The Energy Charge of the Adenylate Pool as a Regulatory Parameter. Interaction with Feedback Modifiers*

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ABSTRACT: The energy charge of the adenylate system, half of the average number of anhydride-bound phosphate groups per adenine moiety, has been proposed as a metabolic regulatory parameter. For several reactions that participate in biosynthesis or other adenosine triphosphate utilizing sequences, plots of enzyme activity against energy charge have positive slopes that increase with charge; thus these curves (type U) are concave upward and steep in the region of high charge. End-product feedback inhibition of the type demonstrated for many biosynthetic regulatory enzymes must be reflected in a decrease in the slope of such curves on the addition of the end product. The area between the curve representing absence of end product and that representing a saturating level of it should indicate the operational range of the regulatory enzyme. Within this range, if either end-product concentration or energy charge is constant the enzyme will respond only to variation in the other; but it may be expected that both parameters affect the behavior of the enzyme in the intact cell. For several reactions that participate in adenosine triphosphate regenerating sequences, plots of enzyme activity against energy charge have negative slopes that increase with charge; thus these curves (type R) are concave downward and steep in the region of high charge. Such sequences also supply primary metabolic intermediates needed as starting points in biosyntheses, and in some cases have been shown to be regulated also by the concentration of one or more of these intermediates. Inhibition of this type should be reflected in an increase in the negative slope of such curves on addition of the regulatory metabolite. As in the case of type U curves, a regulatory area will exist between the curves representing zero concentration and saturating concentration of the modifying metabolite. Experimental examples of both types of pattern are provided in the following two papers. It is proposed that the overlap of such regulatory type R and type U patterns illustrates graphically some of the ways in which energy charge and the concentrations of primary intermediates and of biosynthetic end products interact to stabilize the energy charge and to adjust the partitioning of substrates among competing metabolic functions in response to changing metabolic situations in the cell.

Several enzymes that are involved in energy-yielding sequences, or that catalyze reactions at metabolic branch points, are affected by adenine nucleotides. The pattern of the responses suggests that they participate in the regulatory interactions that must underlie orderly metabolic integration. Thus Krebs (1964; Gevers and Krebs, 1966) suggested that the level of AMP[1] may be an important factor in determining whether glycolysis or gluconeogenesis will predominate, and we (Hathaway and Atkinson, 1963; Ramaiah *et al.*, 1964; Atkinson, 1965, 1966) proposed that the balance among concentrations of the adenine nucleotides may be a major regulatory factor at every point where a metabolite is partitioned between energy-yielding and energy-demanding or energy-storing processes. In recent communications (Atkinson and Walton, 1967; Atkinson and Fall, 1967) this proposal has been stated more explicitly in terms of the energy charge of the adenylate pool, a parameter that is intended to furnish a quantitative estimate of the energy state of the cell.

This paper presents the energy charge concept more fully and discusses possible regulatory interactions between energy charge and the concentrations of products or metabolic intermediates.

The Energy Charge. For systems that regulate the pool levels of metabolic "building blocks," such as amino acids or nucleotides, the negative feedback control of enzyme behavior first explicitly proposed by Umbarger (1956) and Yates and Pardee (1956) is appropriate, since the parameter to be controlled in each case is the concentration of a specific compound. However, control of ATP-regenerating sequences, and especially regulation of the interactions between ATP regeneration and processes that consume ATP, clearly must respond to the energy balance of the cell, rather than to the concentration of a single metabolite.

