Evidence That a low- M_r Diffusible Factor is Involved in Communication between Compatible Mating-types of the Slime Mould *Physarum polycephalum*

By PAUL T. SHARPE¹* AND E. M. GOODMAN²

¹Department of Molecular Embryology, Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ, UK

²Biochemical Research Institute, University of Wisconsin-Parkside, Kenosha, Wisconsin 53141, USA

(Received 10 April 1986; revised 10 July 1986)

Two compatible mating-types of *Physarum polycephalum*, RSD4 and MA185, have recently been shown to have different surface properties by using aqueous two-phase partition. Changes in the surface properties of RSD4 amoebae were detected when in contact with cell culture medium from MA185 amoebae. The ability of MA185 culture medium to alter the surface of RSD4 amoebae was not affected by autoclaving or passage through a filter that removed molecules of M_r greater than 10000. Activity was detected in medium from MA185 amoebae that had not previously been in contact with RSD4 amoebae. These results provide evidence for the existence of a low- M_r factor produced by MA185 amoebae which affects the surface of RSD4 amoebae.

INTRODUCTION

In the slime mould *Physarum polycephalum*, diploid plasmodium formation occurs when two haploid amoebae fuse (Youngman *et al.*, 1977; Collins, 1979; Gorman & Wilkins, 1980; Dee, 1984). Fusion of haploid amoebae is controlled by two mating-type loci, *matA* and *matB* (Youngman *et al.*, 1981). Differences at the *matB* locus are required for fusion while differences at the *matA* locus facilitates the amoebal-plasmodium transition (Youngman *et al.*, 1981).

Previous studies have shown that an inducer of the amoebal-plasmodium transition is produced by growing amoebae (Nader *et al.*, 1984). In these studies cell-free culture medium from dense amoebal cultures was able to accelerate plasmodium formation compared to that from a less dense culture. This diffusible inducer was postulated to be a glycoprotein of M_r about 120000 (Nader *et al.*, 1984). Amoebae from compatible mating types appear to respond to their own (self-produced) inducer or to an inducer produced by other mating types.

Reiskind & Aldrich (1984) isolated and characterized the plasma membrane from *P. polycephalum* strains RSD4 and MA185, which are of compatible mating-type. A comparison of the electrophoretic profiles suggested five high- M_r bands (M_r 119000–215000) that were unique to MA185 and one (M_r 19500) that was unique to RSD4. Both qualitative and quantitative differences in concanavalin A-binding glycoproteins were also observed. Using the technique of aqueous two-phase partition, a non-destructive method of identifying subtle differences in cell surface (Walter, 1977), we have recently shown that strains RSD4 and MA185 have different surface properties (Sharpe & Goodman, 1986). In this paper we report that the surface character of RSD4 amoebae changes when in contact with medium in which MA185 amoebae have been growing. The molecule(s) involved in producing these cell surface changes appear(s) to be unrelated to the previously reported inducer of the amoebal-plasmodium transition.

Abbreviation: SMF, surface-modifying factor(s).

METHODS

P. polycephalum strains. Amoebal strains RSD4 (*matA1 matB1*) and MA185 (*matA3 matB3*), normally maintained in different axenic media, were subcultured and maintained in a medium consisting of 5 g Casamino acids, 5 g yeast extract, 10 g Bacto Soytone, 7 g glucose, 100 ml basal salts, 100 µg thiamin. HCl and 100 µg biotin to 1 litre of distilled water (McCullough & Dee, 1976). The hydrolysates were all purchased from Difco. After adaptation to this medium for 14 d, amoebae from both mating-types grew with doubling times of about 24 h.

Phase system. The phase system used in all experiments consisted of: dextran T-500 (Pharmacia), 5% (w/w); poly(ethylene glycol) 8000, 4.8% (w/w); NaCl, 0.5 mol kg⁻¹; and potassium phosphate buffer pH 7, 0.01 mol kg⁻¹. This phase system was made up more concentrated such that addition of 1 ml suspension of amoebae in water produced the desired final polymer composition. The phases were mixed by inverting a 5 ml phase-cell suspension (in 15 ml tubes) 30 times; the system was allowed to re-partition in an upright position for 30 min at 25 °C, a 1 ml sample was removed from the centre of the top phase and the number of cells was counted in a ZBI Coulter counter. The 'partition coefficient' of the cells is expressed as the total number of cells in the top phase as a percentage of the total cells added.

