Phosphate Incorporation in Desheathed Nerves: Effects of Potassium and Calcium Ions

Abstract. *In the presence of Ringer's solution containing a high concentration of potassium ions, the incorporation of P^{32}-orthophosphate into adenosine triphosphate and other organophosphates of the desheathed sciatic nerve of the bullfrog was inhibited by more than 75 percent. In calcium-free Ringer's solution containing ethylenediaminetetraacetic acid, the inhibition was about 30 percent. Both solutions depolarized the nerves, and the depolarization probably interfered with the mechanisms of phosphate transfer rather than directly with phosphorylation. The inhibition may have been a consequence of the decreased adsorption of phosphates which occurs at the excitable surface during depolarization.*

A number of years ago it was proposed that depolarization and excitation of excitable tissues caused a decrease in the rate of synthesis of adenosine triphosphate (ATP) and other phosphorylated derivatives (*1*). As a possible explanation for the thesis, which was not in conformity with prevailing hypotheses (*2*), it was suggested that structural changes induced in the excitable membrane during depolarization were in some way responsible. From the standpoint of the ionic hypothesis, this phenomenon can be interpreted on the basis that the energy-requiring mechanism for maintaining intracellular K^+ must be interrupted to permit the entry of Na^+ (*1*). To further substantiate and clarify this concept, studies were conducted with bullfrog nerves immersed in Ringer's solution containing a high concentration of potassium (K^+ medium) or in calcium-free Ringer's solution containing ethylenediaminetetraacetic acid (Ca-free medium). Both media induce rapid and complete depolarization.

The sciatic nerves were removed from their surrounding connective tissue sheaths (perineurium and epineurium) so that they maintained their electrical excitability as well as did sheathed nerves, while the fine structure of individual fibers, as revealed by electron microscopy, appeared to be essentially normal. Each nerve weighed about 70 mg. The composition of the various incubation media and the procedures for washing the nerves after incubation and for analyzing the phosphorylated derivatives by chromatography have been described elsewhere (*3*). Electrical recordings were made to establish that both the K^+ medium and the Ca-free medium caused depolarization of the desheathed nerves.

Nerves immersed in the K^+ medium showed a significant decrease in their phosphocreatine content compared with those exposed to normal Ringer's solution, while the levels of other derivatives remained essentially constant (Table 1). No significant change in the total amount of any derivative was observed in nerves exposed to the Ca-free medium. The specific activity of the isotope in all of the derivatives, however, was greatly reduced when nerves were exposed to either test solution.

In K^+ medium, the incorporation of P^{32}-orthophosphate into the organophosphates was inhibited by 74 to 80 percent, and in Ca-free medium the range of inhibition was 28 to 50 percent. Although the specific activity of orthophosphate was also reduced in the K^+ medium and to a lesser extent in the Ca-free medium, the specific activity of the organophosphates relative to orthophosphate was still considerably less than the control values.

The inhibitive effect of external potassium ions on the incorporation of P^{32}-orthophosphate into the organophosphates of the desheathed nerve might be due to an inhibition of phosphorylative mechanisms or to an interference with phosphate entry or transport, or to both. Since mitochondria of neural tissue will conduct oxidative phosphorylation equally well in the presence of potassium and sodium ions, it does not seem that a direct effect upon mitochondrial phosphorylative enzymes could account for the difference. Glycolysis also proceeds equally well in the presence of either cation, as do the associated reactions involved in the synthesis of ATP. Studies with whole homogenates of nerve and with isolated nerve mitochondria fail to show any differential response to sodium and potassium ions. The more plausible explanation is that the high external concentration of potassium ions interferes with phosphate transfer somewhere between the exterior and the main region of ATP synthesis.

For the purpose of discussion, one may consider that the desheathed nerve bundle contains three distinct compartments—the neurilemmal sheath, the multilayered myelin, and the axoplasm—separated by a discrete axonal membrane. In view of the fact that the specific activity of orthophosphate in nerves exposed either to K^+ medium or to Ca-free medium is not appreciably altered, it appears as if the overall entry of P^{32}-orthophosphate is not sufficiently impeded to explain the differences. What seems more probable is that there exists some "compartmentalization" of the ATP within the nerve fiber, as reported previously (*4*), and that communicative channels between the exterior and such compartments

Table 1. The effect of a high concentration of potassium on the rate of incorporation of P^{32}-orthophosphate into the desheathed sciatic nerve of the bullfrog. One pair of nerves was incubated in 2 ml of normal Ringer's solution (control), another in 2 ml of Ringer's solution with 112m*M* sodium chloride replaced by an equivalent amount of potassium chloride, and a third in 2 ml of Ca-free Ringer's solution containing 5m*M* EDTA. The medium contained 50 μc P^{32}-orthophosphate and incubation was for 3 hours at 27°C. The values are presented as the mean of four experiments ± standard deviation.

Amount (μmole/g)			Specific activity*			Inhibition of specific activity (%)	
Control	K^+medium	Ca-free medium	Control	K^+medium	Ca-free medium	K^+medium	Ca-free medium
			Orthophosphate				
1.5 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	190 ± 25	98 ± 35	160	48	16
			Phosphocreatine				
0.8 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	90 ± 15	18 ± 2	45	80	50
			ATP†				
1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	100 ± 15	25 ± 3	70	75	30
			ADP				
0.3 ± 0.25	0.3 ± 0.07	0.3 ± 0.05	90 ± 12	20 ± 3	65	78	28
			Glucose-6-phosphate				
0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	65 ± 10	17 ± 4	45	74	31

* Radioactive counts per minute per μmole P × 10^{-3}. † The two terminal phosphorus atoms are included in the measurement of radioactivity in ATP, but only the terminal phosphorus atom in ADP.

are altered by high concentrations of potassium ions and the removal of calcium ions.