The adenylate system AMP + ADP + ATP resembles an electrochemical storage cell in its ability to accept, store, and supply energy. Because components of the adenylate system differ in extent of phosphorylation rather than in oxidation state, this system would not interact readily with any feasible technical energy-exchanging mechanism. In the context of the living cell, however, the adenylate system serves admirably as an

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[1] For a list of abbreviations, see *Biochemistry 5*, 1445 (1966).

energy-mediating system. Any chemical energy-storage system functions stoichiometrically; that is, a given amount of energy put into the system causes a proportional chemical change in the system. Thus the charge of a lead accumulator cell (eq 1) is a function of the

$$2PbSO_4 + 2H_2O = Pb + PbO_2 + 2H_2SO_4 \quad (1)$$

relative amounts of the three oxidation states of lead, and in a properly functioning cell the change in charge can be expressed quantitatively either in terms of the amount and direction of current that has passed through the cell or of the amount of chemical change that has occurred. The cell voltage, in contrast, is a nonlinear function of charge (as given by the Nernst equation), and change in voltage is not stoichiometric with chemical change.

The adenylate energy-storage system may be described by eq 2. This equation represents the sum of the

$$AMP + 2P_i \rightleftarrows ATP + 2H_2O \quad (2)$$

processes indicated in eq 3 and 4. Equation 4 describes

$$2ADP + 2P_i \rightleftarrows 2ATP + 2H_2O \quad (3)$$

$$ATP + AMP \rightleftarrows 2ADP \quad (4)$$

the reaction catalyzed by adenylate kinase, and eq 3 symbolizes ATP regeneration, by electron transport phosphorylation or otherwise, or the utilization of ATP in metabolism. (Metabolic uses of ATP seldom involve direct hydrolysis, but this fact does not affect the present discussion.)

It follows from the analogy with an electrochemical storage cell that a linear charge function, stoichiometric with chemical change, should be conceptually useful in considering metabolic functions of the adenylate system. One appropriate function is seen from eq 2 to be the number of anhydride-bound phosphates per adenosine moiety, which varies from 0 at complete discharge (only AMP present) to 2 at full charge (only ATP present). Division by 2 gives a parameter varying between 0 and 1, which has been named the *energy charge* of the adenylate system (Atkinson and Walton, 1967). Since the number of anhydride-bound phosphates is 2(ATP) + (ADP), the energy charge is defined in terms of actual concentrations as [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)]. When only ATP and AMP are present, the expression for energy charge reduces to the simple mole fraction of ATP, that is, (ATP)/[(ATP) + (AMP)]. This expression for charge is obviously compatible with eq 2.

The relation between free energy of hydrolysis and charge of the adenylate system is similar to that between voltage and charge of an electrochemical cell (Atkinson, 1968b). The two relations are not identical because the concentrations of three substances, ATP, ADP, and AMP, contribute to energy charge, but only two of these, ADP and ATP, are involved in hydrolysis (eq 3).

The Energy Charge in Metabolic Regulation. The funneling of energy metabolism through the adenylate system clearly is functionally advantageous to the living cell; otherwise it would have been neither developed to such an extent nor retained throughout the evolution of all types of organisms. Among the obvious advantages of a "currency" (ATP) metabolic economy in contrast to a "barter" economy are requirements for a smaller number of enzymes and flexibility in coupling energy-yielding to energy-demanding processes. When the regulatory needs of the cell are considered, it seems likely that an additional and equally important advantage of the adenylate system is that it facilitates control of metabolism on the basis of the cell's energy balance. The energy charge of the adenylate system is an appropriate input parameter in the control of biosynthetic pathways, energy-storage processes, and degradative sequences involved in regeneration of ATP. The central role of the adenylate system in nearly all metabolic energy-coupling processes must have greatly facilitated the evolutionary development of mechanisms for keeping metabolic sequences of these diverse types in balance.

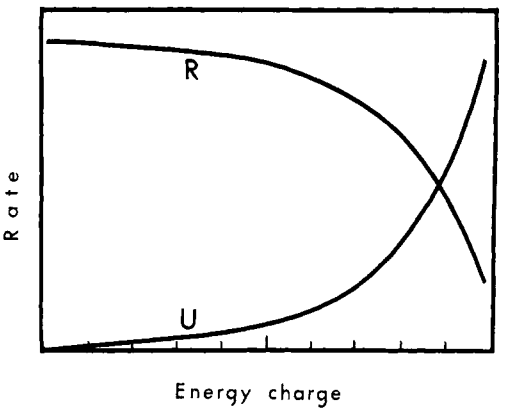

FIGURE 1: Generalized response to the energy charge of enzymes involved in regulation of ATP-regenerating (R) and ATP-utilizing (U) sequences.

