of 0.2 m*M* DNP. In normal ASW membrane potential is dependent on the external K^+ concentration to a much greater extent in light than in darkness, as reported earlier (*4*). In the presence of DNP this disparity is greatly reduced, and membrane potentials both in the dark and in light show a strong dependence on the external K^+ concentration. The same kind of increased dependence of membrane potential in darkness on the external K^+ concentration was observed when 2 m*M* cyanide was used instead of DNP. We have not done experiments to determine whether the slight reduction in the potential reached by the peak of the light response in the presence of DNP or cyanide is due to a decrease in internal K^+ concentration or to interference with some earlier step in the transduction process.

The hyperpolarization of membrane potential, the increase in membrane conductance, and the increased responsiveness of the membrane to changes in the external K^+ concentration indicate that the P_K of the photoreceptor membrane increases in the presence of DNP or cyanide. We cannot completely exclude the possibility that these compounds have a direct effect on membrane permeability since DNP, but not cyanide, has been reported to increase the P_K of nerve cell membranes (*7*). However, the finding that both DNP and cyanide increase P_K in our cells makes an alternative hypothesis more likely.

The similarity of the effects of DNP and cyanide, and the lack of effect of ouabain or IAA, suggest that the observed permeability changes result from interference with the production of adenosine triphosphate (ATP) by oxidative phosphorylation. The small size and large surface-to-volume ratio of these photoreceptors make it likely that ATP stores are quickly depleted when DNP or cyanide are applied. An increase in intracellular Ca^{2+} concentration has been observed in other cells following exposure to either DNP or cyanide (*8*). This is to be expected since active uptake of Ca^{2+} from the cytoplasm by the mitochondria is an ATP-dependent process (*9*). In several types of cells there is evidence that increasing the intracellular Ca^{2+} concentration, either directly or by treatment with metabolic inhibitors (*5, 10*), causes an increase in P_K. We suggest, therefore, that the hyperpolarization and increase in K^+ conductance observed in the presence of DNP and cyanide is due to an increase in intracellular Ca^{2+} concentration. Since the receptor potential in these cells is also generated by an increase in P_K, it is possible that light-induced changes in intracellular Ca^{2+} concentration control P_K in those photoreceptors whose hyperpolarizing response is due to an increase in conductance (*11*).

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12. Much of this work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts. Supported in part by NIH grant EY01157.

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Blepharmone: A Conjugation-Inducing Glycoprotein in the Ciliate Blepharisma

Abstract. *Gamone 1 of* Blepharisma intermedium *was isolated, identified as a slightly basic glycoprotein (molecular weight, 2×10^4), and designated as blepharmone. At the concentration of 6×10^{-8} milligram per milliliter, it specifically transforms mating type 2 cells, so that they can conjugate in about 2 hours.*

Conjugation of *Blepharisma intermedium* is induced by a few hours of interaction between cells of complementary mating types (*1, 2*). Type 1 cells excrete gamone 1, which transforms mating type 2 cells so that they can unite and, at the same time, induces them to produce and excrete gamone 2. This gamone in turn transforms type 1 cells so that they can unite, and it also promotes the production and excretion of gamone 1. Transformed cells can unite in all three possible combinations of mating types but only heterotypic pairs (1–2) complete conjugation. Homotypic pairs (1–1, 2–2) may persist for days, but the process of conjugation appears to stop at the stage of pair formation. Gamone 2, blepharismone (*3*), was isolated, identified as calcium-3-(2′-formylamino-5′-hydroxybenzoyl)lactate (*4*), and synthesized (*5*). Gamone 1 was suggested to be a protein with a molecular weight of about 2×10^4 (*2*). We have isolated and partially characterized this gamone, designated as blepharmone.

