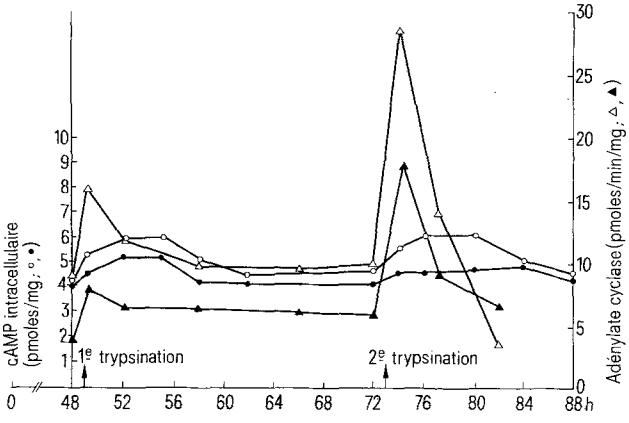
remarquer également que les trypsinations activent d'une manière brusque mais brève, l'activité de l'adénylate cyclase dans tous les cas mais davantage dans les cellules infectées que dans les cellules témoins, l'action de la 2e trypsination étant plus forte que celle de la 1re. Les répercutions au niveau des taux intracellulaires de cAMP sont très fortement atténuées.

Discussion. L'étude du mécanisme d'activation de l'adénylate cyclase sous l'effet de la toxine cholérique[14] a conduit à imaginer que les récepteurs spécifiques de cet enzyme pouvaient se disposer selon plusieurs configurations correspondant à des structures différentes de la membrane plasmatique, ainsi, la modulation de l'activité de l'adénylate cyclase serait l'expression fonctionelle des relations spatiales entre l'enzyme et ses récepteurs.

Dans les cellules infectées, les antigènes viraux, de part leur présence et leur mobilité dans la membrane plasmatique[6] induisent des changements morphologiques chez cette dernière, ce qui peut expliquer l'activité accrue de l'adénylate cyclase.

Les trypsinations activent l'adénylate cyclase d'une manière brusque mais limitée dans le temps. Nous avons précédemment montré[8], qu'elles augmentaient l'affinité enzyme substrat ATP, probablement en faisant apparaître de nouveaux sites de fixation de substrat sur l'enzyme grâce à la protéolyse limitée qu'elles occasionnent. La trypsine est également connue pour augmenter la perméabilité cellulaire[15,16]. Ainsi, le cAMP synthétisé pourrait être évacué rapidement par les cellules dans le milieu de culture, mais il est difficile de vérifier cette évacuation, car le milieu de culture contient de grandes quantités de cAMP apportées par le sérum de veau. Le cAMP peut être aussi dégradé par la phosphodiestérase dont la plus grande partie est soluble dans les cellules. Il existe toutefois une fraction membranaire de cet enzyme qui peut être activée par les protéolyses ou la présence des antigènes viraux. Toutefois, les travaux récents ne sont pas en faveur de cette hypothèse, en effet: Russel et Pastan[17] ont montré une altération de phosphodièstérase membranaire des fibroblastes après traitement par la trypsine et Krizanova[18] a montré une diminution d'activité de la phosphodièstérase membranaire immédiatement après la fixation du virus de la grippe.

Il est toutefois indéniable que la 2e trypsination qui occasionne la libération de virus infectieux est suivie d'une augmentation du taux intracellulaire de cAMP. L'étude de l'éventuelle influence du cAMP sur les réactions cytopathogènes des cellules infectées constituera la suite de ce travail.