Several enzymes that catalyze reactions in glycolysis or the citrate cycle are either inhibited by ATP or stimulated by AMP (for review, see Atkinson, 1966). These findings suggested that such enzymes should respond to the energy charge as indicated by curve R in Figure 1. Reactions in which ATP is used for biosynthesis or the production of storage compounds, on the other hand, might be expected to respond as shown by curve U. Several enzymes of each type have been found to give curves of the expected shape. Phosphofructokinase and pyruvate dehydrogenase (Shen *et al.*, 1968), and citrate synthase and isocitrate dehydrogenase (Atkinson, 1968b) respond as indicated by curve R. The citrate cleavage enzyme (Atkinson and Walton, 1967), phosphoribosyl pyrophosphate synthetase (Atkinson and Fall, 1967), and aspartokinase and phosphoribosyl-ATP synthetase ("pyrophosphorylase") (Klungsøyr *et al.*, 1968) respond as indicated by curve U. A plot similar to Figure 1 was presented in a recent abstract (Atkinson, 1967).

In Figure 1, the intersection of the two curves represents a stable metabolic steady state. Any decrease in the energy charge will tend to increase the rates of reactions that control ATP-regenerating sequences (curve

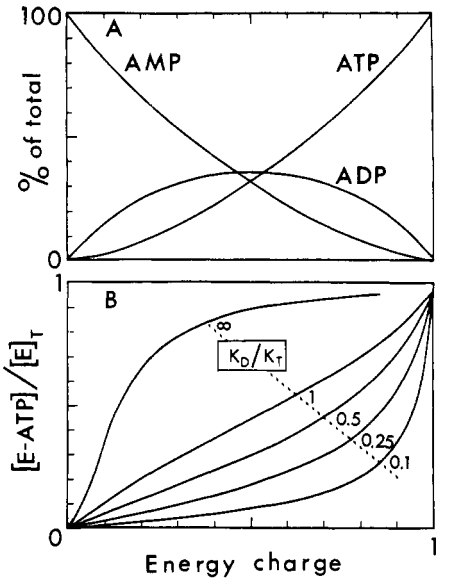

FIGURE 2: Relative concentrations of the adenine nucleotides and per cent saturation of an ATP binding site as a function of energy charge. (A) Relative concentrations of ATP, ADP, and AMP when the adenylate kinase reaction is at equilibrium, assuming an equilibrium constant of 0.8 (Markland and Wadkins, 1966). (B) Calculated per cent saturation, as a function of energy charge, of an enzymic ATP binding site, assuming a total adenylate pool (ATP + ADP + AMP) of 5 mM, Michaelis constant for ATP, K_T, of 0.2 mM, and various Michaelis constants for ADP, K_D, as specified by the K_D/K_T ratios identifying the curves.

R) and to decrease the rates of reactions controlling ATP-consuming processes (curve U). Both of these changes counteract the decrease in charge. It is obvious that an increase in the value of the energy charge would be similarly resisted; thus the system is strongly poised.

