The Energetic Cost of Limbless Locomotion

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CTX will allow these ideas to be tested through site-directed mutagenesis, and the structure of such mutants can be routinely determined by 2-D NMR spectroscopy.

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6. All 2-D NMR experiments were performed by collecting 2048 (t_2) by 960 (t_1) points: the data were zero-filled twofold in each dimension to give a Fourier-transformed data set containing 2048 by 1024 points. Experiments were done with 32 to 96 acquisitions per t_1 point, and data were processed with moderate convolution difference in both dimensions. COSY experiments used a 2-s relaxation delay, and H_2O COSY used saturation of the H_2O resonance during the delay. D_2O HOHAHA was performed with a 32-ms mixing period, 1-ms trim pulses, and a 2-s relaxation delay. H_2O HOHAHA experiments used a selective observation pulse and a spoil pulse after the 48-ms mixing period (with 1-s relaxation delay). The double-quantum experiment was performed in H_2O with a 22-ms coherence delay, and water was saturated for 0.6 s of the 1-s relaxation delay period. The D_2O NOESY was performed with 300-ms mixing time and a 2-s relaxation delay. H_2O NOESY experiments used mixing periods of 100, 150, 300, and 600 ms.
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18. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and Z, pyGlu.
19. The atomic coordinates of the structure shown in Fig. 4 have been deposited in the Brookhaven Protein Data Bank. We thank D. Ringe for use of a Silicon Graphics computer, I. Papayannopoulos of the MIT mass spectroscopy facility for performing FAB-MS of CTX, and H. M. Massefski for drawing Fig. 3. Supported by NIH grants GM-31768 (C.M.) and GM-20168 (A.G.R.). The FAB-MS facility is supported by the NIH Division of Research Resources Grant RR0031.

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The Energetic Cost of Limbless Locomotion

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The net energetic cost of terrestrial locomotion by the snake *Coluber constrictor*, moving by lateral undulation, is equivalent to the net energetic cost of running by limbed animals (arthropods, lizards, birds, and mammals) of similar size. In contrast to lateral undulation and limbed locomotion, concertina locomotion by *Coluber* is more energetically expensive. The findings do not support the widely held notion that the energetic cost of terrestrial locomotion by limbless animals is less than that of limbed animals.

SPECIES WITH REDUCED LIMBS OR NO limbs and elongate bodies have evolved independently from limbed antecedents in several groups of vertebrates: salamanders, caecilians, amphisbaenians, lizards, and snakes (*1*). An important factor proposed to explain the evolution of limblessness is its presumptively low energetic cost, such that energetic expenditure during locomotion by limbless animals is expected to be less than that of limbed animals of similar size (*1, 2*). Biomechanical arguments advanced in support of the low energetic cost of limbless locomotion include no costs associated with vertical displacement of the center of gravity (*1, 3, 4*), no costs to accelerate or decelerate limbs (*3*), and low cost for support of the body (*1*). A preliminary study, published only as an abstract, reported that the energetic cost of locomotion of the garter snake (*Thamnophis sirtalis*) was only 30% of that predicted for a quadrupedal lizard of similar size (*5*). Although that study was preliminary, it has been widely cited in review articles (*1–3, 6, 7*) and textbooks (*4, 8*) in support of mechanical arguments for the low cost of limbless locomotion. We sought to test the generality of these conclusions by examining the energetic cost of locomotion in a snake, the black racer (*Coluber constrictor*).

A snake may utilize a variety of locomotor modes, depending on both speed and surface encountered (*4, 9–11*). Lateral undulation and concertina locomotion are two common modes that use lateral vertebral movements to generate propulsive forces. During lateral undulation on the ground, snakes move along an approximately sinusoidal trajectory. Bends in the body contact with the substrate and push posteriorly on projections from the ground, propelling the body forward. All parts of the body move simultaneously with the same overall speed, while forward and lateral components of velocity change as a result of the sinusoidal trajectory (*10, 11*). Snakes moving with lateral undulation experience only sliding contact with the ground (*4, 12*). In narrow passageways such as tunnels, snakes often perform concertina locomotion exclusively (*4*). Snakes performing concertina locomotion stop periodically, and certain parts of the body are moved forward while others maintain static contact with the ground. In passageways, snakes alternately press themselves against the sides by forming a series of bends and then extend themselves forward from the region of static contact (*4, 13*). In comparison to lateral undulation, concertina locomotion involves higher momentum changes (*4*), resistance due to static (as well as sliding) friction (*4*), and usually slower forward speed (*11*) and, therefore, probably entails higher energetic costs.

