Reports

Changes in Mean Concentration, Phase Shifts, and Dissipation in a Forced Oscillatory Reaction

JAMES G. LAZAR AND JOHN ROSS

Experiments are presented that confirm earlier predictions that the mode of supply of reactants to a nonlinear (bio)chemical reaction determines or controls concentrations at steady states far from equilibrium. The oxidation of nicotinamide adenine dinucleotide (NADH) catalyzed by the enzyme horseradish peroxidase with continuous input of oxygen was studied; NAD^+ is continuously recycled to NADH through a glucose-6-phosphate dehydrogenase system. A comparison of steady-state concentrations is made with an oscillatory oxygen input and a constant input at the same average oxygen input for both modes. By varying the frequency and amplitude of the perturbation (O_2 influx), the following may be changed: the average concentration of NADH; the Gibbs free energy difference ΔG of the reactants and products at steady state; the average rate of the reaction; the phase relation between the oscillatory rate and ΔG; and the dissipation. These results confirm the possibility of an "alternating current chemistry," of control and optimization of thermodynamic efficiency and dissipation by means of external variation of constraints in classes of nonlinear reactions and biological pumps, and of improvements of the yield in such reactions (heterogeneous catalysis, for example).

NONLINEAR BIOCHEMICAL REACTIONS operating under highly nonequilibrium conditions are currently being studied because of their importance to the understanding of many biological processes. Critical and complex biological functions often turn out to be controlled or regulated by an enzyme or biochemical system of high nonlinearity. Autocatalysis, feedbacks from substrates or product or both, and diffusional (delay) effects may all be sources of nonlinearity in biochemical systems. As a result of such mechanisms, there may occur bistability, damped and sustained oscillations, and complex or chaotic oscillations, and observations of these processes have been made in chemical and biological systems (*1, 2*). For example, in anaerobic glycolysis in yeast cells, oscillations arise out of the interaction between two nonlinear reactions catalyzed by the enzymes phosphofructokinase and pyruvate kinase, respectively (*3, 4*). Damped oscillatory behavior has been observed in an adenosine triphosphate (ATP)–driven proton pump (*5*). Ester hydrolysis by immobilized papain causes *p*H oscillations at the electrode upon which the papain is coated (*6*).

Predictions have been made that the power output, the dissipation (for isothermal reactions, the product of ΔG and the rate) and hence the efficiency of nonlinear biochemical systems may be changed depending upon the mode of supply of reactants (steady or oscillatory) (*7, 8*), with the same average consumption of reactants in either the oscillatory or steady mode of supply. We show experimentally that the mode of supply of reactants, steady or oscillatory, to a nonlinear, non-equilibrium biochemical reaction can determine the steady-state concentrations of the reaction. That is, the average concentrations of reactants and products with constant input of reactant may differ from the average concentrations of reactants and products with an oscillatory input of reactants [for an earlier measurement of this effect, see (*9*)]. A change in the steady state of a non-equilibrium chemical reaction is generally equivalent to a change in the average of the rate of the reaction. If a (bio)chemical reaction is in a steady state and an oscillatory perturbation is applied to the input of reactant, then the temporal behavior of ΔG and the rate can also change, depending upon the interaction of the perturbation with the mechanism of the reaction. Furthermore, these interactions may result in a shift of the temporal relation of ΔG relative to the rate, that is a phase shift, which we show experimentally; there exists the possibility of changes in the dissipation of the reaction, in power output, and in the efficiency of coupled processes (*10, 11*).

There is an analogy, to some extent, in comparing reactions in stationary states versus driven or autonomous oscillatory reactions to comparing dc and ac circuits (*12, 13*); ΔG is analogous to a voltage V, and the rate is analogous to a current I. In a stationary state, ΔG and the rate are constant, and so are the voltage and current at points in a dc circuit. Both the dissipation [$\Delta G(\star\text{rate})$] and $V\star I$ are constant. In an ac circuit, V and I vary sinusoidally, and the phase relation as well as the average values of these quantities are important in determining properties such as dissipation or power output. Similarly, in driven or autonomous oscillatory reactions in which concentrations of chemical species vary in general nonsinusoidally, the phase relation of ΔG and the rate is a new important quantity and is one factor in determining the dissipation, power output, and efficiency; other factors are the average values of ΔG and the rate.