Assays of cell-cell communication. RSD4 and MA185 amoebae were grown in separate sides of U-tubes (Bellco) divided by a 0.45 µm Millipore filter; sterile aluminium foil was used to separate the tubes in control experiments. Amoebae were inoculated to a density of 10⁶ cells ml⁻¹ (total 10 ml) and allowed to grow for 48 h before partitioning.

Cell-free medium was prepared from 48 h MA185 cultures by centrifugation (500 g, 4 °C). Exponential-phase 24 h RSD4 amoebae were harvested and resuspended at 10⁶ cells ml⁻¹ in 48 h MA185 cell-free medium, or in new medium (control experiment) or in RSD4 cell-free medium. The amoebal suspensions were shaken at 25 °C on a rotary shaker at 80 r.p.m. Samples of amoebae were removed after 1, 2 and 4 h, and partitioned.

Treatment of MA185 cell-free medium. Samples of freshly prepared MA185 cell-free medium were autoclaved at 120 °C for 20 min. Samples were also filtered through an Amicon 10000 filter system, which removes molecules of *M_r* above 10000.

Reproducibility. All the results reported are means of three experiments, ± the standard error of the mean.

RESULTS

Evidence of cell-cell communication

Control experiments using aluminium foil to prevent contact between the two cultures in the U-tubes confirmed that after 48 h growth, MA185 amoebae had a higher partition coefficient ($42 \pm 2.6\%$, mean ± SEM) than RSD4 amoebae ($20.3 \pm 1.5\%$). In the experiments where the two amoebal cultures were in contact via the filter, the partition coefficient of the RSD4 amoebae was higher than in the controls ($38.6 \pm 4\%$) and very close to the partition coefficient of the MA185 amoebae ($39.5 \pm 2.2\%$). No changes in the partition of MA185 amoebae were observed, nor were any significant changes in RSD4 amoebae detected after 24 h growth (data not shown).

To determine the time required for the RSD4 surface change to occur, the amoebal cultures grown in the U-tubes for 48 h were reversed. Thus RSD4 experimental amoebae were placed in contact with MA185 control amoebae connected via a filter. The experimental MA185 amoebae were similarly placed in contact with the RSD4 control amoebae. The tubes were shaken for a total of 4 h, samples being removed at 1, 2 and 4 h for partition (Table 1). There were no changes in the partition coefficients of any of the amoebae after 1 h. However, the partition of RSD4 amoebae (in contact with MA185 amoebae) increased from 20% at 1 h to 36% after 2 h contact. The partition of all other amoebae remained the same. After a total of 4 h, the partition of RSD4 amoebae in contact with MA185 amoebae had further increased to 40%, whereas no significant changes in partition for the other amoebae were observed. RSD4 experimental amoebae (i.e. amoebae previously in contact with MA185) did show a slight increase in partition, from 34 to 41%, with increasing time in contact with MA185 amoebae. These results suggested that MA185 amoebae were secreting a 'factor' that was able to pass through the filter and change the surface character of RSD4 amoebae within 2 h.

To determine whether MA185 amoebae would produce this 'factor' in the absence of contact with RSD4 amoebae, cell-free medium was prepared from MA185 amoebal cultures. RSD4 amoebae were then shaken in this medium for a total of 4 h and their partition was measured.

Table 1. *Partition coefficients of RSD4 and MA185 amoebae after being in contact with 48 h conditioned media from the compatible mating-type for different times*

See text for details.