Variations d'activité de l'adénylase cyclase (△, ▲) et du taux intracellulaire de cAMP (○, ●) des cellules témoins (●, ▲) et des cellules infectées (○, △) au cours des étapes permettant la libération de virus infectieux. Valeurs moyennes de 4 expériences. Erreur maximum cAMP intracellulaire (±9%), activité adénylate cyclase (±11%). Les cellules KB sont entretenues sur un milieu de Eagle BME enrichi en sérum de veau comme nous l'avons précédemment décrit[8]. Le virus Sendaï entretenu sur œuf de poule embryonné est inoculé après 24 h de culture à raison de 10^{-2} DI_{50} par cellule (calcul sur papier log probit[12] selon la méthode de Reed et Muench[13]. Les trypsinations sont effectuées par incubation des tapis cellulaires 5 min avec 0,1% de trypsine. La 1e n'entraine ni réaction cytopathogène ni production de virus infectieux (titre inférieur à $10^{1,1}$ DI_{50}/ml), mais les cellules hemadsorbent les hématies de poulet. La 2e entraîne la formation de syncytiums qui s'accroissent et évoluent vers la dégénérescence de la culture ainsi que la production de virus infectieux (titre compris entre $10^{5,2}$ et 10^{6} DI_{50}/ml 6 h plus tard - 4 expériences).

1 M. Homma, J. Virol. *9*, 829 (1972).
2 M. Homma et S. Tamagawa, J. gen. Virol. *19*, 423 (1973).
3 M. Ohuchi et M. Homma, J. Virol. *18*, 1147 (1976).
4 A. Scheid et P.W. Choppin, Virology *57*, 475 (1974).
5 L. Colobert et A. Berkaloff, Annls Inst. Pasteur *106*, 581 (1964).
6 Y. Okada, J. Kim, Y. Maeda et I. Koseki, Proc. natl Acad. Sci. USA *71*, 2043 (1974).
7 W.L. Ryan, M.A. Short et G.L. Curtis, Proc. Soc. exp. Biol. Med. *150*, 699 (1975).
8 A. Guiraud-Simplot et L. Colobert, Experientia *33*, 899 (1977).
9 A.A. White et T.V. Zenzer, Analyt. Biochem. *41*, 372 (1971).
10 A. Guiraud-Simplot et L. Colobert, Biochem. biophys. Res. Commun. *76*, 963 (1977).
11 A.G. Gilman, Proc. natl Acad. Sci. USA *67*, 305 (1970).
12 P. Bonet-Maury, A. Jude et P. Servant, Rev. Immun. *18*, 21 (1954).
13 L.J. Reed et H. Muench, Am. J. Hyg. *27*, 493 (1938).
14 V. Bennet, E. O'Keefe et P. Cuatrecasas, Proc. natl Acad. Sci. USA *72*, 33 (1975).
15 H.J. Phillips et J.E. Terryberry, Exp. Cell Res. *13*, 341 (1957).
16 H.J. Phillips, Can. J. Biochem. *45*, 1495 (1967).
17 T. Russel et I. Pastan, J. biol. Chem. *248*, 5835 (1973).
18 O. Krizanova, D. Lacinova et J. Knopp, Acta virol. *21*, 97 (1977).

Some observations on bipolar filaments formed by non-muscle myosins[1]

J.E. Hesketh, N. Virmaux and D. Aunis

Centre de Neurochimie du CNRS, INSERM, 11, rue Humann, F-67085 Strasbourg (France), 19 April 1978

Summary. Adrenal medullary and retinal myosins formed bipolar filaments in vitro. These filaments showed features suggesting flexibility in the rod region of the myosin molecules composing such filaments; in certain cases the myosin heads spread away from the filament backbone, in others the backbone itself was twisted. In addition the bare central backbone showed transverse striations.

Both muscle and non-muscle myosins are long, asymmetric proteins consisting of 2 globular heads and a long, helical rod[2,3]. One of the characteristic properties of purified myosin is its ability to form, in vitro, typically bipolar filaments under conditions of specified ionic strength. This property is exhibited equally by skeletal muscle[4], smooth