Although these considerations logically suggest differential costs of the two locomotor modes, only if we directly determine the metabolic rates of moving animals can these be verified and compared to anticipated values for limbed animals. In the current study, we measured energy expenditure as the rate of oxygen consumption of snakes at rest ($\dot{V}O_{2\ rest}$), in the moments just before locomotory exercise ($\dot{V}O_{2\ pre\text{-}ex}$), and during locomotion at several speeds (0.2 to 1.0 km hour^{-1} for lateral undulation, 0.06 to 0.14 km hour^{-1} for concertina locomotion) on motorized treadmills (*14, 15*). Endurance, measured as time sustained on tread, as a function of speed and locomotor mode was also determined (*16*). Videotapes were used to verify locomotor mode and to correlate frequency of movement with energy expenditure. By dividing oxygen consumption by frequency of movement, we estimated energetic costs of single cycles of lateral undulatory and concertina movement.

The metabolic response of $\dot{V}O_2$ to speed in *Coluber constrictor* [mass = 102.8 ± 6.1 (SE) g, n = 7] locomoting by lateral undulation is similar to that observed in many terrestrial vertebrates with limbs (*17*): $\dot{V}O_2$ increases as a linear function of speed (*18*) throughout the range of sustainable speeds (0.2 to 0.5 km hour^{-1}), above which $\dot{V}O_2$ is constant and endurance decreases (speeds greater than 0.5 km hour^{-1}) (Fig. 1, A and B). Oxygen consumption also increased as a

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linear function of speed during concertina locomotion by *C. constrictor* (*19*) but with a greater slope than that of lateral undulation ($t = 3.67$, df = 28, $P < 0.001$, Fig. 1A). The point of intersection between the increasing and constant phases of the metabolic response plot defines the maximal rate of oxygen consumption ($\dot{V}O_{2\ max}$) and the lowest speed at which $\dot{V}O_{2\ max}$ is achieved, often termed the maximum aerobic speed (MAS) (*20*). *Coluber constrictor* performing lateral undulation achieves $\dot{V}O_{2\ max}$ = 0.83 ml of O_2 per gram per hour ± 0.06 ($n = 5$) at MAS = 0.5 km hour^{-1} (Fig. 1A). This $\dot{V}O_{2\ max}$ is nine times the resting rate and is similar to the maximum rate previously reported for this species (*21*). The MAS is one-tenth of the maximum burst speed (*22*) of these animals (mean maximum burst speed = 5.5 ± 0.4 km hour^{-1}). Endurance decreased too precipitously during concertina locomotion (Fig. 1B) to permit us to determine $\dot{V}O_{2\ max}$ and MAS for this locomotor mode.

Rates of oxygen consumption extrapolated to zero speed (the y intercept) are often elevated above resting rates, and earlier investigators have interpreted this increment as the energetic cost of postural support (*20*). For *C. constrictor* performing lateral undulation, the y intercept is elevated above zero ($t = 2.61$, df = 21, $P < 0.02$) but is indistinguishable from the resting ($t = 1.39$, df = 21, $P > 0.10$) or pre-exercise ($t = 0.33$, df = 21, $P > 0.50$) rates of oxygen consumption (Fig. 1A). The y intercept for the metabolic response during concertina locomotion is indistinguishable from zero ($t = 0.89$, df = 7, $P > 0.40$), $\dot{V}O_{2\ rest}$ ($t = 1.50$, df = 7, $P > 0.10$), and $\dot{V}O_{2\ pre\text{-}ex}$ ($t = 2.36$, df = 7, $P > 0.50$). The y intercepts for the metabolic responses during concertina and lateral undulation are also indistinguishable from each other ($t = 1.84$, df = 28, $P > 0.05$).