We studied the highly nonlinear oxidation of NADH catalyzed by the enzyme horseradish peroxidase (HRP) under conditions of continuous oxygen supply (either steady or oscillatory) (*14, 15*). NAD^+ is simultaneously recycled to NADH by a glucose-6-phosphate dehydrogenase (G6PDH) system (*16, 17*).

$$\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \xrightarrow{\text{HRP}} \text{NAD}^+ + \text{H}_2\text{O} \quad (1)$$

$$\text{NAD}^+ + \text{G6P} \xrightarrow{\text{G6PDH}} \text{6PGL} + \text{NADH} + \text{H}^+ \quad (2)$$

The products of reaction 2, H^+ and 6-phosphogluconolactone (6PGL), do not interfere with the HRP reaction. The HRP reaction is convenient for study because the NADH concentration can be monitored continuously with a spectrophotometer and the O_2 concentration with a microelectrode. Oxygen was supplied to the system by an arrangement involving two computer-linked mass flow controllers that precisely mix and regulate any combination of gas mixture (O_2 and N_2) and flow rate (*18*). Waveforms used to produce periodic perturbations in the oxygen concentration were produced by a computer-controlled waveform generator. Time series measurements from the spectrophotometer, O_2 electrode, and two mass flow controllers were read and recorded by a computer every 0.5 s.

About 5 min after the start of a run (*18*), sustained oscillations in NADH and O_2 concentration commence, and a stable oscillatory state was reached after approximately 15 min. A perturbation in the O_2 concentration in the gas inflow was then applied with a period integrally related to the period of the autonomous system. Perturbations were done at one, two, three, and four times the

Department of Chemistry, Stanford University, Stanford, CA 94305.

