, B., Davids, M., Tiedemann, H. and Tiedemann, H. (1990) FEBS Lett. 273, 68–70.
[32] Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. and Melton, D.A. (1990) Cell 63, 485–493.
[33] Becker, U., Tiedemann, H. and Tiedemann, H. (1961) Embryologia 6, 185–203.
[34] Born, J., Tiedemann, H. and Tiedemann, H. (1969) FEBS Lett. 2, 251–254.
[35] Tiedemann, H., Tiedemann, H. and Born, J. (1969) Science 164, 1175–1177.
[36] Born, J., Davids, M. and Tiedemann, H. (1987) Cell Diff. 21, 131–136.
[37] Ueno, N., Ling, N., Ying, S.-Y., Esch, F., Shimasaki, S. and Guillemin, R. (1987) Proc. Natl. Acad. Sci. USA 84, 8282–8286.
[38] Weinberg, R.A. (1990) Trends Biochem. Sci. 15, 199–202.