Snakes increase speed by increasing the number of cycles of movement per unit time (Fig. 2A). The frequency of lateral undulation continues to increase beyond the range of sustainable speed. Thus, undulation at nonsustainable speeds is probably supported by both aerobic metabolism and increasing contributions of anaerobic metabolism. The energetic cost of a single cycle of lateral undulation does not change within the range of aerobically sustainable speeds [$F(3, 9) = 2.57$, $P = 0.12$] (Fig. 2B) but does vary significantly among individual snakes [$F(3, 26) = 33.9$, $P < 0.001$] (*23*).

Concertina locomotion has an increased energetic cost compared to that of lateral undulation. The oxygen consumption of animals performing concertina locomotion exceeds $\dot{V}O_2$ predicted for snakes performing lateral undulation at similar speeds (Fig. 1A). Furthermore, endurance during concertina locomotion is much less than for lateral undulation at similar speeds (Fig. 1B). The elevated energetic cost of concertina locomotion is attributable to two factors: (i) snakes performing concertina locomotion require more cycles of movement to sustain the same speed than they do during lateral undulation [two-tailed paired t test comparing the rate (in cycles per minute) of animals locomoting at 0.2 km hour^{-1}, $n = 5$, $t = 8.37$, $P = 0.001$, Fig. 2A], and (ii) the energetic cost of a single cycle of concertina locomotion is greater than that of a single cycle of lateral undulation [Mann-Whitney U test, $U(9, 23) = 179$, $P < 0.001$, Fig. 2B].

The energetic cost of locomotion by *C. constrictor* is expressed by the slope of the line relating $\dot{V}O_2$ to speed within the range of aerobically sustainable speeds (*17*) (Fig. 1A). This slope, often termed the net cost of transport (NCT), indicates the amount of energy required to move a unit mass of animal a given distance (*20*) and is frequently used for comparisons among taxa and locomotor modes (*24, 25*). The NCT of

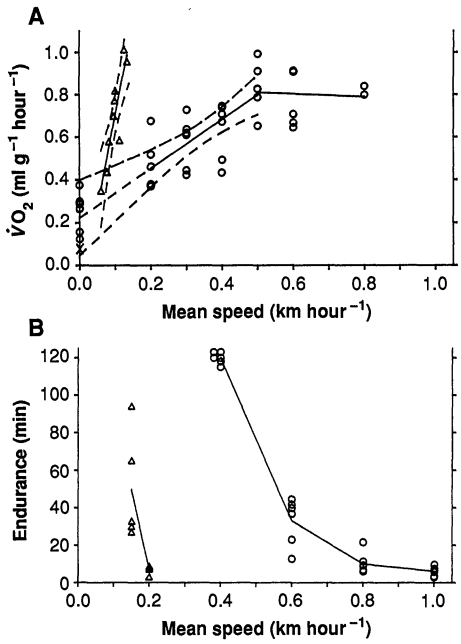

Fig. 1. (**A**) Steady-state rate of oxygen consumption ($\dot{V}O_2$) as a function of speed for seven individuals performing lateral undulation (○) and three individuals performing concertina locomotion (△). Mean speed is reported, because animals moving by concertina locomotion periodically stop. The mean resting rate of oxygen consumption is indicated by the × at zero speed; pre-exercise rates of oxygen consumption are indicated by circles at zero speed. The net cost of transport for lateral undulation is represented by the slope of the line in the increasing region of the plot (0.2 km hour^{-1} ≤ speed ≤ 0.5 km hour^{-1}). The least-squares estimate of the equation for this line is $\dot{V}O_2$ = 1.153 (±0.205 SE) × speed + 0.222 (±0.085 SE), $n = 23$, $P = 0.0001$ (curved dashed lines represent ±95% confidence limits of predicted values of $\dot{V}O_2$). An alternative method is to calculate separately a regression for each snake and then determine the mean slope and intercept of these individual regressions. This method indicated a relation similar to that of the model I regression: $\dot{V}O_2$ = 0.916 (±0.199 SE) × speed + 0.285 (±0.069 SE) ($n = 6$). Oxygen consumption is not related to speed at ≥0.5 km hour^{-1}. The regression equation for this region is $\dot{V}O_2 = -0.069 \times$ speed + 0.845, $P = 0.84$. The equation relating $\dot{V}O_2$ to speed during concertina locomotion is $\dot{V}O_2$ = 8.494 (±1.679 SE) − 0.151 (±0.170 SE), $n = 9$, $P = 0.002$. (**B**) Endurance (time to exhaustion) as a function of speed. All snakes performing lateral undulation at 0.4 km hour^{-1} sustained locomotion for 120 min; the trials were stopped at that point.