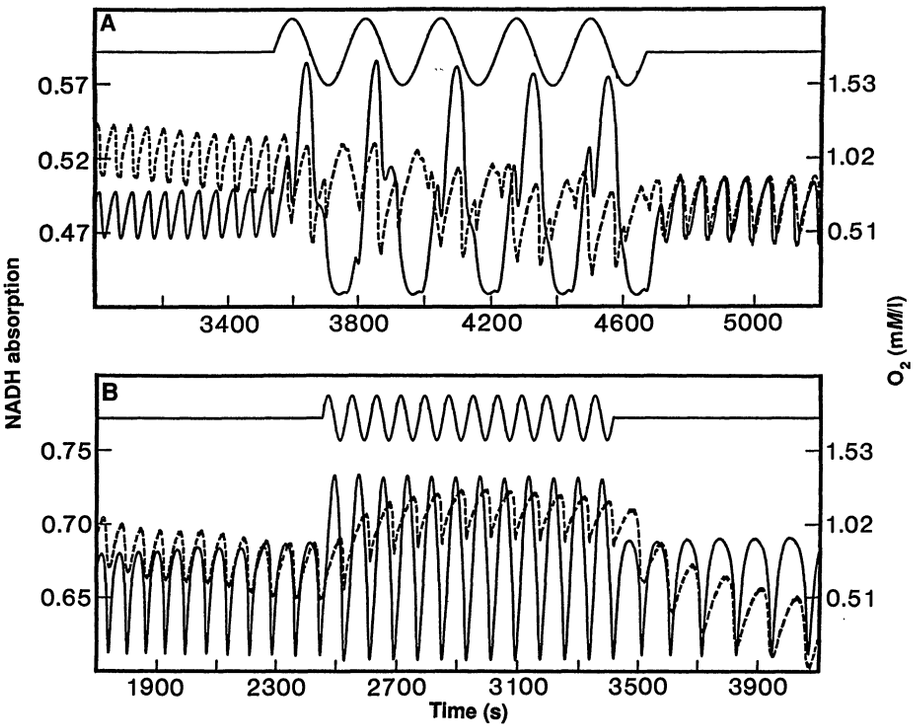

Fig. 1. (**A**) Plot of NADH (dashed line) absorption and O_2 (solid line) concentration in solution versus time. Oxygen input (not to scale) is shown above. The O_2 perturbation is ±50% of its original value, and its period of 60 s. (**B**) Plot of NADH (dashed line) absorption and O_2 (solid line) concentration in solution versus time. Oxygen input (not to scale) is shown above. The O_2 perturbation is ±75% of its original value, and its period is 230 s.

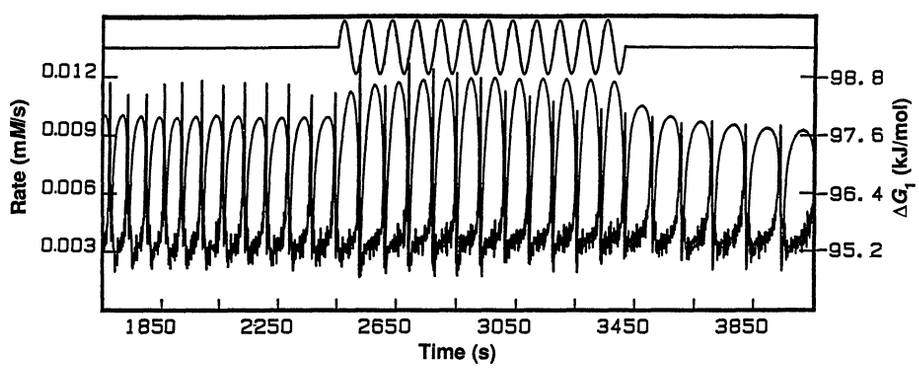

Fig. 2. Plot of ΔG_1 and the rate of reaction 1 versus time for the data shown in Fig. 1A. Oxygen input (not to scale) is shown above.

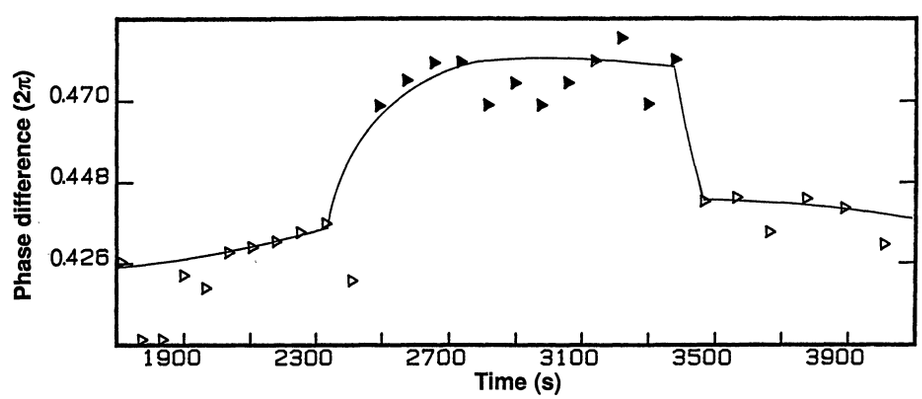

Fig. 3. Phase difference between ΔG_1 and the rate of 1 in units of 2π for the data presented in Fig. 1A. Darkened symbols designate values calculated during the perturbation in O_2 influx.

natural period of the system with magnitudes of ±50% or ±75% of the original O_2 concentration (*19*). The total flow rate during a perturbation was kept constant by continuous computer adjustment of the N_2 flow. The perturbation was applied for an integral number of cycles, typically 8 to 15, depending upon the period of the perturbation (*20*).