The relative proportions of the individual adenine nucleotides as functions of the energy charge are shown in Figure 2A (Atkinson and Walton, 1967). Modulation of enzymic behavior cannot, of course, depend directly upon an abstract concept like energy charge. The enzyme must respond to the concentration of one of the nucleotides or to some function of the concentrations of two or all three. Response to a ratio of two concentrations is especially readily available, since it can be produced by evolutionary modification of the affinities at a single site for the two compounds. In the case of enzymes that catalyze reactions in which one adenylate nucleotide is converted into another, a considerable range of responses to the energy charge can be evolved merely by adjustment of the relative affinities of the catalytic site for substrate and product. Figure 2B illustrates, for the important case of kinases, the spectrum of responses to energy charge that may be produced by alteration of the relative affinities of the catalytic site for ATP and ADP. In calculating the curves of Figure 2B, the parameters of an assumed kinase were fixed, except that the affinity for ADP at the catalytic site was varied. It is clear that variation of this parameter alone leads to behavior ranging from simple Michaelis response to ATP concentration to an exceedingly sharp response to the energy charge, and that surprisingly large changes in response can result from small changes in relative affinities for ATP and ADP. Observed behaviors resemble the curves for K_D/K_T ratios of 0.25 and 0.1 (Atkinson and Walton, 1967; Atkinson and Fall, 1967; Shen *et al.*, 1968).

Control of the type illustrated in Figure 2B requires only adjustment of affinities at the catalytic site for compounds that, because they participate in the reaction, are of necessity bound at that site in any case. Development of such a response thus appears to be much simpler than the evolution of a wholly new property, for example, a discrete regulatory site. Since kinases are ATP-utilizing enzymes, the direction of response illustrated in Figure 2B (and by curve U of Figure 1) will in a great majority of cases be the appropriate direction, and it seems reasonable to predict that competition between ADP and ATP will be found to contribute to the regulation of most kinases.

The metabolic roles of a few kinases would make response of the type just discussed inappropriate. Phosphofructokinase, for example, although it catalyzes an ATP-using reaction, participates in a sequence (glycolysis) that is directed toward the *regeneration* of ATP. Thus its response to energy charge should resemble curve R of Figure 1, rather than curve U. The discovery that ATP, although a reactant, inhibits phosphofructokinase (Lardy and Parks, 1956), and that this inhibition is overcome by AMP (Mansour, 1963; Passonneau and Lowry, 1962) or ADP (Atkinson, 1966), provided preliminary evidence for the expected type of response, which is shown directly in an accompanying paper (Shen *et al.*, 1968).

Interactions between Feedback Modifiers and Energy Charge. ENZYMES INVOLVED IN BIOSYNTHETIC SEQUENCES OR IN PRODUCTION OF STORAGE COMPOUNDS. Following the initial discoveries of Umbarger (1956) and Yates and Pardee (1956), product feedback inhibition has been found to participate in the regulation of most, if not all, biosynthetic sequences. It is thus of interest to consider how product feedback and energy charge regulation may interact. A preliminary discussion of this point has been presented (Atkinson, 1969).

In the case of an ATP-utilizing enzyme that is known to be regulated both by product feedback and by energy charge, the type of interaction is predictable (Figure 3). Since ATP is a reaction component, the standard assay system is at an energy charge of 1.0, and product inhibition in the standard assay is represented by points along the right-hand vertical coordinate of the energy charge plot. When the enzyme is known to respond to energy charge in the absence of product inhibitor as shown by curve l of Figure 3, it follows that its response to variation in charge at fixed levels of product must be as shown by curves n and h. Curve n represents behavior in the presence of a metabolically normal level of product, and curves l and h refer to low and high concentrations, respectively. Phosphoribosyl pyrophosphate synthetase, aspartokinase, and phosphoribosyl-ATP synthetase have been shown to respond to variation in energy charge and in the concentration of a feedback modifier approximately as shown in Figure 3 (Klungsøyr *et al.*, 1968).

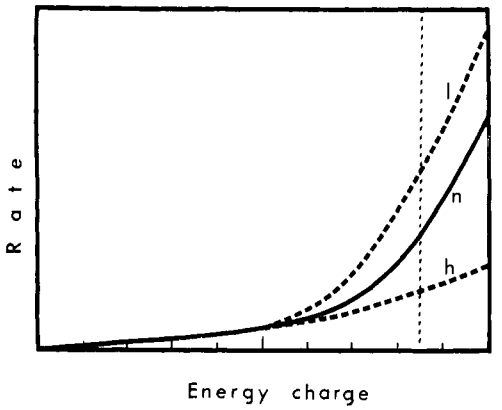

FIGURE 3: Generalized interaction between energy charge and end-product concentration in the control of a regulatory enzyme in a biosynthetic sequence. The curves correspond to low (l), normal (n), and high (h) concentrations of the end-product modifier.