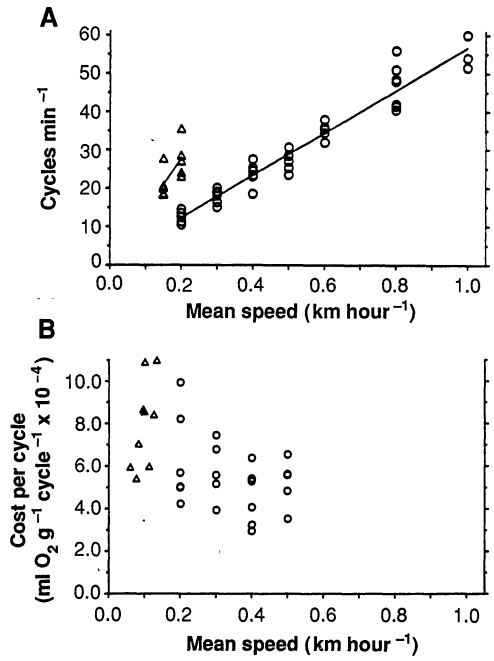

Fig. 2. (**A**) Frequency of movement as a function of speed. For lateral undulation (○) the regression equation relating frequency of movement (f) (in cycles per minute) to mean forward speed (speed) (in kilometers per hour) is: $f = 54.9 \times$ speed + 1.6, $n = 41$, $P < 0.001$. Sample sizes for lateral undulation are as follows: 0.2 km hour^{-1}, $n = 6$; 0.3 km hour^{-1}, $n = 6$; 0.4 km hour^{-1}, $n = 6$; 0.5 km hour^{-1}, $n = 5$; 0.6 km hour^{-1}, $n = 7$; 0.8 km hour^{-1}, $n = 7$; 1.0 km hour^{-1}, $n = 3$. The regression equation for concertina locomotion (△) is $f = 132 \times$ speed + 1.2, $n = 10$, $P < 0.05$. Sample sizes for concertina locomotion are 0.15 km hour^{-1}, $n = 5$ and 0.2 km hour^{-1}, $n = 5$. (**B**) Gross energetic cost of a single cycle of movement as a function of speed. We calculated these values by dividing $\dot{V}O_2$ at a particular speed by the frequency of movement over the same time interval. Sample sizes are as in Fig. 1A for corresponding speeds.

lateral undulatory locomotion for *C. constrictor* (1.15 ± 0.21) is virtually equivalent to that predicted for a limbed lizard (*26*) of similar mass (predicted NCT = 1.14, Fig. 3). In fact, the NCT of *C. constrictor* is similar to that predicted for terrestrial locomotion of birds, mammals, and arthropods of similar mass (*25*). Furthermore, the NCT of *C. constrictor* performing concertina locomotion (8.49 ± 1.68) is seven times the NCT for *C. constrictor* lateral undulation and substantially greater than that predicted for limbed animals of similar mass (Fig. 3). In contrast to previous observations (*5*) and widely held opinions concerning the energetic cost of limbless locomotion (*1–4, 6–8*), lateral undulatory and concertina locomotion by *C. constrictor* are not more economical than walking or running by limbed animals. Because the data for garter snakes (*Thamnophis sirtalis*) were published only as an abstract (*5*), it is difficult to assess this discrepancy. For our study, we verified locomotor mode using videotape; and we used a snake, *C. constrictor*, that is known to have a relatively high capacity for aerobic metabolism (*21*). Thus, we are certain that the data we used to calculate NCT involved only aerobically sustainable speeds. In the earlier study (*5*), the locomotor mode was not identified, although lateral undulation seems probable, and the snakes in that study were tested at speeds (up to 0.9 km hour^{-1}) that may have elicited extensive anaerobiosis and, therefore, underestimated NCT.