The experiments were analyzed by first converting the NADH and O_2 measurements to concentrations as a function of time. The total concentration of NADH + NAD decreased linearly during the course of the reaction because of the slow decomposition of NADH at *p*H 6.0 (*21*). We calculated the NAD^+ concentration as a function of time by subtracting the NADH concentration from the corrected total (NADH + NAD) concentration, from which we calculated ΔG_1 of reaction 1. The rate of the HRP reaction was determined from the slope of the measurements of NADH as a function of time and the known rate of reaction 2 (*22*). The dissipation of Eq. 1 was calculated at each point from the equation $D = \Delta G_1 * \text{rate}$.

Two time series from typical experimental runs are shown in Fig. 1. Switching a constant input of O_2 to a sinusoidally varying input at the frequency of the autonomous oscillation led to a periodic response of the system (Fig. 1A), whereas switching to a sinusoidally varying O_2 input with a period of four times the autonomous period (Fig. 1B) led to an almost periodic response. Distinct changes occurred in each case in NADH and oxygen concentrations in solution from the steady to oscillatory input of O_2.

The calculated instantaneous time series of the rate and ΔG of reaction 1 for the time series presented in Fig. 1A are shown in Fig. 2. The spiked time dependence of the rate is due to the on-off nature of the HRP mechanism. The temporal variation of ΔG_1 reflects the changing concentrations of NAD^+, NADH, and O_2. The phase between the thermodynamic force (ΔG_1) and flux (HRP rate) of the reaction is changed by perturbing the O_2 supply. For a constant O_2 supply, ΔG_1 and the rate of the HRP reaction peak almost simultaneously. During the perturbation, ΔG_1 of the reaction peaks ahead of the rate of 1. In Fig. 3, we plot the measured phase shift between ΔG_1 and the rate of 1 before, during, and after the periodic perturbation in O_2 supply. During the perturbation, the phase difference between ΔG_1 and the rate of 1 increases noticeably.

In Fig. 4A, we show the average NADH concentration and dissipation of the HRP reaction obtained from the measurements given in Fig. 1A, averaged over complete

cycles of oscillation. At the beginning of the perturbation of O_2 inflow, the NADH concentration immediately begins to rise and the dissipation decreases sharply. As the system approaches its asymptotic state (its new attractor), the average NADH concentration is greater than that before the perturbation, while the dissipation approaches an average value near that of the unperturbed system. The NAD^+ concentration evolves to a lower average value during the perturbation since, at any time, the sum of NADH and NAD^+ is constant (after a small correction). When the oscillatory perturbation is removed, the average NADH concentration begins to decrease and the average dissipation increases sharply as the phase shift between ΔG_1 and the rate 1 decreases. As the system relaxes back to its autonomous state, the dissipation approaches its value before the perturbation. The rapid change in phase between ΔG_1 and the rate 1 (Fig. 3) upon the application of the periodic perturbation causes a rapid decrease in the dissipation. The slower increase in NADH concentration, however, raises the average ΔG, thus increasing the dissipation.

A similar treatment of the time series of Fig. 1B is shown in Fig. 4B. The average NADH concentration during the perturbation is less than that without the perturbation, and the dissipation during the perturbation is less than before the perturbation because of the phase shifting of ΔG_1 and the rate 1 (time series not shown). In this case, both phase shifting and change in ΔG_1 contribute to lowering the dissipation.

The reproducibility of the results described above has been verified. Although absolute numbers may vary from day to day, the same effects are always observed when the constant influx of oxygen is switched to an oscillatory influx. In order to assure ourselves that the same average flux of O_2 was reaching the solution during a perturbation, the average flow rate of O_2 into the reaction vessel was measured before, during, and after a perturbation. The average influx of O_2 during a perturbation was found to deviate by less than 0.8% from the constant influx, and this deviation is uncorrelated with observed phase shifts, increases or decreases in dissipation, average concentrations, and so forth.