Mating type 1 cells were grown (*2*), concentrated, washed with SMB (1.5 m*M* NaCl, 0.05 m*M* KCl, 0.4 m*M* $CaCl_2$, 0.1 m*M* $MgSO_4$, 2×10^{-3} m*M* EDTA, 2 m*M* sodium phosphate buffer of *p*H 6.8), and suspended in SMB containing 32 units of gamone 2 per milliliter (*6*) and 0.01 percent albumin (bovine serum albumin, Behringwerke) at the density of 0.01 ml of packed cells per milliliter (about 10^4 cell/ml). Albumin was added to protect the gamone activity of blepharmone (*2*). After the suspension was kept for 1 day at 25°C, the cell-free fluid was obtained by removing cells by centrifugation. The cells were resuspended as described above, and the process was repeated for several days. The gamone 1 activity of the cell-free fluid was 0.8×10^4 to 12.8×10^4 unit/ml (*6, 8*); the cell-free fluid could be stored frozen for a few months without change in the activity.

The cell-free fluid (20 liters) having

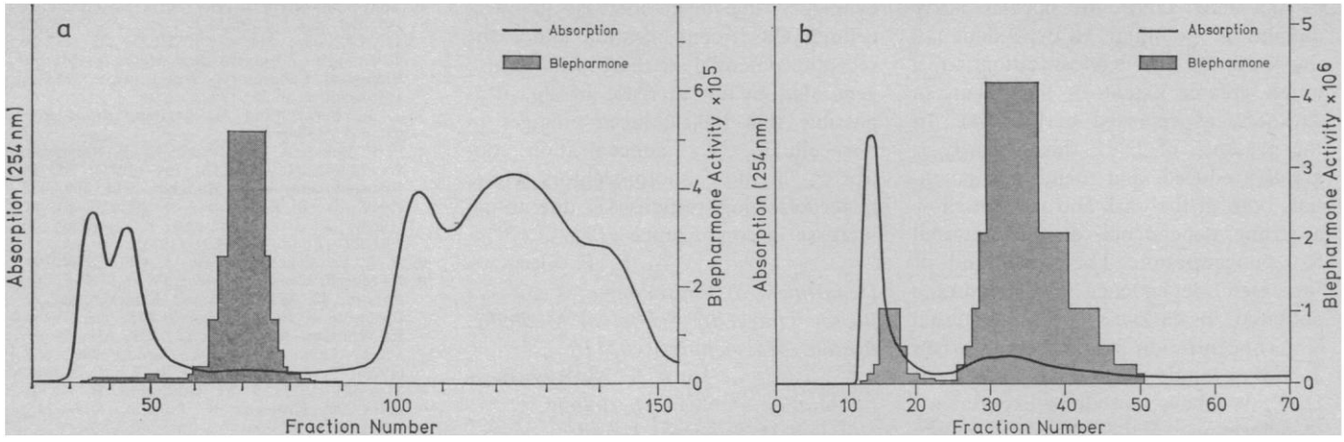

Fig. 1. Elution patterns of crude blepharmone samples. Chromatography at 4°C; the fraction size was 6 ml. (a) Bio-Gel P-150 (100 to 200 mesh) was used. The column size was 5 × 40 cm, and the elution medium contained SMB plus 0.01 percent albumin. (b) Carboxymethyl cellulose (Whatman, CM-52) was used. The column size was 2.5 × 35 cm. The equilibration medium was SMB, *p*H 5.8 (the NaCl was omitted, and the phosphate buffer was increased to 4 m*M*). The elution medium consisted of the equilibration medium plus 0.01 percent albumin. Before the sample application, the column was eluted with 2 liters of elution medium.

an average activity of 3.2×10^4 unit/ml was concentrated at reduced pressure to 1 liter at 25°C or below, and lyophilized. The lyophilized material was dissolved in 100 ml of H_2O, and centrifuged at 10,000*g* for 30 minutes. The supernatant was divided into five portions, each of which was separately chromatographed on Bio-Gel P-150 (Fig. 1a). The fractions in the gamone activity peaks of the five portions were pooled, lyophilized, and chromatographed again as described above. The fractions in the activity peak were lyophilized, dissolved in SMB (*p*H 5.8) in which NaCl was omitted and phosphate buffer was increased to 4 m*M*, and dialyzed for 1 day at 4°C against this buffer. The dialyzate (20 ml) was chromatographed on carboxymethyl cellulose (Fig. 1b). The blepharmone was eluted in two peaks, but about 90 percent of the activity was found in the second peak. The fractions in this peak were lyophilized, dissolved in SMB (*p*H 7.6), and dialyzed for 1 day at 4°C against this buffer. The dialyzate (10 ml) was applied to a column (2.5 by 34 cm) of diethylaminoethyl-cellulose (Whatman DE 52) equilibrated with SMB (*p*H 7.6) and eluted with the same buffer. Blepharmone was eluted at the front with a small absorption peak recorded at 254 nm. The blepharmone sample obtained by lyophilizing the fractions in this peak contained 2.0 mg of protein (*7*) and showed an activity of 3.2×10^7 units. On the assumption that the real activity of blepharmone in the starting material was 8×10^7 units (*8*), the recovery was 40 percent (*9*).