Why is terrestrial limbless locomotion not energetically less expensive than limbed locomotion despite plausible biomechanical arguments for its low cost? Equally plausible arguments suggest that certain energetically costly features used during limbless locomotion may neutralize proposed energetic benefits associated with limblessness. For example, limbless animals probably encounter greater external frictional resistive forces than limbed animals (*3*). Lateral accelerations of the body during limbless movement should also add to the energetic cost. Finally, limbless locomotion is not necessarily without energetic cost for body support, either from muscular activity to maintain rigidity of the ribs or to elevate the head and anterior regions of the body above the ground as the animal moves.

In addition to multiple independent origins of limblessness in amphibians and reptiles, many fossorial lizards and salamanders are characterized by elongate bodies and small but fully functional limbs (*1*). One explanation for the presence of small limbs in such taxa is that they represent a transitional stage toward the evolution of limblessness (*27*). In this view, limbs are seen as encumbrances during locomotion through narrow tunnels or crevices. Our results concerning the high cost of concertina locomotion suggest an alternative hypothesis. Many limbless lower vertebrates must switch to energetically costly concertina locomotion within tunnels. In contrast, small but fully functional limbs enable animals to perform limbed locomotion in narrow tunnels, which may also convey an energetic benefit that favors their evolutionary retention.

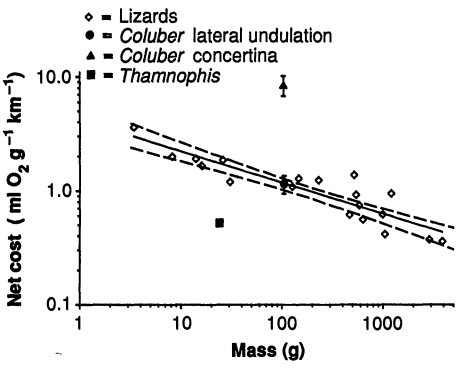

Fig. 3. Net cost of transport (NCT) as a function of body mass plotted on a log-log scale. Data for limbed lizards were compiled by John-Alder *et al.* (*26*). The solid line indicates the regression line calculated for the lizard data (curved dashed lines indicate ±95% confidence limits for the predicted values of NCT). Vertical bars on the lateral undulation and concertina NCTs for *Coluber* indicate ±1 SE. The garter snake (*Thamnophis*) datum is from Chodrow and Taylor (*5*).