The specific observations presented are indicative of a wide range of possible changes resulting from an oscillatory input of reactant: lower or higher on-average concentrations of a product, rates of reaction, ΔG, and dissipation. For such changes to occur, the reaction under study need not necessarily be in an oscillatory state; a reaction in a stable focus (a stable steady state that, when perturbed, decays with an oscillatory component) shows similar effects [see (*23*) for experimental confirmation of some of the concepts presented here in a combustion reaction and thermal engine]. Such changes are manifestations of "alternating current" chemical kinetics: ΔG is the analog of the voltage, the rate is the analog of the current, and the power output and dissipation depend on several factors including phase shifting between the forces and fluxes and changes in the average values of the forces and fluxes. Many heterogeneous reactions are highly nonlinear and are amenable to changes in steady-state concentrations as discussed here (*24*). For reactions with linear or near-linear kinetics, an oscillatory input of reactants increases the dissipation.

A decrease in dissipation in a chemical reaction that acts as an energy transduction device may lead to an increased power output and efficiency (*25*). Consider the reaction of a proton pump,

$$ATP + H^+(\mu_1) \rightarrow ADP + H^+(\mu_2) + P \quad (3)$$

where P denotes phosphate. The chemical potential of protons μ_2 on the side of the membrane to which the protons are pumped is greater than μ_1. The dissipation of the overall process may be changed by an oscillatory reactant input, and the power output, $(\mu_2 - \mu_1)$ times the rate of proton pumping, may be increased for the same power input (the difference in ΔG for the hydrolysis of ATP times its rate) (*26, 27*). Thus the efficiency of energy utilization and transduction may be controlled through the modulation of the input of reactants (or other constraints). In addition, an external periodic perturbation may also alter the final state of the reacting system, which changes ΔG. Changes in efficiency may come about through a combination of these factors.

These results are of interest to the biological process of producing and maintaining concentration gradients by the continuous consumption of a chemical fuel. The ability to change an average steady-state concentration, by means of an oscillatory versus constant input of fuel, without changing the average input of fuel, gives the system a means to adjust to changing demands on the concentration gradient without radical changes in fuel supply; only the mode of supply of the fuel need be changed, which may be a less costly option. A system may thereby be able to adjust rapidly to a change in the concentration requirement for a biological product without changing the average amount of fuel consumed.

