ACTIVATION OF A MORPHOGENETIC FACTOR BY ELECTROPHORESIS

J. BORN, Heinz TIEDEMANN and Hildegard TIEDEMANN
Institut für Physiologische Chemie der Freien Universität Berlin

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1. Introduction

From amphibian embryos of the gastrula stage as well as chicken embryos of different age a factor can be isolated which initiates in ectoderm of early gastrula stages of Triturus the differentiation of structures, which are derivatives of the mesoderm (trunk and tail with muscle, notochord and renal tubules) [1]. The factor is called mesodermalizing factor. In chicken muscle homogenate it is preferentially bound together with a neuralizing factor to particles which can be sedimented at 105,000 × *g* [2]. The supernatant has almost no inducing activity. It contains, however, some of the mesodermalizing factor which can be activated by treatment with phenol. The proteins extracted from the supernatant with phenol induce trunk and tail structures. The aqueous phase on the other hand contains a substance which inhibits the action of the mesodermalizing factor. If the inducing protein fraction is recombined with the aqueous phase in the presence of 6 M urea and dialyzed against water a complex is formed which has no inducing activity [3]. The inhibitor is also present in chick embryo brain and amphibian embryos. In the experiments to be described the supernatant was electrophoretically separated into a protein and a ribonucleoprotein fraction (which includes acidic glycoproteins). It is shown that the protein fraction induces trunk and tail structures.

2. Methods

Frozen trunks of 9 day chicken embryos were thawed, homogenized with two volumes 0.085 M NaCl or with NaCl-phosphate buffer (table 2) and centrifuged for 2 h at 105,000 × *g*. The floated lipids were removed and the supernatant dialyzed for 2–4 h against 2 × 500 ml electrophoresis buffer (table 2). Electrophoresis of about 3 ml (~ 30 mg protein) dialyzed supernatant was performed in the apparatus shown in fig. 1. The electrode vessels contained 1 M

Table 1
Inducing capacity of fractions obtained from a 0.085 M NaCl homogenate of 9 day chick embryo trunks.

	Number of cases	Positive (%)	Size of inductions (%)			Induced region (%)			
			large	medium	small	fore-head	hind-head	trunk-trail	not specificable
Sediment (washed with 0.085 M NaCl)	46	100	50	35	15	0	67	50	15
Supernatant (2 h 105,000 × *g*)	111	27	2	5	21	7	4	1	15
Phenol extracted proteins from supernatant	39	77	26	23	28	8	10	51	18

Table 2
Inducing capacity of electrophoretically separated fractions and controls.

a) Electrophoretically separated protein fraction

Medium for homogenization	Electrophoresis	Number of cases	Positive (%)	Size of inductions (%)			Induced region (%)			
				large	medium	small	forehead	hindhead	trunk-tail	not specifiable
H 11/2 0.05 M phosph. pH 8.0	Sephadex G 25 0.03 M phosph. pH 7.5, 9 h, 400 V, 50 mA	19	68	21	26	21	0	21	*42*	21
H 13/1 0.05 M phosph. pH 8.0	Sephadex G 25, 0.03 M phosph. pH 7.5, 5 h, 500 V, 70 mA	26	58	15	19	23	8	19	*23*	19
H 40/1 0.085 M NaCl	Sephadex G 25, 0.01 M phosph. + 0.01 M NaCl pH 7.5, 4 h, 600 V, 55 mA	24	67	21	29	17	4	25	*38*	12
H 52/2 0.085 M NaCl	Sephadex G 25, 0.01 M phosph. + 0.01 M NaCl pH 7.5, 5 h, 550 V, 60 mA	25	76	24	20	32	0	20	*28*	28
H 17/5 0.01 M phosph. + 0.075 M NaCl pH 7.5	5–25% sacch. 0.006 M phosph. + 0.03 M NaCl pH 7.5, 2 h 20′, 800 V, 60–75 mA	20	60	35	10	15	5	5	*45*	15
b) Electrophoretically separated ribonucleoprotein fraction										
H 13/2 0.05 M phosph. pH 8.0	Same as H 13/1	26	12	0	4	8	0	0	0	12
c) Controls										
H 11/K		17	76	29	23	23	12	24	*6*	47
H 13/K		21	62	0	29	33	24	14	*0*	29
H 40/K		26	39	4	15	19	4	12	*8*	19
H 17/K		22	27	0	14	14	0	14	*9*	14

The control fractions were dialyzed against electrophoresis buffer, diluted to the same extent as the protein peak after electrophoresis, incubated in an ice bath and then prepared for testing together with the electrophoresis fractions. The difference of the inducing capacity for trunk and tail of electrophoretically separated protein fractions as compared to the control fractions is highly significant ($\chi^2 = 97; p < 0.001$).