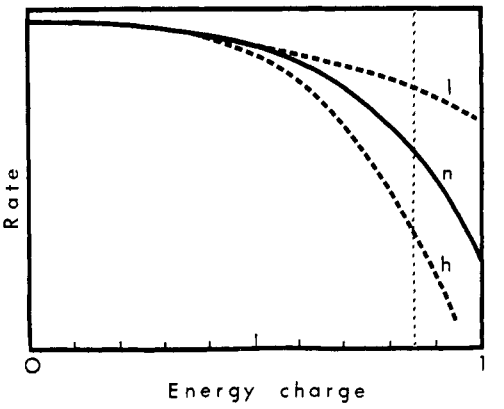

FIGURE 4: Generalized interaction between energy charge and the concentration of a metabolite modifier in the control of a regulatory enzyme in an amphibolic sequence. The curves correspond to low (l), normal (n), and high (h) concentrations of the metabolite modifier.

If the response patterns of enzymes in the intact cell are similar to those observed in extracts, Figure 3 provides a basis for consideration of interactions among biosynthetic pathways. In a cell with the energy charge poised at a value near 0.85 (the vertical line in Figure 3), each biosynthetic sequence will be controlled primarily by the concentration of its regulatory end product. This control is represented by motion up or down the vertical line. The effectiveness with which each biosynthetic process competes with other synthetic sequences for ATP and primary intermediates will thus depend upon the momentary concentration of its product. In functional terms, the cell's needs determine how its resources will be partitioned among competing demands. When the supply of exogenous substrate is adequate, any tendency for the charge to fall will be counteracted by an increase in the rate of ATP-regenerating sequences (curve R, Figure 1). The steady-state metabolizing system will thus be well regulated and relatively stable.

When the supply of exogenous substrate is limited, the rate of ATP regeneration may be expected to decrease (curve R of Figure 1 will move downward). The resulting imbalance between the rates of utilization and regeneration of ATP will cause the energy charge to decrease, and because of the changed position of curve R (that is, limitation on rate of ATP regeneration), a new steady-state situation at a lower value of energy charge will result. For all biosynthetic curves, intersection with the new energy charge line will be lower than before; thus all biosyntheses, and consequently macromolecular synthesis and growth, will be decelerated. Competition among biosynthetic sequences should continue, as before, on the basis of the relative concentrations of feedback modifiers.

INTERACTION BETWEEN ENERGY CHARGE AND FEEDBACK MODIFIERS IN REGULATION OF SEQUENCES THAT REGENERATE ATP AND SUPPLY PRIMARY BIOSYNTHETIC INTERMEDIATES. The metabolic sequences that lead to regeneration of ATP contain most of the primary intermediates that are used in biosyntheses. Thus replenishment of the pools of these intermediates cannot be separated from ATP regeneration. Davis (1961) emphasized this overlap of function by terming such sequences *amphibolic*. It is clear that overlapping function must be accompanied by overlapping controls. In a cell metabolizing carbohydrate, glycolysis is such an amphibolic sequence. Regulation by the energy charge alone would have the consequence that the replenishment of primary biosynthetic intermediates (such as pyruvate, acetyl coenzyme A, α-ketoglutarate, and oxalacetate) would be severely limited when the energy charge was high. Thus a plentiful energy supply would depress biosynthetic activity. In order to avoid this undesirable result, energy charge regulation of such sequences must be supplemented by feedback modulation similar in principle to the product feedback inhibition by which biosynthetic sequences are regulated. A few effects have already been reported that seem to serve this function. Thus citrate is a negative modifier for phosphofructokinase (Passonneau and Lowry, 1963; Garland *et al.*, 1963; Parmeggiani and Bowman, 1963) and acetyl coenzyme A for pyruvate dehydrogenase (Garland and Randle, 1963). Both of these effects are related explicitly to energy charge control in an accompanying paper (Shen *et al.*, 1968).