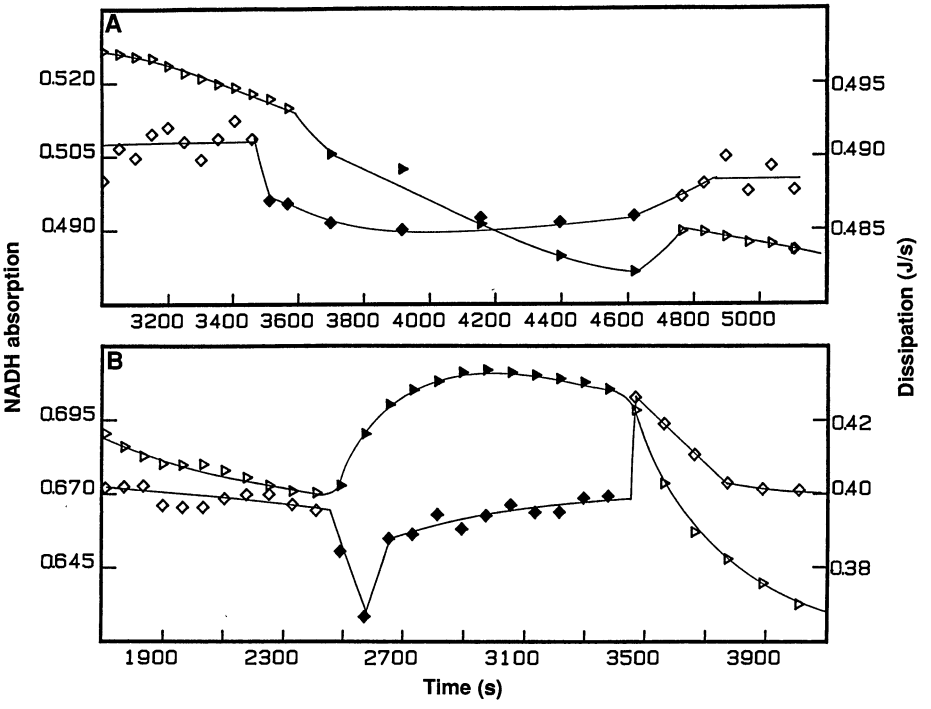

Fig. 4. Plots of average NADH concentration (▷ and ▶) and average dissipation (◇ and ◆) for the data shown in (**A**) Fig. 1A and (**B**) Fig. 1B, respectively. Averages are calculated over each complete cycle of oscillation and plotted at the center of each peak (calculated from the first moment). Darkened symbols designate averages calculated during the perturbation in O_2 input.

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Mountains and Arid Climates of Middle Latitudes

S. MANABE AND A. J. BROCCOLI

Simulations from a global climate model with and without orography have been used to investigate the role of mountains in maintaining extensive arid climates in middle latitudes of the Northern Hemisphere. Dry climates similar to those observed were simulated over central Asia and western interior North America in the experiment with mountains, whereas relatively moist climates were simulated in these areas in the absence of orography. The experiments suggest that these interior regions are dry because general subsidence and relatively infrequent storm development occur upstream of orographically induced stationary wave troughs. Downstream of these troughs, precipitation-bearing storms develop frequently in association with strong jet streams. In contrast, both atmospheric circulation and precipitation were more zonally symmetric in the experiment without mountains. In addition, orography reduces the moisture transport into the continental interiors from nearby oceanic sources. The relative soil wetness of these regions in the experiment without mountains is consistent with paleoclimatic evidence of less aridity during the late Tertiary, before substantial uplift of the Rocky Mountains and Tibetan Plateau is believed to have occurred.

THE MECHANISMS CONTROLLING the distribution of the earth's arid climates are only partially understood. While the existence of subtropical deserts can be explained by their location beneath the subsiding branch of the Hadley circulation, mid-latitude aridity is more problematic. Sizable dry regions stretch across the interior of Asia from Turkestan east to the Gobi Desert and across North America from the Great Basin to the western Great Plains. Some climatologists have speculated that distance from oceanic moisture sources is the major cause of such mid-latitude dry regions, accentuated locally by the presence of mountain barriers upwind (*1*). If this were the case, such dry regions would occur even in the absence of orography.

One way to separate the effect of orography on climate from other effects is through the use of atmospheric general circulation models (GCMs). In a number of studies, GCMs have been used to simulate the earth's climate with and without mountains (*2*). Most of these studies concentrated on the effects on atmospheric circulation, with less attention given to changes in the global distribution of climate brought about by the presence of mountains. Manabe and Terpstra (*3*) briefly discussed the impact of orography on the precipitation distribution, on the basis of simulations of the January climate with and without mountains. In their simulations, a zonal belt of moderate precipitation formed in the mid-latitudes of the Northern Hemisphere in the case without mountains, but interruptions in the belt over the interiors of Eurasia and North America developed in their experiment with orography. In a subsequent study using a model with orography and incorporating seasonal variation, Manabe and Holloway (*4*) compared the simulated and observed distributions of global climate. Their model simulated the mid-latitude dryness of the Eurasian interior, and they suggested that the presence of the Tibetan Plateau is important in maintaining central Asian aridity.

Recently, a series of studies was conducted in which large-scale uplift of the Tibetan Plateau and the western United States was linked to the changes in climate during the past 30 to 40 million years (*5–7*). As part of that work, climate model experiments were run with the Community Climate Model of the National Center for Atmospheric Research. In these experiments, a global model with prescribed soil moisture and snow cover was used to simulate climate with and without mountains. Although the model was not integrated through a complete sea-

Geophysical Fluid Dynamics Laboratory–National Oceanic and Atmospheric Administration, Post Office Box 308, Princeton University, Princeton, NJ 08542.