Since both the excitability and the rate of incorporation of P^{32}-orthophosphate return to normal upon reimmersion of the nerves in ordinary Ringer's solution, it seems unlikely that any structural damage to the nerve fiber occurred during our experiments. A high external concentration of potassium ions does not significantly alter the internal K^+ concentration in the excitable tissues (*5*), so that the effect of K^+ probably occurs at the external surface. The surfaces most accessible to the exterior are the outer layer of the neurilemmal sheath and the axonal membrane at the region of the nodes of Ranvier. Since mitochondria are located in the axoplasm as well as in the neurilemmal sheath (*1*), and since the inhibitory action of potassium ions upon phosphorylation is almost complete, both regions must be affected, and a common inhibitory mechanism is suggested.

It has been postulated that a release of calcium ions from the excitable membrane occurs during depolarization and that this release is accompanied by an outflux of orthophosphate and ATP (*4*). The net flow of phosphates in the outward direction would indicate that phosphate adsorption at the interface is diminished by depolarization. It remains to be seen whether this outflux of phosphates and inhibition of phosphorylation accompanying depolarization is due to loss of Ca^{2+} or to the change in the K^+ concentration at the surface of the excitable membrane (*6*).

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References and Notes

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Feedback Control of Purified Deoxycytidylate Deaminase

Abstract. *The activity of purified deoxycytidylate deaminase obtained from chick-embryo extracts is dependent upon the presence of deoxycytidine triphosphate and magnesium ions. The stability of the enzyme at 37°C is markedly enhanced by deoxycytidine triphosphate, and less so by the other deoxyribonucleotides. The inhibition by* p*-chloromercuribenzoate, urea, and deoxythymidine triphosphate, is reversed by deoxycytidine triphosphate. This reversal suggests that the regulation of enzyme activity is effected through configurational changes in the enzyme structure.*

We have recently described (*1*) the activating effect of dCTP (*2*) on deoxycytidylate deaminase, and the marked inhibition of the enzyme by dTTP. In view of the end-product regulatory influence of these nucleotides on the enzyme, and a similarity to the studies on aspartate transcarbamylase (*3*), a feedback mechanism was suggested which might possibly be invoked through conformational changes in the enzyme structure. These changes could explain the apparent increase in affinity of the enzyme for dCMP and its reversal by dTTP, and they suggest a control mechanism that may be important to numerous other enzymes. Subsequent to our original observations with extracts from chick and rat embryos, the enzyme has been highly purified from 6-day-old chick embryos and some of its properties have been described (*4*). The present report on studies with the purified enzyme supports the concept of a feedback control. Monod *et al.* (*5*) have recently reviewed a number of enzymes that appear to be activated or inhibited at a site (allosteric) other than the substrate site.

The activity of the purified enzyme is markedly dependent on the presence of dCTP and Mg^{++} when the substrate concentration was 2m*M* and less (Table 1). This observation would explain the activating effect of dCTP when added either to aged preparations or those incubated at 37°C (*1*), in which the endogenous dCTP had probably been depleted through enzymic hydrolysis. Similar results were recently reported (*6*) on a partially purified spleen preparation of deoxycytidylate deaminase. However, no apparent requirement for dCTP and Mg^{++} was described, which may be the result of differences in purity of the two preparations. The enzyme preparation was purified approximately 100-fold from the crude extract of 6-day-old chick embryos. The essential steps in the purification were calcium phosphate gel absorption of inert protein, precipitation of nucleic acids by protamine, saturation with ammonium sulfate, and fractionation on a cellulose phosphate column. A unit is defined as the amount of enzyme that will deaminate 1 μmole of dCMP in 10 minutes at 37°C. The initial specific activity was 0.50 unit per milligram of protein in a crude extract of chick embryos and the final purified enzyme preparation was capable of deaminating, in 10 minutes at 37°C, 50 to 60 μmoles of dCMP per milligram of protein when the concentration of dCMP in the substrate was 2m*M*.

When the substrate concentration of dCMP was 10m*M*, charcoal treatment of the purified enzyme preparation results in a partial requirement for dCTP, whereas the requirement was absolute at 2m*M* dCMP. There was a 30-percent decrease in activity at a concentration of 10m*M* dCMP, which could be partially restored by the addition of dCTP and Mg^{++}. It is not known whether this results from the stabilizing effect of dCTP (*7*) on the deaminase (Table 2), its role as a cofactor, or possibly an increase in affinity of the enzyme for its substrate. An increase in the affinity should be reflected in a decrease in the Michaelis-Menten constant (K_m) for the monophosphate in the presence of the triphosphate; however, as indicated in Table 1, little or no activity could be measured in the absence of the triphosphate and Mg^{++} at 2m*M* or less of dCMP, thus making it difficult to evaluate K_m accurately. Since this inability to detect significant activity may be due to the insensitivity of the assay, the question of whether a change in K_m is being effected by dCTP and Mg^{++} can possibly be resolved through the use of dCMP-2-C^{14} in the assay (*8*). It is also conceivable that two enzymes are present in the purified deaminase preparation, one with a low affinity for dCMP and the