Amoebae	Partition (% amoebae in top phase, $\pm$ SEM)		
	1 h	2 h	4 h
RSD4 control (+ MA185 exp.)	20 $\pm$ 2.3	36 $\pm$ 1.7	40 $\pm$ 2.1
MA185 control (+ RSD4 exp.)	36 $\pm$ 1.9	39 $\pm$ 3.1	38 $\pm$ 3.8
RSD4 exp. (+ MA185 control)	34 $\pm$ 2.3	36 $\pm$ 2.2	41 $\pm$ 1.7
MA185 exp. (+ RSD4 control)	34 $\pm$ 3.2	37 $\pm$ 1.8	39 $\pm$ 2.7

Table 2. *Partition coefficients of RSD4 amoebae after being in contact with various preparations of cell-free medium for different times*

Medium preparation*	Partition (% amoebae in top phase, $\pm$ SEM)		
	1 h	2 h	4 h
Fresh medium	19 $\pm$ 2.8	20 $\pm$ 1.9	21 $\pm$ 3.1
RSD4 medium	21 $\pm$ 2.1	19 $\pm$ 2.7	21 $\pm$ 1.9
MA185 medium	22 $\pm$ 3.2	38 $\pm$ 3.1	41 $\pm$ 1.7
Heat-treated MA185 medium	19 $\pm$ 2.8	39 $\pm$ 2.1	40 $\pm$ 2.7
Amicon-filtered MA185 medium	23 $\pm$ 1.2	39 $\pm$ 1.8	43 $\pm$ 2.3

* Fresh medium, no previous amoebal growth; RSD4 medium, 48 h growth of RSD4 amoebae; MA185 medium, 48 h growth of MA185 amoebae; heat-treated MA185 medium, 48 h growth of MA185 followed by autoclaving for 20 min at 120 °C; Amicon-filtered MA185 medium, 48 h growth of MA185 followed by passage through an Amicon 10000 filter to remove molecules of M_r above 10000.

The partition of RSD4 amoebae shaken in the MA185 cell-free medium increased (from 21.6 $\pm$ 1.5%) to a level similar to that obtained (39 $\pm$ 2.0%) when the cells were grown in contact with MA185 amoebae. The partition of the RSD4 amoebae was the same when they were shaken in MA185 cell-free medium obtained from cultures either grown in the absence of any contact with RSD4 amoebae (40.3 $\pm$ 0.57%) or grown in contact via U-tubes connected with a 0.45 μ m filter (39.6 $\pm$ 2.5%). Thus MA185 contact with RSD4 amoebae is not an obligatory event in producing this surface-active agent.

Partial characterization of the surface-modifying substance

Fresh cell-free MA185 medium was either heat treated or passed through an Amicon filter that removed molecules of M_r greater than 10000. RSD4 amoebae were then shaken in these two medium preparations; as controls, the same RSD4 amoebae were shaken either in fresh axenic medium or in cell-free medium obtained from RSD4 amoebal cultures. The partition coefficients of the amoebae after 1, 2 and 4 h shaking are shown in Table 2. Neither heat treatment nor the removal of molecules of M_r above 10000 had any appreciable effects on the ability of the medium to promote an increase in the partition coefficient of RSD4 amoebae.

DISCUSSION

Aqueous two-phase partition is a simple, yet sensitive, method for detecting small differences in cell surface character. Differences or changes in the partition coefficient as reported here reflect differences or changes in cell surface properties. When RSD4 and MA185 amoebae were

grown separately, they had different partition coefficients in the same phase system, indicating that they inherently have different surface properties (Sharpe & Goodman, 1986). However, when compatible amoebae were grown such that their culture media were in contact via a filter, the partition of RSD4 amoebae increased to a level similar to that of the MA185 amoebae. Thus MA185 culture medium appears to have an influence on the surface character of RSD4 amoebae. Since there was no cell-cell contact between the amoebae, these results suggest that a diffusible factor is present in the MA185 culture medium that is capable of changing the surface of RSD4 amoebae.

When the two mating types were in contact via a filter, changes in the cell surface of RSD4 amoebae were not detected during the first 24 h of growth, but were clearly detectable at 48 h. This suggests that the density of MA185 amoebal cultures needs to be above a certain level before an effect is induced. If MA185 amoebae are continually secreting a factor into the medium during growth that can affect RSD4 amoebae, it might be expected that the activity of this factor would be more readily detected in high-density cultures. A similar effect has been reported regarding the production of a high- M_r factor believed to be involved in promoting the amoebal-plasmodium transition (Nader *et al.*, 1984). In these experiments increased activity of this high- M_r factor was detected in medium from cultures grown to high density.

