# A study of invertebrate actins by isoelectric focusing and immunodiffusion[1]

H.G. de Couet[2], K.-D. Mazander and U, Gröschel-Stewart

*Institut für Zoologie, Technische Hochschule Darmstadt, Schnittspahnstr. 10, D-6100 Darmstadt (Federal Republik of Germany), 2 July 1979*

*Summary.* Actin isolated from various invertebrate phyla comigrates with the $\beta$-form of vertebrate smooth muscle actin. However, invertebrate actins are not identical, since antibodies to insect-actin will not crossreact with the other species.

Actin has long been considered a highly conservative protein, unable to stimulate the production of antibodies. When actin-antibodies finally became available, they did not seem to discriminate between actins from smooth and striated muscle or from non-muscle tissue[3]. This seemed surprising in view of subsequent findings that actins can be separated by isoelectric focusing in at least three discrete species, named $\alpha$ (being representative for cardiac and skeletal muscle), $\beta$ and $\gamma$ (being present in both smooth muscle and non-muscle tissue in varying proportions). $\beta$-actin is the only species found in human erythrocyte membranes[4] and in the liver of *Torpedo marmorata*[5]. Small but pronounced differences were also found in the primary structure of actins from various sources[6–10].

Studies on the isoforms of actin have also included invertebrate tissue. Thus, actins from *Physarum polycephalum*[5] and from *Dictyostelium discoideum*[11] were shown to consist of a single species, more acidic than vertebrate $\alpha$-actin. The most alkaline isoelectric point was found in $\delta$-actin from *Acanthamoeba castellani*[12]. The nematode *Caenorhabditis elegans* has a single actin species, which has not been correlated to vertebrate species[13]; whereas 3 different actins (I, II and III) were described in embryonic insect muscle[14] which are supposed to be metabolic percursors of a single stabile form.

We have isolated highly purified actins from the helical body muscle of the mollusc *Sepia officinalis,* from the obliquely striated muscle of the marine annelid *Nereis virens,* and the striated, synchronous flight muscle of the cricket (*Gryllus* spec.) either by our standard procedure[15] or by dissociation from the actomyosin-complex[16]. An actin-enriched fraction was also obtained from the epithelial muscle of the sea-anemone *Actinia equina.* All actins had the same molecular weight of 42,000 (by SDS-PAGE), and they migrated as a single species in isoelectric focusing, all showing the same isoelectric point. Co-electrofocusing with vertebrate actins furthermore indicated that all invertebrate actins studied here behave like vertebrate $\beta$-actin (figure 1). It was therefore rather surprising to find out that these invertebrate $\beta$-actins possess a pronounced species specifity. Thus, antibodies raised in rabbits to insect- and annelid-actin (for method see Gröschel-Stewart[15]) will not cross-react with each other nor with the actins from other invertebrate sources. Only a faint crossreaction was noted between actins from the closely related arthropod insect and crayfish actin; but we never observed a reaction of the invertebrate antibodies with antigens of vertebrate origin (smooth and striated muscle, thromocytes).