This sample was subjected to acrylamide gel electrophoresis by a modified method of Gordon and Louis (*10*) and the method of Davis (*11*), which gave different band patterns for a less purified sample of blepharmone. For each run, 1/40 of the purified sample described above was used. In both methods, only a single band was strongly stained by amido black (Fig. 2a), and most of the gamone activity was found there. The rest of the activity was at the origin. If stained by the periodic acid–Schiff's reagent (PAS) for the detection of carbohydrate (*12*), a single band was seen at the position stained by amido black. Comparing the intensity of the PAS reaction of the band with that of orosomucoid and β_2-glycoprotein 1 (Behringwerke), the carbohydrate in the electrophoresed material was estimated to be 5 percent of the protein. The sample was also subjected to isoelectric focusing. For each run, 1/40 of the sample was used. Only a single band was strongly stained. In addition, two or three hardly visible bands in the more acidic region were observed (Fig. 2b). The peak of the blepharmone activity was at the position of the strongly stained band. By measuring the *p*H and blepharmone activity of a gel cut into 3-mm thick slices the isoelectric point of blepharmone was determined to be *p*H 7.5. This value is consistent with the chromatographic

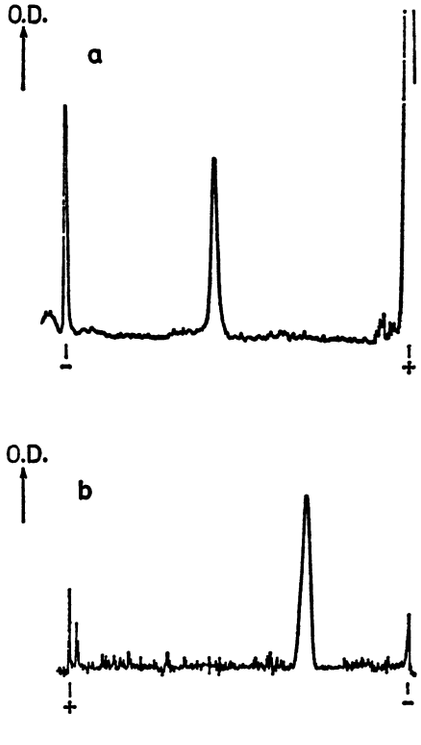

Fig. 2. Electrophoresis and isoelectric focusing of purified blepharmone. The optical density (*O.D.*) was measured with a microdensitometer (Joyce, Loebl MK IIIC) with a red filter (No. 620). The symbols + and — indicate the anodal and cathodal ends of the gel. (a) Electrophoresis in a polyacrylamide gel column (5 × 62 mm) with a modified system of Gordon and Louis (*10*). The desalted sample was applied in 0.2 ml of 0.05*M* tris·HCl buffer, *p*H 6.8, containing 25 percent Ficoll (Pharmacia). Fixation and staining: 7 percent acetic acid, 1 percent amido black. (b) Isoelectric focusing in a polyacrylamide gel column (5 × 100 mm) with a modified system of Righetti and Drysdale (*17*); 4 percent Ampholine (LKB), *p*H 3 to 10, was used. After fixation and removal of Ampholine (*17*), the gel was stained with 0.05 percent Coomassie blue in 10 percent trichloroacetic acid (*18*).

behavior of blepharmone on the ion exchange celluloses described above.