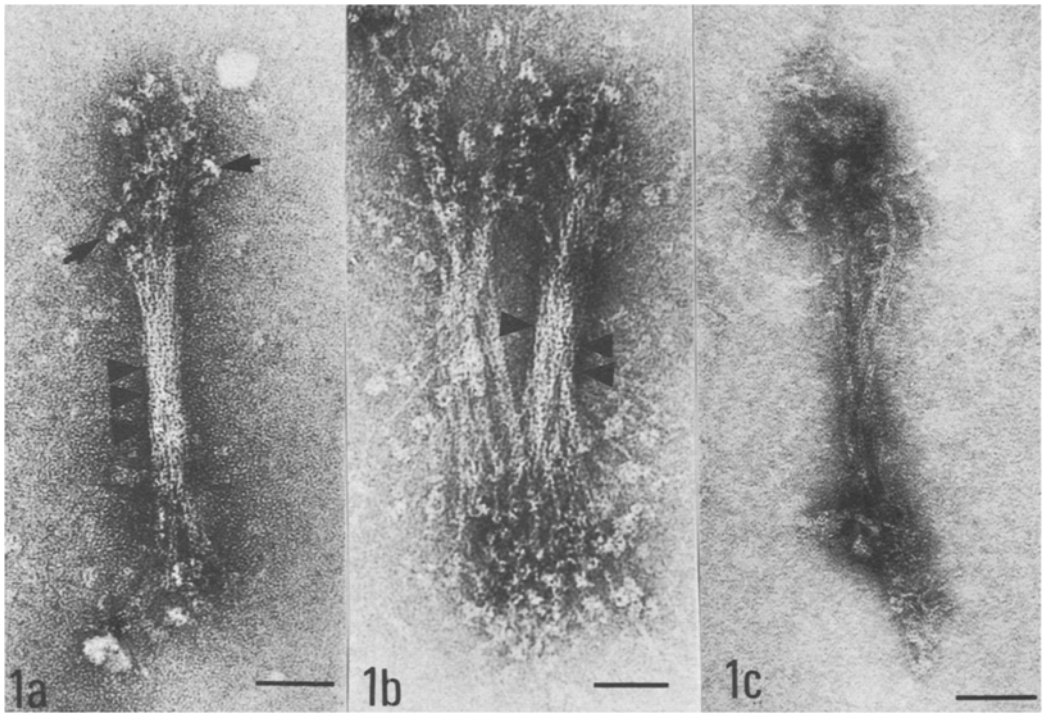


Fig. 1. Electron micrographs of bipolar filaments formed by retinal and adrenal medullary myosins. *a* and *b* Retinal myosin, *c* adrenal medulla myosin. Magnification × 172,000. Bar (1 cm) represents 58 nm. Note in figure a certain heads (→) at the ends of rods which can be seen to bend away from the filament backbone. The distance from these heads to the bends in the rods is 35–50 nm. The filaments in a and b show faint transverse striations (►) at approximately 15 nm intervals. Note in figure c the twist in the central bare region of the filament.

muscle[5] and non-muscle[6-8] myosins. Such filaments are thought to arise through antiparallel interactions between the rod regions of the myosin molecules such that the heads are arranged at either end of the filaments, thus giving the bipolar structure when observed by electron microscopy following negative staining. In the case of skeletal muscle myosin filaments, the heads project from the side of the filaments with a periodicity of 14–15 nm[4] whereas in the case of non-muscle myosins the heads seem to be less regularly arranged. During the characterization of myosins from the retina[9] and the adrenal medulla[10], we noticed certain interesting features of the bipolar filaments formed by these myosins.

Methods. Myosin was purified from bovine retinae and bovine adrenal medullae, as we have recently described elsewhere[9,10]. Samples of the purified myosins were dialyzed against 0.1 M KCl, 10 mM imidazole-HCl, 0.1 mM dithiothreitol, 0.2 mM $MgCl_2$, pH 7.0 for either 3 h (retina) or 14 h (adrenal medulla), centrifuged at 180,000 × g 30 min and the pellet resuspended in a small volume of the above buffer. Resuspended material was negatively stained with 1% aqueous uranyl acetate and examined on copper grids at 80 kV with a Phillips EM 300 electron microscope.

