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事業内容

- 基礎研究用試薬・体外診断用医薬品・動物用医薬品・化学工業薬品の販売
- 理化学機器・医療用機器・分析用機器・その他機器、器具の販売・修理
- 家電製品・コンピュータおよび医療関連ソフトウェアの開発・販売

swimming speed and direction of the individual cells with respect to the gravitational vector was analyzed.
Gravikinesis, defined as the active control of the propulsive thrust depending on the swimming direction, was observed when the magnitude of gravity was more than 0.2xg. The extent of gravikinesis increased with increase in the magnitude of the gravitational force. On the other hand, gravitactic characteristics assessed by the vertical distribution of the cells did not change significantly during partial gravity.
Downward plume of convective motion disappeared when the magnitude of gravity was less than 0.6xg, although we suspect that the plume might have disappeared even in the grater magnitude of partial gravity, if the partial gravity had been prolonged beyond the limit of flight maneuver. These results may imply that gravity plays different roles in collective motion and in individual swimming.

497 [Physiology] [Protozoa]

Re-exploring gravitactic mutant strains of the unicellular green alga Chlamydomonas reinhardtii

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How negative gravitaxis, biased swimming against gravity, emerges in unicellular protists has been controversial in the fields of protozoology, fluid mechanics and gravitational biology for more than a century. The "passive" mechanisms are pure mechanics due to the fore-aft asymmetry of the body, and emerged even in the non-living, self-propelled colloid particles (ten Hagen et al., 2014), while the "active" physiological mechanism has also been proposed. For relatively large protists such as *Paramecium* and *Euglena*, the "active" mechanism seems to take place (Häder et al., 2005). The problem now I am focusing on is how about *Chlamydomonas reinhardtii*: a unicellular green alga with the cell body of just 10 μm in diameter and with rigid cell wall. Previously we showed that two kinds of "passive" mechanisms actually worked in *C. reinhardtii*: combining the experimental and the computational approaches, we dissected which passive mechanism worked more in the actual *C. reinhardtii* (Kage et al., submitted; ZSJ2014). How about the "active" physiological mechanism? We do have a clue.
Yoshimura et al. (2003) isolated two gravitactic mutant strains of *C. reinhardtii*, which they named *gtx1* and *gtx2*. Except weaker negative gravitaxis than that of the wild type, they reported that both of the strains do not have anomalies in morphology or most of the major swimming phenotypes. Unexpected from the original description, the strains having been kept in the Mogami Lab, Ochanomizu University (Tokyo, Japan), have "obtained" negative phototaxis or "agging" phenotype, and the *gtx2* strain sometimes could not be distinguished from the wild type in gravitaxis when reassessed in 2015. From the PCR analysis, it was turned out that the "agging" mutation in the *gtx* strains was different from the *agg1* mutation in the CC124 strain, which is sometimes treated as the "wild type" of *C. reinhardtii* in motility. The unindentified "agging" mutation was segregated from *gtx1*. In addition, our data so far suggested that the *gtx2* strain has a tendency of temperature-sensitivity, which might have confused our initial reassessment. The relationship between *gtx1* and *gtx2* is being investigated.

498 [Physiology] [Protozoa]

Arginine kinase (AK) and arginine phosphate shuttle in Paramecium tetraurelia.

