Purification and Preliminary Characterization of an Aggregation-Sensitive Chemoattractant of Dictyostelium minutum

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Aggregative amoebae of Dictyostelium minutum are not attracted by cyclic AMP; they are sensitive to various attracting sources from which yeast extract was chosen to purify the chemoattractant. A small acrasin-like species-specific molecule which contains glycine and C₅H₈N₆ has been purified 30,000-fold. Several characteristics of this chemotactic molecule, which is inactivated by an enzyme that is not species specific, are described.

To understand the mechanism of chemotactic movement during cell aggregation, identification of the attractants (acrains) (1) is urgently needed. Only one acrasin, cyclic AMP, has been identified (5). Cyclic AMP attracts the sensitive amoebae of the larger species of Dictyostelium (3). Wurster et al. purified the Polysphondylium acrasin and concluded that the chemotactic molecule in this genus probably is a peptide (8). The chemotactic activity of folic acid and its derivatives seems to be limited to a food-seeking device for the amoeba (7). Recently, we started to isolate and purify acrasins of the more simple species of Dictyostelium, in which there is no evidence of signal relay and pulsating cell movement during aggregation. The preliminary characterization of the Dictyostelium lacteum acrasin indicates that this molecule is small sized, heat stable, negatively charged, aromatic, and different from all other known attractants for amoebae of the genera Dictyostelium and Polysphondylium (6). In this paper, characteristics of an acrasin-like substance for Dictyostelium minutum will be described.