The studies presented here not only support the postulation

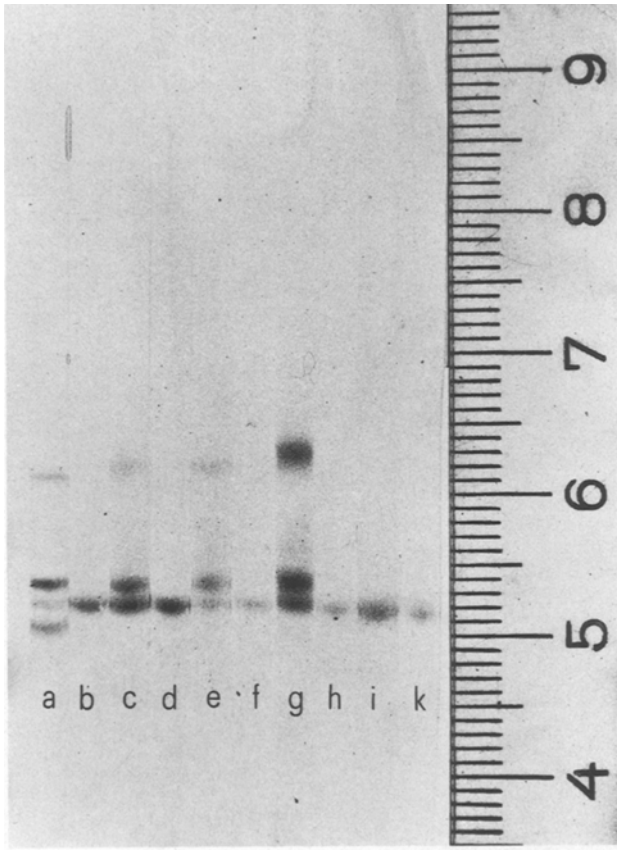


Fig. 1. 1-dimensional isoelectric focusing of different actin species. Top of the picture represents basic side of the gel. Original length of gels in 13 cm, diameter about 2 mm. Staining with Coomassie Brilliant Blue. Between 3 and 6 µg protein were loaded on the gels. Mixing experiments were carried out with comparable quantities. *a* Mixture of chicken gizzard actin and bovine heart actin as a reference system. The most acidic band represents the alpha-form of actin from bovine-heart. *b* Insect flight-muscle actin. *c* chicken gizzard actin plus insect flight-muscle actin. Note the increase of protein migrating with the betaform of chicken gizzard actin. *d* Cuttlefish actin *(Sepia officinalis). e* Cuttlefish actin plus chicken gizzard actin. *f* Sea anemone actin *(Actinia equina). g* Sea anemone actin plus chicken gizzard actin. *h* Polychaete actin *(Nereis virens). i* Polychaete actin plus insect flight-muscle actin. *k* Cuttlefish actin plus insect flight-muscle actin.

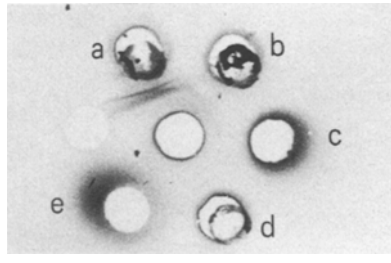


Fig. 2. Immunodiffusion of different actin species and anti insect actin in a system containing 0.6 M KCl and 2% Agar. Central well contains anti insect actin from rabbit serum (IgG-enriched fraction, 28 mg/ml). *a* Insect flight-muscle actomyosin. *b* Crustacean tail-muscle actomyosin. *c* Cuttlefish actomyosin. *d* Polychaete actomyosin. *e* Anthozoan actomyosin. A weak crossreaction is seen between crustacean actomyosin and anti insect actin, but only when using high $\gamma$-globulin concentrations. When using serum of immunized rabbits a single precipitation arc occurred between insect antigen and antibody. Antigen concentrations were between 3 and 6 mg/ml.

that isoelectric focusing alone is not the most useful tool to distinguish between actins of various sources, they also corroborate recent findings by our group[15] and by others[17], that actins may well be distinguished by their antigenic differences. It is hoped that combined studies on the chemistry and the immunology of vertebrate actins will further elucidate the plylogenetic origin of this conservative ubiquitous structural protein.

1 This work was supported by the Deutsche Forschungsgemeinschaft (Ste 105/19+20).
2 We wish to thank Dr. R. Whalen, Inst. Pasteur, Paris, for introducing us to the technique of I.E.F.

3 E. Lazarides and K. Weber, Proc. natl Acad. Sci. USA *71*, 2268 (1974).
4 J.C. Pinder, E. Ungewickel, D. Bray and W.B. Gratzer, J. supramolec. Struct. *8*, 439 (1978).
5 K. Zechel and K. Weber, Eur. J. Biochem. *89*, 105 (1978).
6 M. Elzinga and J. Kolega, Fedn Proc. *37*, 2341 (1978).
7 J.H. Collins and M. Elzinga, J. biol. Chem. *250*, 5915 (1975).
8 R.C. Lu and M. Elzinga, Biochemistry *16*, 5801 (1977).
9 J. Vandekerckhove and K. Weber, Proc. natl Acad. Sci. USA *75*, 1106 (1978).
10 J. Vandekerckhove and K. Weber, Eur. J. Biochem. *90*, 451 (1978).
11 D.G. Uyemura, S.S. Brown and J.A. Spudich, J. biol. Chem. *253*, 9088 (1978).
12 D.J. Gordon, J.L. Boyer and E.D. Korn, J. biol. Chem. *252*, 8300 (1977).
13 F.H. Schachat, H.E. Harris and H.F. Epstein, Biochim. biophys. Acta *493*, 304 (1977).
14 R.V. Storti, S.J. Horovitch, M.P. Scott, A. Rich and M.L. Pardue, Cell *13*, 589 (1978).
15 U. Gröschel-Stewart, S. Ceurremans, J. Lehr, C. Mahlmeister and E. Paar, Histochemistry *50*, 271 (1977).
16 T.P. Stossel and T.D. Pollard, J. biol. Chem. *248*, 8288 (1973).
17 S.A. Fellini, G.S. Bennett and H. Holtier, J. Cell Biol. *79*, 266a (1978).

## Effects of araC and aphidicolin on DNA chain elongation rate in HeLa $S_3$ cells

E. Wist

*Institute of Medical Biology, University of Tromsø, N-9001 Tromsø (Norway), 11 July 1979*

*Summary.* The effects of araC and aphidicolin on DNA chain elongation rate were tested. The rate was markedly reduced at low concentrations. Total DNA synthesis was more inhibited, indicating a role of DNA polymerase $\alpha$ in replicon initiation.