The factor (or factors) identified in this report, hereafter referred to as surface-modifying factor(s) (SMF), influences the surface of RSD4 amoebae; it does not appear to be related to the high- M_r factor described by Nader *et al.* (1984), which is a glycoprotein of M_r around 120000, whereas SMF has an M_r below 10000. It is possible that SMF is a degradation product of the high- M_r glycoprotein, but this would appear to be unlikely, since the SMF is heat stable. Another possibility is that the SMF is the carbohydrate moiety of the high- M_r glycoprotein, and that this is the active part of the amoebal-plasmodium transition factor.

SMF appears to be produced only by MA185 amoebae, and not by RSD4 amoebae. When RSD4 amoebae are exposed to SMF, their surface changes such that their partition is the same as MA185 amoebae. This does not necessarily imply that the surfaces of the two types of amoebae are now the same, since different surface characteristics could conceivably produce similar partition. However, it is possible that the surfaces of the two mating-types do become similar as a result of the action of SMF. As a consequence, the ability of the two amoebae to fuse may be increased. The phase system used was a 'low potential' type which detects non-charge-related surface differences, usually referred to as changes in surface hydrophobicity, with an increase in partition coefficient reflecting an increase in surface hydrophobicity (Walter, 1977). The increased partition of RSD4 amoebae in contact with SMF represents an increase in surface hydrophobicity, which may be a prerequisite for fusion. MA185 amoebae are continually producing and secreting SMF and hence would be constantly under its influence, which may imply that the difference in surface character between the two amoebal types during normal axenic growth is due to the presence or absence of SMF.

The effects of SMF are relatively rapid, surface changes being detected after only 2 h. It is conceivable that transcription, translation or transport of specific surface proteins could be affected within this time. It is also possible that the SMF-induced surface changes may result directly from the binding or adsorption of SMF to the cell surface. Several differences in plasma membrane protein composition between MA185 and RSD4 have been reported (Reiskind & Aldrich, 1984), and we are currently attempting to ascertain whether SMF can specifically alter cell surface protein composition by affecting transcription or translation.

REFERENCES

- COLLINS, O. R. (1979). Myxomycete biosystematics: some recent developments and future research opportunities. *Botanical Review* **45**, 146-201.
- DEE, J. (1984). Genetics of *Physarum polycephalum*. In *Cell Biology of Physarum and Didymium*, pp. 211-251. Edited by H. Aldrich & J. Daniels. New York: Academic Press.
- GORMAN, J. A. & WILKINS, A. S. (1980). Developmental phases in the life cycle of *Physarum* and related myxomycetes. In *Growth and Differentiation in Physarum polycephalum*, pp. 157-202. Edited by E. F. Dove & H. P. Rusch. Princeton NJ: Princeton University Press.
- MCCULLOUGH, C. H. R. & DEE, J. (1976). Defined and

- semi-defined media for the growth of amoebae of *Physarum polycephalum*. *Journal of General Microbiology* **95**, 151–158.
- NADER, W. F., SHIPLEY, G. L., HUETTERMANN, A. & HOLT, C. E. (1984) Analysis of an inducer of the amoebal-plasmodial transition in the Myxomycetes *Didymium iridis* and *Physarum polycephalum*. *Developmental Biology* **103**, 504–510.
- REISKIND, J. B. & ALDRICH, H. H. (1984). Comparison of isolated plasma membranes of compatible mating strains of *Physarum polycephalum* myxamoebae. *Experimental Mycology* **8**, 105–116.
- SHARPE, P. T. & GOODMAN, E. M. (1986). Differences in the surface properties of the mating types of *Physarum polycephalum*. *European Journal of Cell Biology* **40**, 248–250.
- WALTER, H. (1977). Partition of cells in two-polymer phases: a surface affinity method for cell separation. In *Methods of Cell Separation*, vol. 1, pp. 307–354. Edited by N. Catsimopoulos. New York: Plenum Press.
- YOUNGMAN, P. J., ADLER, P. N., SHINNECK, T. M. & HOLT, C. E. (1977). An extracellular inducer of asexual plasmodium formation in *Physarum polycephalum*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 1120–1124.
- YOUNGMAN, P. J., ANDERSON, R. W. & HOLT, C. E. (1981). Two multiallelic mating compatibility loci separately regulate zygote formation and zygote differentiation in the myxomycete *Physarum polycephalum*. *Genetics* **97**, 513–536.