The obvious choice for isolating acrasin, the amoebae themselves, did not produce sufficiently large quantities of attractant for purification and characterization. From other active sources such as bacterial extracts, yeast, urine, and milk, the easily available and very active source, yeast extract, was chosen. The 100-fold higher activity of the purified yeast extract attractant at the aggregative stage than at the vegetative stage gave strong evidence that chemotaxis was due to an acrasin-like substance. The chemotaxis was measured by deposition of small drops of pregrown, suspended D. minutum amoebae on a hydrophobic agar surface (2). The cells were incubated at 22°C in darkness. Within 1 h after deposition and shortly (less than 0.5 h) before aggregation, 0.1-μl drops of crude or purified active material were placed twice, with a 5-min interval, at a distance of 100 to 300 μm from the amoebal populations. The response of the amoebae was measured 15 and 30 min after the second deposition of the active extract and was marked positive when at least twice as many cells were pressed against the side closest to the attracting drop as against the opposite side (2). Amoebae close to the aggregative stage were used for testing the fractions because of their high sensitivity to the chemoattractant(s). Batches of 150 g of yeast extract (Difco) in 250 ml of water were cooled to 4°C, precipitated with 250 ml of 90% ethanol, and centrifuged at 2,400 × g for 10 min. The supernatant was concentrated under low pressure at 40°C to 250 ml. Fifty-milliliter samples of this fraction were mixed with 50 g of diethylaminoethyl(DEAE)-Sephadex A-25 and shaken at 22°C for 30 min at pH 5.0. The gel was washed on a filter with 150 ml of water, shaken with 150 ml of 0.05 M HCl, and washed again with 150 ml of 0.05 M HCl (pH 3.0). By washing the gel two times with 150 ml of 0.1 M HCl (pH 1.0), the chemotactic activity dissociated from the gel. The two washings were pooled, concentrated to dryness at 40°C under low pressure, dissolved in 3 ml of water, and chromatographed on a Sephadex G-10 column (bed volume, 210 ml; 48 ml/h; 8 ml/tube) equilibrated with water at pH 6.0. The chemoattractant(s) came off shortly after the void volume (V₀), indicating a size less than 700 daltons. The chemotactically active fractions were pooled, freeze-dried, solved in 3 ml of 10 mM phosphate buffer, and chromatographed on a DEAE-Sephadex A-25 column with a linear sodium chloride gradient. Much UV-active non-
chemotactic material was removed this way (Fig. 1). The pooled active tubes containing 0.36 M NaCl, pH 6.0, were bound to a Dowex AG-1X2 ion-exchange column equilibrated with water to remove the salts. The gel had been washed with 100 ml of water and 100 ml of 0.001 M HCl. The active material had been removed by passing 200 ml of 0.1 M HCl through the column. The eluate was concentrated and passed through a Dowex AG-1X2 column with a pH gradient (Fig. 2). The active fraction, which came off in tubes 25 to 28 at a pH of around 1.6, was pooled and concentrated. Thin-layer chromatography (silica gel IB2-F, Baker-flex) with different solvents revealed that in water this fraction contained at least four different UV spots from which one (Rf, 0.56) contained the chemotaxant(s). Chromatography with other solvents resulted in fewer UV spots except with n-butyl alcohol-acetic acid-water (50:15:35), which solvent also gave four UV spots but with the active spot close to the origin. The active UV spot obtained with water as solvent was removed and used for further characterization of the attractant. The threshold activity (0.53 μg/ml) of the final active product(s) was lower than the threshold activity of the purified D. lacteum acrasin, which indicates that chemotaxis by this D. minutum attractant occurs at physiological concentrations. The chemotactic response was species specific. Vegetative and aggregative amoebae of Dictyostelium discoideum, D. roseum, D. purpureum, D. mucoroides, D. lacteum, D. polyccephalum, and P. violaceum were not attracted by the purest fraction. Only the first four species reacted positively to cyclic AMP. Both tested strains of D. minutum, V3 and G, kindly provided by, respectively, K. B. Raper and G. Gerisch, showed close-to-aggregation a similar increase in chemotactic response. Besides this specificity are the chemical characteristics different from known attractants. Loss of activity (96%) during purification may be due to attractants in minor activity peaks, which were not further characterized, and to folic acid or its analogs, which are particularly attractive to D. minutum amoebae (4, 8). Incubation with beef heart cyclic nucleotide phosphodiesterase (Boehringer; 0.2 mg/ml, final concentration) at 37°C for 90 min did not inactivate the purified acrasin-like attractant from yeast extract. Incubation with its own acrasinase for the same period at 37°C destroyed the chemotactic activity. The acrasinase of D. minutum was obtained by shaking 10⁸ cells per 10 ml in 1% Bonner salt solution (8) at 22°C for 90 min and centrifuging and freeze-drying the supernatant. The 10-μl samples inactivated 50 μl of the purified attractant (5 μg/ml, pH 7.5) after incubation at 22°C for 1 h. The chemotactic activity was not destroyed by a boiled amoebal supernatant. Besides D. minutum, D. discoideum also secreted an inactivating enzyme of the attractant. Incubation with Pronase (Merck; 0.2 mg/ml, final concentration) for 60 min at 37°C did result in loss in activity, which means that the attractant may be a small peptide of a size similar to the Polysphondylium acrasin (8). The attractant was ninhydrin test negative, but, after 6 N HCl hydrolysis for 24 h at 110°C, it became ninhydrin positive. The amino acid analysis of such a hydrolyzed sample revealed that the attractant contained glycine. Perhaps the D. min-
utum acrasin has, similar to Polysphondylium (8), a peptide structure. The characteristics of the ninhydrin reaction indicate that the amino terminal may be blocked. The UV activity may be due to the blocking group. The attractant of D. lacteum also seems to be an aromatic moiety bound to the amino terminal of a glycine molecule (J. M. Mato, P. J. M. van Haastert, F. A. Krens, and T. M. Konijn, unpublished data). Mass spectrography of D. minutum attractant showed, besides other peaks, an electronic mass peak of 135.0542, which represents a C\textsubscript{5}H\textsubscript{5}N\textsubscript{5} group.

The similarities of the acrasin-like substance of D. lacteum and its other characteristics suggest that the purified attractant is chemotactically functional during cell aggregation in D. minutum.

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