Results and discussion. Figure 1, a and b shows typical bipolar filaments formed by purified retinal myosin; total length 420 nm with a bare central region of 160 nm. The heads of the individual myosin molecules can be seen (slightly elongated, length approximately 20 nm) and they form a bouquet type structure rather than a periodic array. In addition, retinal myosin also formed bipolar filaments in which the heads did not form a bouquet but formed a much more compact structure (results not shown, see Hesketh et al.[9]) similar to that we found for adrenal medulla myosin filaments (figure 1, c). Interestingly adrenal medulla myosin has been found by others to form filaments similar to those in figure 1, b where the heads are far less compact[11]. Platelet myosin has also been observed to form bipolar filaments in which the arrangement of the heads can differ[6]. Thus it seems that non-muscle myosins can form a variety of bipolar filaments varying in the exact arrangement and disposition of the heads. Niederman and Pollard[6] suggested that such differences in structure might be explained by a flexible 'joint' in the myosin molecule between the rod and head portions. However, the dimensions of the bouquet-like filaments in figure 1 make such an explanation insufficient, since the heads spread away from the bare

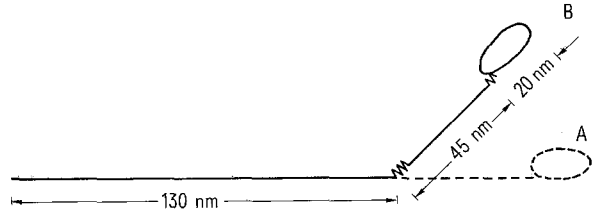


Fig. 2. Model of non-muscle myosin. The rod region is divided into sections of approximately 130 nm and 45 nm by a flexible joint (≈). A 2nd flexible joint may also be present close to the heads[2,12] (for clarity only one head is shown in the figure). Flexibility in the rod region might allow the heads to adopt 2 extreme positions, one where the heads are almost in line with the rod so giving a 'compact' filament (a), and a 2nd where the rod is bent at the flexible point (b) so giving a bouquet-type filament. As with muscle myosin, there may also be movement at the 2nd joint. Calculation of the distances given in the diagram was carried out using the dimensions of the filaments in figure 1; the heads (1a) were found to be approximately 20 nm in length (compare Offer and Elliott[2]) and the flexible joint in the rod was situated approximately 45 nm from the heads. The length of the whole molecule was calculated from the lengths of the filaments[6], and the distance from the end of the rod to the flexible joint then obtained by subtraction (120–140 nm). The model illustrated in the figure resembles that of Offer and Elliott[2] for muscle myosin.

central region to such an extent that the arrangement cannot be explained by flexibility of the molecules between the head and rod regions. Several myosin rods can be seen to bend away from the filament backbone suggesting a flexible 'joint' within the rod region. In certain cases, the distance between the heads and such a flexible point could be measured and it was found to be 35–50 nm. Thus if there are flexible joints in the non-muscle myosin molecules responsible for the filament structure, they must be situated approximately 45 nm from the heads. The presence of a flexible joint in such a position is supported by the observation of adrenal medulla myosin filaments with twisted bare central regions (figure 1, c); such a twist must reflect a flexible part of the rod region of the myosin molecules. Skeletal muscle myosin has been suggested to possess 2 flexible joints[2], one between the heads and the rod region and another in the rod region 43 nm from the heads, and in addition cardiac myosin has been shown to possess flexibility in the rod region[12]. Our results suggest that a flexible joint comparable to that found in the rod of skeletal muscle myosin[2] is also present in non-muscle myosins, and that it may be responsible for the variation in the disposition of the heads found in non-muscle myosin bipolar filaments[6,9]. The heads appear as compact or diffuse structures and the necessary flexibility involved probably derives from a flexible joint in the myosin rod as shown in figure 2. Models of bipolar filament structure should take into account such flexibility in the rod region of the molecule.

The twisted filament in figure 1, c exhibits a 2nd interesting feature, namely a bare central region which is split into 4 separate 'rods'. The existence of such split backbones was common in the adrenal medulla myosin preparations and it suggests that head-head interactions are important, as well as rod-rod interactions, in the formation and conservation of the bipolar filament structure. Head-head interactions have been previously suggested to be important in controlling the length of non-muscle myosin bipolar filaments[6] and in the formation of large assemblies of bipolar filaments[8,10].

The filaments of retinal myosin (figure 1, a and b) show a 3rd feature, the presence of faint transverse striations in the bare central region; in some cases, but not all, they traverse the width of the filaments. The distance between the striations varies from 6 to 20 nm, the average separation being 15 nm and there is some evidence for a regular arrangement. At present, the structural basis of the striations is not clear but they may represent the arrangement of the ends of the myosin rods composing the filament backbone. Perhaps this reflects some specificity in the interactions of the myosin rods during filament formation.