The expected interaction between energy charge and feedback modifier in amphibolic metabolism is illustrated in Figure 4. The solid line n represents response to energy charge when the feedback modifier (an indicator of the concentration level of biosynthetic intermediates supplied by the amphibolic sequence) is at the midpoint of its regulatory range. A decrease in this concentration causes the response curve to change toward that labeled l; the rate of the sequence increases at any given energy charge. Conversely, an increase in the concentration of the feedback modifier shifts the response toward that labeled h. In the extreme case of abundant availability of the feedback intermediate from another source, the amphibolic sequence becomes only an ATP-regenerating sequence; under these conditions it is maximally sensitive to regulation by energy charge (curve h).

If response to energy charge is modulated by feed-

back inhibitors as proposed in Figures 3 and 4, the simple intersection of two response curves shown in Figure 1 must be replaced by overlap of regulatory regions. Regulation of energy metabolism may be considered to result from simultaneous interaction between many enzymes that behave as indicated by Figure 3 and several that respond as shown in Figure 4. Such a regulatory system is complex in terms of multiplicity, but extremely simple in principle. It should allow maximal flexibility in regulating the rates of individual synthetic sequences in response to metabolic need for their products, maintaining suitable levels of the primary intermediates that serve as starting points for biosynthetic pathways, stabilizing the energy charge when the supply of utilizable substrate is adequate, and adjusting the over-all rate of ATP expenditure in response to the cell's ability to regenerate ATP when the supply of substrate is limited.

General Discussion

The generalizations proposed in this paper, like any discussion of metabolic interrelations at present, are necessarily oversimplified. Little satisfactory information is available as to the extent of functional compartmentation in the living cell, or of the relative importance of barrier compartmentation (resulting from membranes) as compared with kinetic compartmentation (resulting from association of enzymes in ways that facilitate movement of a product to the active site of the next enzyme, and hinder diffusion between the vicinity of the active sites and the general cell sap). It seems likely that the selective permeabilities and transport properties of membranes are modulated by the energy charge and by the concentrations of specific metabolites on both sides of the barrier, but definitive evidence is lacking.

The concepts illustrated in Figures 3 and 4 are oversimplified also with regard to the effects immediately involved. The figures show *rates* of enzyme-catalyzed reactions as functions of energy charge and the concentration of a feedback modifier. In most cases, however, the property most strongly modulated is the *affinity* of the enzyme for its substrate. The typical effect of energy charge and modifier concentration is probably to determine the effectiveness with which an enzyme competes with other enzymes that utilize the same substrate.

The fundamental chemical needs of a living cell are primary biosynthetic intermediates, energy (ATP), and reducing power (TPNH). This paper discusses properties of enzymes, as studied *in vitro*, that suggest that the regeneration and expenditure of ATP may be controlled by the cell's energy balance (the energy charge of the adenylate system). Because of the unique importance of the adenine nucleotides in energy metabolism, this control may directly affect nearly all metabolic processes. Figure 4 illustrates how the level of primary intermediates may interact with energy charge control. It would be surprising if the supply of the third fundamental requirement, reducing power, were not regulated in a similar manner. Thus we may predict that the charge of the TPNH system, the mole fraction (TPNH)/[(TPN^+) + (TPNH)], will be found to be an important control parameter for those processes whose metabolic function is the regeneration of TPNH. Indeed, the discovery of enzymes that are so regulated may be of value in solving the currently open question of which sequences, among the several possibilities, are actually important in the regeneration of TPNH.

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