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Arginine kinase (AK) is the enzyme that catalyzes reversible transfer of gamma phosphoryl group of ATP to arginine yielding ADP and a phosphorylated arginine.
The ciliate *Paramecium tetraurelia* contains four AK genes (AK1, AK2, AK3 and AK4). In this study, we confirmed that three of the four genes (AK1, AK2 and AK3) are expressed in the cell by detecting their mRNAs. The recombinant enzymes of the four AKs were expressed in *Escherichia coli* and their kinetics parameters were determined.
The AK3 of *P. tetraurelia* showed a typical substrate inhibition toward arginine, namely that the enzyme activity was markedly decreased when the concentration of the substrate arginine was increased. This is the first finding of substrate inhibition in AKs. To explore the mechanism of substrate inhibition, the site-directed mutagenesis was introduced in the amino acids sequence D-D-S-Q-V at position 77-81 in AK3, and the kinetics parameters of the mutants were determined. The three mutants, D78A, S79A and V81A showed a strong substrate inhibition, like the wild-type AK3, but the substrate affinity for arginine was increased about 10-fold, while the substrate inhibition of the S79A mutant was almost disappeared. These results indicate that the residue S79 is responsible for the substrate inhibition mechanism in AK3.
AK3 and AK4 show 91% identity in the amino acid sequences. However, the catalytic constant, k_{cat}, (or V_{max}) of AK4 was extremely low when compared with that of AK3 (only 3%). From the sequence alignment of AKs, the unusual amino acid replacement of the conservative residue Gly at position 298 by Arg was observed in the AK4. We constructed two mutants, G298R in AK3 and R298G in AK4. The k_{cat} of the R298G in AK4 was remarkably increased and the value was almost the same as that of wild-type AK3, while the k_{cat} the G298R in AK3 was considerably reduced. Thus we concluded that the low k_{cat} value in AK4 is due to the R298.
Interestingly, the amino acids sequences of AK1, AK3 and AK4 were found to have typical prenylation signal sequences in C-terminal regions. In order to determine whether the enzymes are prenylated in the native form or not, the AK1 and AK3 were synthesized by cell-free protein synthesis system in the presence of farnesyl diphosphate, and their tryptic peptides were analyzed by peptide mass fingerprinting (PMF). Although the prenylated peptide of AK1 was not observed, the target peptide of AK3 was obtained by PMF analyses. Thus, *P. tetraurelia* AK3 may be farnesylated also in vivo and would be anchored to membrane. Western blot analyses indicated that the cilia of *P. tetraurelia* contain AK3.
Noguchi et al. (2001) demonstrated that phosphoarginine supplies energy for ciliary beating in the ciliate *P. caudatum*, suggesting that it functions not only reservoir of energy but also as a transport of energy in conditions that continuously consume energy (phosphoarginine shuttle). They prepared an intact ciliated cortical sheet from live *P. caudatum*. The AK activity was clearly observed on this sheet, indicating that some of the AK enzymes are attached to the membrane matrix fraction. Our work suggests that the attached AK is the AK3 in *P. tetraurelia* and the AK enzyme is anchored to the membrane by farnesylated group.

499 [Behavior] [Mammalia]

Biphonic vocalizations in canids: considering anatomical sources of frustration calls

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Biphonation, i.e. two independent fundamental frequencies in a call spectrum, is a prominent feature of vocal activity in dog-like canids: African wild dogs *Lycaon pictus*, Asiatic wild dogs or dholes *Cuon alpinus*, timber wolves *Canis lupus*, domestic dogs *C. lupus familiaris*, dingos *C. lupus dingo* and red wolves *C. rufus*. Dog-like canids can produce a low (f0) and a high (g0) fundamental frequencies simultaneously. In our study of domestic dog frustration whines, the biphonic calls comprised 451 (17.0%) of 2643 whines recorded from nine individual dogs, from 0 to 39% of calls depending on individual. In our study of dhole vocalizations, the biphonic calls comprised 583 (44.3%) of 1317 contact calls recorded from 14 individual dholes, from 20 to 92% of calls depending on individual. In domestic dogs, the range of the low fundamental frequency is 0.4-1.4 kHz and the range of the high fundamental frequency is 3.1-11 kHz. In the dhole, the range of the low fundamental frequency is 0.5-1.4 kHz and the range of the high fundamental frequency is 5.5-10.7 kHz. In contrast to dog-like canids, biphonic calls are lacking in all studied fox-like canids: red fox *Vulpes vulpes*, swift fox *V. velox* and Arctic fox *V. lagopus*. The fox-like canids are only capable of producing the low fundamental frequency (f0). In our study of red fox, a detailed analysis of 12,964 whines recorded from 75 individuals did not reveal one single biphonation. In red fox, the range of the low fundamental frequency is 0.32-1.21 kHz, while the high fundamental frequency is missing. To reveal macroscopic structures potentially responsible for canid biphonation, we used a comparative anatomical approach. We investigated the vocal anatomy for 4 (1 male, 3 female) captive dholes and for 2 (1 male, 1 female) wild red fox. In addition, we analyzed the acoustic structure of vocalizations in the same dholes that served postmortem as specimens for the anatomical investigation. All study dholes produced both high-frequency and biphonic calls. The anatomical reconstructions revealed that the vocal morphologies of the dhole are very similar to those of red fox. These results suggest that the high-frequency and biphonic calls in dog-like canids can be produced without specific anatomical adaptations of the sound-producing structures. We discuss possible production modes for the high-frequency and biphonic calls involving laryngeal and nasal structures. This study was supported by the Russian Science Foundation, grant 14-14-00237 (to IAV, EVV and SSG).

500 [Behavior] [Mammalia]

Anatomical and vocal divergence between sexes in a ungulate species with prominent and descended larynx in males: parallels with "Adams apple" of humans

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Male goitred gazelles *Gazella subgutturosa* bear a handicap of an en-