1 We thank G. Devilliers for assistance with electron microscopy, D. Thiersé for technical assistance during purification of adrenal medulla myosin and Professor P. Mandel for his interest and support. J.E.H. gratefully acknowledges receipt of a Royal Society European Exchange Fellowship and a grant from INSERM. N.V. is chargée de recherches au CNRS, D.A. is chargé de recherches à l'INSERM.

2 G. Offer and A. Elliott, Nature *271*, 325 (1978).
3 A. Elliott, G. Offer and K. Burridge, Proc. R. Soc. B *193*, 43 (1976).
4 H.E. Huxley, J. molec. Biol. *7*, 281 (1963).
5 R. Craig and J. Megerman, J. Cell Biol. *75*, 990 (1977).
6 R. Niederman and T.D. Pollard, J. Cell Biol. *67*, 72 (1975).
7 T.D. Pollard and R.R. Weihing, CRC Critical Rev. Biochem. *2*, 1 (1974).
8 K. Burridge and D. Bray, J. molec. Biol. *99*, 1 (1975).
9 J.E. Hesketh, N. Virmaux and P. Mandel, Biochim. biophys. Acta *542*, 39 (1978).
10 J.E. Hesketh, D. Aunis, P. Mandel and G. Devilliers, Biol. cell., in press.
11 J.M. Trifaro and C. Ulpian, Neuroscience *1*, 483 (1976).
12 S. Highsmith, K.M. Kretzschmar, C.T. O'Konski and M.F. Morales, Proc. nat. Acad. Sci. USA *74*, 4986 (1977).

The effect of bisamidines of 2,6-diaminoanthraquinone on *Entamoeba histolytica* infections in rats and hamsters

E.J. Burden[1,2], S.G. Carvajal, P.F. Fabio, T.L. Fields, Yang-I Lin, K.C. Murdock and S.A. Lang, Jr[1,2]

Infectious Disease Research Section, Medical Research Division, American Cyanamid Company, Pearl River (New York 10965, USA), 3 April 1978

Summary. Bisamidines of 2,6-diaminoanthraquinone have demonstrated potent activity against cecal and hepatic *Entamoeba histolytica* infections in rats and hamsters, respectively. A number of these compounds compared favorably, in overall drug efficacy, with metronidazole and other standard agents.

A variety of drugs for the treatment of *Entamoeba histolytica* infections is available but indications and effectiveness differ considerably depending upon the severity of the disease. The organism may be present in the bowel lumen, the bowel well, extraintestinal tissues (primarily in the liver), or in both intestinal and extraintestinal sites. The modes of action and principal sites of action of various drugs differ. Asymptomatic intestinal infections can usually be treated successfully with halogenated hydroxyquinolines, however, optic atrophy and loss of vision have caused these drugs to be withdrawn from use in many parts of the world[3,4].

More severe intestinal infections have been treated with metronidazole or with varying sequential or concomitant treatment including the diloxanide furoate and metronidazole[5]. Hepatic abscesses were treated with metronidazole or sometimes by metronidazole followed by other therapy diiodohydroxyquin, or by dehydrometine or emetine followed by chloroquine and/or diiodohydroxyquin. Costs, mode of action, degree of toxicity and regional preferences among drugs with similar modes of action have been instrumental in governing the selection of the drug or combination of drugs used for treating amebiasis in different parts of the world. The nitroimidazoles are now generally recognized as the principal drugs of choice for amebiasis since they are effective against infections in all sites and are usually well tolerated. However, some have been reported carcinogenic in animals and a well tolerated product with at least equivalent efficacy and with no carcinogenic potential should provide a highly competitive substitute.

A number of bisamidines of 2,6-diaminoanthraquinone have displayed antiamebic activity against experimental *E. histolytica* infections in test animals[6]. These novel non-nitro compounds were non-mutagenic when tested in the Ames test and the Dominant-lethal test. Metronidazole, the leading marketed agent has been shown to be mutagenic and carcinogenic in test animals[7]. The compounds de-