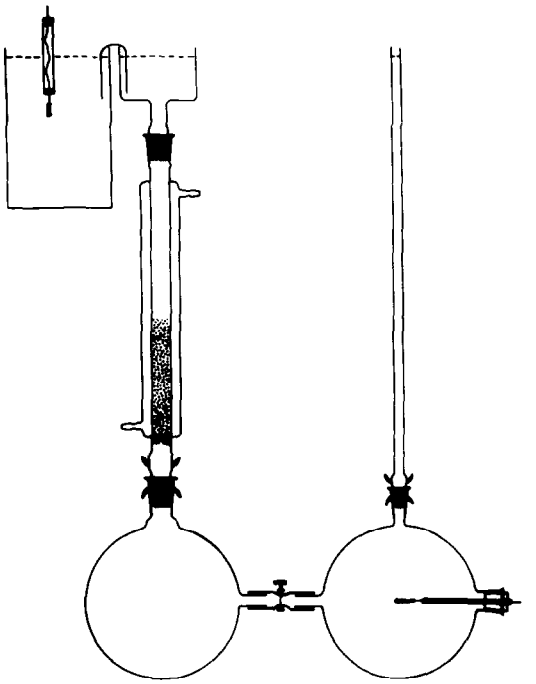

Fig. 1. Apparatus for zone electrophoresis (1:7.5).

Dotted area: Sephadex layer.

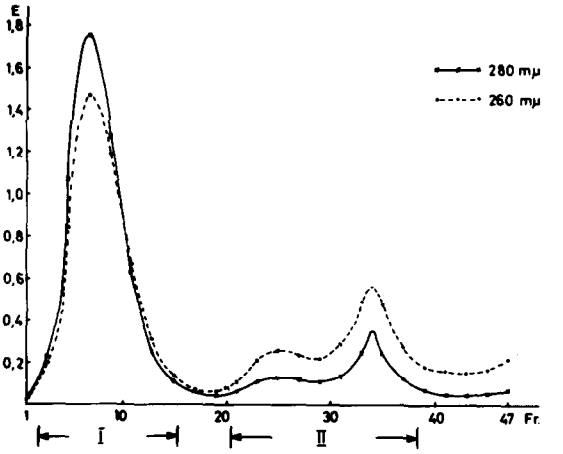

Fig. 2. Electrophoresis of supernatant (H 13) on Sephadex G 25.

NaCl (electrodes: Ag/AgCl). The red colored sample was washed into the lower end of the short (~ 8–12 cm) Sephadex layer before the current was applied. After electrophoresis the column was disconnected from the anode (upper) buffer vessel, closed at the upper end, then disconnected from the cathode buffer vessels and eluted with electrophoresis buffer (2–3 ml/20 min). In experiment H 17 a short Sephadex layer was used only to brake the buffer flow. On top of Sephadex a 25%–5% sucrose-gradient (20 ml) was layered. The cathode buffer contained 35% sucrose and the sample (30% sucrose) was carefully layered in between the cathode buffer and the gradient by means of a capillary, which was also used to fractionate the gradient after the run. The fractions were combined as indicated (fig. 2) and precipitated with 2 vol ethanol. The sediment was dissolved in 1–2 ml H_2O and dialyzed 24–36 h against 3 X 5 l H_2O, 0.05 vol 10% NaCl was added and then precipitated with 2 vol ethanol. The precipitate was washed twice with 66% ethanol, evacuated 1 min at 40 Torr before the final centrifugation to remove air bubbles and dried in a desiccator on the water pump for 2 h. Pieces of almost equal size were tested by the implantation method on Triturus alpestris [4]. The embryos were raised for 14–16 days in Holtfreter solution.

Phenol extraction was carried out for 7 min at 60°C.

3. Results

The inducing activities of sediment and supernatant as outlined in the introduction are shown in table 1.

The 105,000 X *g* supernatant was separated by electrophoresis into a ribonucleoprotein fraction (which contains also acidic polysaccharides) and a protein fraction. The mesodermalizing factor, which is included in the protein peak (fig. 2, I) is thereby at least partially activated (table 2a, H 11/2, H 13/1, H 40/1, H 52/2, H 17/5). This is true whether Sephadex G 25 was used as a supporting medium or the buffer column was stabilized by a sucrose gradient. The ribonucleoprotein fraction (fig. 2, II and table 2b, H 13/2) has no inducing activity at all. A part of the supernatant which was not electrophoretically separated and served as a control (table 2c, H 11/K, H 13/K, H 40/K, H 17/K) showed only a very weak inducing capacity for trunk and tail structures.

In another series of experiments the supernatant was filtered through Sephadex G 25 without electro-

Table 3
Inducing capacity of supernatant after filtration on Sephadex G 25

	Number of cases	Positive (%)	Size of inductions (%)			Induced region (%)			
			large	medium	small	fore-head	hind-head	trunk-tail	not specificable
Exclusion peak after filtration through Sephadex G25 (H 35/3, H 69/3)	53	38	2	21	15	2	9	9 *	19
Control: supernatant without filtration (H 35/2, H 69/2)	49	33	0	8	25	0	4	6 *	25

* Only mesenchymatic tails without muscle and notochord.

phoresis. An activation of the mesodermalizing factor was not observed under these conditions. The exclusion peak, which consists of proteins, ribonucleic acid and polysaccharides (table 3, H 35/3, H 69/3) has only a very weak inducing capacity for trunk- and tail-structures which corresponds to the very weak inducing capacity of the supernatant which was not filtered through Sephadex but only diluted to the same extent as the exclusion peak and then directly precipitated with ethanol (table 3, control H 35/2, H 69/2). The result of the Sephadex filtration experiments agrees with the result of dialysis experiments which have shown that dialysis of the supernatant does not lead to an activation of the mesodermalizing factor.

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