From these results it is concluded that blepharmone is a slightly basic glycoprotein and that it has been isolated almost free of other proteins. Although the purity of the sample was not rigorously tested for organic substances other than protein, the purification procedures described above are likely to have removed most of them. Assuming that the 2.0 mg of protein detected in the purified sample is all active blepharmone, we calculate that its specific activity is 1.6×10^7 unit/mg; that is, this glycoprotein can induce cell union at 6×10^{-8} mg/ml in mating type 2 cells (10^3 cell/ml).

The molecular weight of blepharmone was estimated by the pressure filtration method. Blepharmone dissolved in SMB passed freely through the Amicon PM-30 membrane but was retained (about 95 percent) by the PM-10. To avoid absorption of blepharmone to the membrane, a preliminary run was made for 5 minutes with SMB containing 1 percent albumin, which did not appreciably change the flow rate. Since these membranes retain globular molecules with a molecular weight larger than 3×10^4 and 10^4, respectively (*13*), the molecular weight of blepharmone should be 1×10^4 to 3×10^4. This value is consistent with the previous value of 2×10^4 measured by the gel filtration method (*2*).

Both components of a pair of gamones of *B. intermedium* have now been isolated and characterized. Unlike gamone 2 or blepharismone, which is calcium-3-(2′-formylamino-5′-hydroxybenzoyl)lactate, a derivative of formylkynurenine (*4*), gamone 1, blepharmone, is a much larger molecule and is a glycoprotein. This striking chemical difference in a pair of specific substances participating in a cell interaction may be compared to that seen in the feedback interaction between thyroid gland and pituitary gland by thyroxine (amino acid) and thyroid stimulating hormone (glycoprotein).

Gamones are known in other species of protozoa (*14, 15*) but gyno- and androgamones of *Chlamydomonas eugametos* (*16*), a chlorophyll-bearing flagellate, are the only other gamones so far isolated and identified in protozoa. They are both large glycoproteins with a molecular weight of 10^8 and are the material basis of the agglutination between complementary mating types. Blepharmone and blepharismone differ from these gamones not only by their molecular size but also by their function. They are not the material basis of agglutination but are hormone-like substances which transform specific types of cells so that they can form a cell union and also induce them to produce their own gamone. Isolation and characterization of both of these gamones are expected to facilitate the study of cell interaction by means of specific substances as well as the study of the molecular mechanism of conjugation.

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7. The protein content was measured by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin as standard.
8. When 0.01 percent of albumin was contained in a sample, its gamone 1 activity was four to eight times higher (*2*).
9. Blepharmone is very unstable in a diluted state, and successful purification was achieved only under the continuous presence of albumin. Thus both Bio-Gel and carboxymethyl cellulose columns were eluted with a buffer containing albumin. When albumin was removed in the last step of purification, blepharmone was no longer so unstable probably because its concentration was high enough to protect itself.
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Neurochemical Correlate of a Spatial Preference in Rats

Abstract. *Spatial (left or right) preferences were determined for rats given foot shock in a T-maze. The animals were killed, and left and right striata were assayed separately for dopamine and left and right teldiencephalic regions were assayed for norepinephrine. Dopamine content was significantly higher (by 12 percent) in the striata contralateral to rats' side preferences than in the ipsilateral striata; there was no such difference for teldiencephalic norepinephrine. The small asymmetry in striatal dopamine content is not due to any learning- or stress-related change induced by the testing procedure but is probably inherent in normal rats. Some spatial behavior appears to be the manifestation of a normal and specific difference in the activity of left and right nigrostriatal systems.*

Electrical stimulation or lesions of the dopaminergic nigrostriatal system disrupt normal motor (*1*) and associative functions (*2*). Unilateral lesions in the nigrostriatal pathway, for example, induce a rotary behavior in rats characterized by circling toward the side of the lesion. Drugs such as amphetamine that release dopamine (*3*) or drugs such as apomorphine that directly activate dopaminergic receptors (*4*) in the striatum potentiate such rotation or elicit rotation when animals have recovered from the initial tendency to rotate spontaneously. The rotation has been attributed to an imbalance of the left and right nigrostriatal systems induced by the unilateral lesion (*3*). Drugs can enhance this imbalance by differentially activating the intact and damaged pathways (*3, 4*). We demonstrated