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14. The resting rate of oxygen consumption was measured for snakes maintained in darkened isolated, cylindrical plastic metabolic chambers. Snakes were placed in the chambers and left undisturbed for 48 hours at 30°C. [All measurements were conducted at 30°C, a typical field active body temperature for *C. constrictor*; H. S. Fitch, *Univ. Kans. Publ. Mus. Nat. Hist.* **15**, 351 (1963); H. F. Hirth and A. C. King, *J. Herpetol.* **16**, 101 (1969); L. J. Vitt, *Copeia* **1971**, 255 (1971).] Fresh air was ventilated through the chambers at a constant flow rate. At 1900 hours on the day of the measurements, an initial 20-ml gas sample was drawn for oxygen analysis with a gas-tight glass syringe. The chamber was sealed and left undisturbed in the darkened temperature cabinet for 3 hours. A second 20-ml gas sample was then withdrawn, and the chamber was ventilated with a constant flow of fresh air. We determined the fractional oxygen content of the samples by injecting the sample at a constant flow rate into an Ametek S3A oxygen analyzer. Gas samples were injected through columns of water (Drierite) and CO_2 (Ascarite) absorbents before entering the analyzer sensor. Gas volumes were corrected to standard temperature, pressure, and density. The rate of oxygen consumption was determined according to the method of D. Vleck [*J. Appl. Physiol.* **62**, 2103 (1987)].
15. We measured rates of oxygen consumption during locomotor exercise at 30°C over a range of speeds on motorized treadmills designed to elicit either lateral undulation or concertina locomotion. A snake at a body temperature of 30°C was fitted with a lightweight, clear plastic mask, which was secured to the head with a small rubber spacer inserted between the dorsal surface of the head and the mask. Room air was drawn through the mask at a constant flow rate of 200 to 300 ml min^{-1} by a length of flexible plastic tubing attached to the end of the mask. The expired air stream passed from the mask through absorbent columns, through the Ametek S3A analyzer, and through a metered air pump. We determined the oxygen consumption by comparing the fractional oxygen content of room air to that of the expired air. The rate of oxygen consumption was calculated according to the method of P. C. Withers [*J. Appl. Physiol.* **42**, 120 (1977)]. In the open-flow mask technique it is assumed that oxygen consumption is dependent on pulmonary and buccopharyngeal, but not cutaneous, respiration. Cutaneous oxygen consumption, however, represents only a small fraction (<8%) of total gas exchange among terrestrial reptiles [M. E. Feder and W. W. Burggren, *Biol. Rev. (Cambridge)* **60**, 1 (1985)]. Pre-exercise rates of oxygen consumption were measured during the last 2 to 3 min of a rest period (30 min to 1 hour) before the exercise trial. The treadmill was then started, and oxygen consumption during movement was recorded. Snakes usually crawled voluntarily, but many required light taps on the tail with our fingers or a soft brush to initiate and encourage continual movement. Data for individuals that did not maintain continual movement were not used in subsequent analyses. To elicit lateral undulation, we used a straight treadmill (21 cm wide by 80 cm long), the surface of which was covered with artificial grass. To provide surface projections, small artificial grass squares (2.5 cm by 2.5 cm) were affixed to the surface 4 cm from the treadmill walls and at 17-cm intervals in two parallel rows 6 cm apart. The concertina treadmill consisted of a circular parallel-sided tunnel (2 m in outside diameter) mounted on wheels, which rotated about the center in the horizontal plane. The floor of the tunnel was 7 cm wide and consisted of smooth plywood. Friction tape was affixed to the vertical sides to provide that surface with a high coefficient of friction. On both treadmills, oxygen consumption was recorded when the snakes had achieved several minutes of steady-state oxygen consumption. During the lateral undulation trials, speed was then increased by 0.1 or 0.2 km hour^{-1} and oxygen consumption was measured again. We proceeded in this fashion until the snake could no longer maintain position on the tread. We attempted to exercise each animal at each speed.
16. Endurance was measured as the time to exhaustion during locomotion at a particular speed and locomotor mode. Exhaustion was determined as the point at which the snake could no longer keep pace with the treadmill.
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18. A total of seven snakes were used in the lateral undulation experiments. However, only one animal performed satisfactorily at all speeds. Therefore, sample sizes at each speed are ≤6 individuals. According to the procedures of J. H. Zar [*Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, NJ, 1984)], a model I regression (multiple values of y for each value of x) was calculated to examine the relation between $\dot{V}O_2$ and speed of locomotion by lateral undulation. A model I regression partitions variability into among-group, regression, deviations from regression, and within-group components.

The test for the significance of the regression was based on a comparison of the regression and deviations from regression mean squares. Confidence limits were based on a residual mean square that pools deviations from regression and within-groups components.
19. Only three snakes performed satisfactory concertina locomotion during the oxygen consumption experiments [mass = 107.1 ± 6.2 (SE) g]. Two of these were used during lateral undulation experiments as well.
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22. Burst speed at 30°C was measured as snakes crawled over a 4-m track, 21 cm wide and covered with artificial grass and surface projections (as above). We vigorously tapped the tail with a soft brush to elicit rapid movement and used the timer feature of the video system to determine velocity. Three trials were conducted for each snake, and the fastest 50-cm interval of all three trials was selected as the snake's maximum burst speed.
23. Using the four individuals with $\dot{V}O_2$ data at each of the four sustainable speeds of lateral undulation (0.2, 0.3, 0.4, and 0.5 km hour^{-1}), we calculated the cost per cycle for each 1-min interval within the 2- to 5-min records of $\dot{V}O_2$ used to calculate the average values shown in Fig. 1A. These values were compared by means of a two-way analysis of variance (ANOVA) with speed as a fixed effect and individual as a random effect [M. J. Norusis, *Advanced Statistics, SPSS/PC+* (Statistical Package for the Social Sciences (SPSS), Inc., Chicago, 1986)]. Following the guidelines of J. H. Zar [*Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, NJ, 1984], we calculated F statistics using comparisons among mean squares (MS) as follows: $F_{speed} = MS_{speed}/MS_{speed \times individual}$ and $F_{individual} = MS_{individual}/MS_{error}$.
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Design, Activity, and 2.8 Å Crystal Structure of a C_2 Symmetric Inhibitor Complexed to HIV-1 Protease