1-$\beta$-D-Arabinofuranosylcytosine (araC) is known to be a potent inhibitor of DNA synthesis[1-3] probably acting through its triphosphate araCTP. The antibiotic aphidicolin produced by the mold *Cephalosporidum aphidicola* Petch is also a very effective inhibitor of semiconservative DNA synthesis[4,5]. Both araCTP and aphidicolin inhibit DNA synthesis in an isolated HeLa $S_3$ nuclear system[3,6,7]. Moreover araCTP and aphidicolin have a marked inhibitory action on DNA polymerase $\alpha$ partially purified from isolated HeLa $S_3$ nuclei. The activities of the DNA polymerases $\beta$ and $\gamma$ are not affected[6,7]. These findings indicate a major role of DNA polymerase $\alpha$ in DNA replication. This is supported by other reports[8,9].

To link the results of our in vitro experiments with the in vivo situation we have examined the effects of araC and aphidicolin on the DNA chain elongation rate in whole HeLa cells.

The method of Painter and Schaefer[10] was used. HeLa cells in suspension culture were synchronized and reversed by addition of thymidine as described earlier[3]. 2.5 h after reversal, araC or aphidicolin was added to give the final concentrations shown in the table. 30 min later, cell suspensions of 20 ml ($2.0–2.5\times10^5$ cells/ml) were pulse labelled with [$^3$H]thymidine (19.5 Ci/mmole, final concentration 5 $\mu$Ci/ml) for the time periods given in the table. The cells were then pelleted, cooled on ice and washed free of soluble radioactivity by 3 washes with ice-cold medium containing amethopterin (1 $\mu$M), adenosine (50 $\mu$M) and bromodeoxyuridine (16 $\mu$M). The cells were resuspended in 20 ml medium containing bromodeoxyuridine (16 $\mu$M) and incubated at 37 °C for 3 h in the dark. The cells were then pelleted (500 × g for 5 min), resuspended in 2 ml SSC and mixed with [$^{14}$C]thymidine-labelled cells (as marker for DNA of normal density). DNA was isolated as described by Painter and Schaefer[10] and 1 M NaOH added to a final concentration of 0.1 M NaOH. DNA was sheared by 4 passages through a 23-G needle or by ultrasonication and analyzed in alkaline sucrose gradients and alkaline CsCl/$Cs_2SO_4$ gradients[11] thereby allowing estimation of the size of the DNA (B) and the fraction of radioactivity in bromo-

Effects of araC and aphidicolin on the rate of DNA chain elongation per growing point and overall DNA synthesis

| Inhibitor | Inhibitor concentration (M) | Size of DNA after shearing (daltons) | [$^3$H]thymidine labelling time (min) | Fraction of radioactivity in BrdUrd-labelled molecules after shearing ultrasonication | | | Overall DNA synthesis (% of control) | Rate of DNA chain elongation | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $f_{sh}$ | $f_u$ | $f=f_{sh}-f$ | | daltons/min | % of control |
| AraC(I) | 0 | $1.59\times10^7$ | 33 | 0.186 | 0.024 | 0.162 | 100 | $1.49\times10^6$ | 100 |
| | $10^{-8}$ | $1.46\times10^7$ | 33 | 0.231 | 0.039 | 0.192 | 59 | $1.15\times10^6$ | 77 |
| | $10^{-7}$ | $1.24\times10^7$ | 33 | 0.340 | 0.099 | 0.241 | 45 | $0.78\times10^6$ | 52 |
| AraC(II) | 0 | $2.06\times10^7$ | 30 | 0.283 | 0.026 | 0.257 | 100 | $1.33\times10^6$ | 100 |
| | $10^{-7}$ | $1.58\times10^7$ | 30 | 0.398 | 0.084 | 0.314 | 43 | $0.84\times10^6$ | 65 |
| | $5\times10^{-7}$ | $1.81\times10^{-7}$ | 30 | 0.546 | 0.040 | 0.516 | 15 | $0.60\times10^6$ | 45 |
| Aphidicolin | 0 | $0.72\times10^{-7}$ | 28 | 0.155 | 0.060 | 0.095 | 100 | $1.35\times10^6$ | 100 |
| | $2.4\times10^{-7}$ | $1.08\times10^{-7}$ | 28 | 0.290 | 0.080 | 0.210 | 55 | $0.92\times10^6$ | 68 |
| | $4.8\times10^{-7}$ | $0.82\times10^{-7}$ | 28 | 0.440 | 0.120 | 0.320 | 23 | $0.46\times10^6$ | 34 |