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A two-fold (C_2) symmetric inhibitor of the protease of human immunodeficiency virus type–1 (HIV-1) has been designed on the basis of the three-dimensional symmetry of the enzyme active site. The symmetric molecule inhibited both protease activity and acute HIV-1 infection in vitro, was at least 10,000-fold more potent against HIV-1 protease than against related enzymes, and appeared to be stable to degradative enzymes. The 2.8 angstrom crystal structure of the inhibitor-enzyme complex demonstrated that the inhibitor binds to the enzyme in a highly symmetric fashion.

HUMAN IMMUNODEFICIENCY VIRUS type–1 (HIV-1) the causative agent of acquired immunodeficiency syndrome (AIDS) (*1*), is a member of the retrovirus family (*2*). The *gag* and *pol* genes of HIV-1 encode the viral structural and replicative enzymes that are translated as polyprotein precursors: Pr55*gag* and the ribosomal frameshift product Pr160*gag-pol* (*3*). The polyproteins are proteolytically processed by the action of a virus-encoded protease (*4*). The activity of the protease is essential for the proper assembly and maturation of fully infectious virions for HIV-1 (*5*) as well as for other retroviruses (*6*). Thus, the HIV-1 protease has become an important target for the design of antiviral agents for AIDS.

Retroviral proteases were tentatively assigned to the aspartic proteinase family on the basis of putative active site sequence homology (*7*), but are only about one-third the size of the two-domain, cellular enzymes (*8*). For this reason, the retroviral proteases were hypothesized to function as dimers in which each monomer contributes one of the two conserved aspartates to the active site (*9*). This hypothesis was verified by the crystal structure determinations of Rous sarcoma virus (RSV) protease (*10*) and recombinant (*11*) and chemically synthesized (*12*) HIV-1 protease. Furthermore, these results firmly established the structural relatedness of the retroviral and cellular enzymes. Both viral enzyme structures are highly twofold symmetric; in the case of HIV-1 protease, the dimer exhibits exact crystallographic, twofold rotational (C_2) symmetry. As predicted, the structural similarity between these enzymes is strongest in the active site region. The cellular proteases contain an extended β-hairpin structure, or so-called flap (because of its flexibility), that tightly embraces the substrate in the active site (*13*). The retroviral proteases contain an analogous region that is disordered in the crystals of RSV protease (*10*). The flap is well ordered in the native HIV-1 protease structure, but crystal packing forces maintain it in a conformation that makes it unavailable for substrate binding (*11, 12*). The crystal structure of HIV-1 protease complexed with a reduced peptide inhibitor has been determined (*14*). The flap has undergone a major structural rearrangement in the complex to make favorable van der Waals and hydrogen-bonding interactions with the inhibitor.

Strategies that have been developed for the design of inhibitors for renin (*15*), an aspartic proteinase that is an important target for the design of antihypertensive agents, are now being applied to the design of inhibitors for HIV-1 protease. Current drug discovery approaches are based on the screening of renin inhibitors against HIV-1 protease (*16*) and on the synthesis of peptide substrate analogs in which the scissile P1-P1′ amide bond has been replaced by a nonhydrolyzable isostere with tetrahedral geometry (*17*). Two hydroxyethylene-containing substrate analogs have been reported to inhibit processing of HIV-1 polyproteins and to inhibit virus infection in tissue culture (*18*). However, the development of peptide-based inhibitors into effective drugs has been hampered by the inherently poor pharmacologic properties of peptides and peptide-like pharmacophores: for example, poor oral absorption, poor stability, and rapid metabolism (*19*). For this reason, we

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