Formation of Active Phosphoenzymes with the Diphosphoglycerate-Dependent Phosphoglycerate Mutases

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It is not clear whether the diphosphoglycerate-dependent phosphoglycerate mutases combine with diphosphoglycerate to give enzyme-diphosphoglycerate complexes or whether phosphoenzymes are formed. In the latter case monophosphoglycerate should be released in stoichiometric quantity during the reaction. 2,3-Diphosphoglycerate was therefore added to substrate quantities of the enzymes in the presence of enolase, pyruvate kinase, lactate dehydrogenase, ADP and NADH. Monophosphoglycerate determined by the change in E_{340} was formed in an initial burst in the quantity expected for phosphoenzyme formation (2mol of phosphate/mol for the rabbit muscle and pig kidney enzymes; >3mol of phosphate/mol for the yeast enzyme); thereafter there was a steady production of monophosphoglycerate until the diphosphoglycerate was completely hydrolysed. Successive additions of diphosphoglycerate indicated that the lifetime of the phosphoenzymes was of the order of minutes, and the instability of the phosphoenzymes accounted for the diphosphoglycerate phosphatase activity. In further experiments with the rabbit muscle and pig kidney enzymes, the amount of enolase was lowered to rate-limiting quantities and only sufficient diphosphoglycerate was added to titrate the enzyme. The initial rate of reaction was the same as that obtained when an equal quantity of 3-phosphoglycerate was substituted for diphosphoglycerate. Reversible association of the phosphoenzymes with monophosphoglycerate to form enzyme-diphosphoglycerate complexes could therefore be excluded. Since the enzymes show Michaelis-Menten kinetics under these conditions, it follows that the phosphoenzymes must be catalytically active.

Incubation of the enzymes (from rabbit muscle, pig kidney and yeast) with [^{32}P]diphospho[^{14}C]glycerate followed by gel filtration yielded phosphoenzymes with no bound ^{14}C . After denaturation with sodium dodecyl sulphate, the phosphoproteins from pig kidney and yeast were stable to alkali and unstable to acid, suggesting the involvement of phosphohistidine, as has been shown for the rabbit muscle enzyme (Rose, 1970).

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Rose, Z. B. (1970) *Arch. Biochem. Biophys.* **140**, 508

Nucleic Acids Spontaneously Released by Living Frog Auricles

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Bacteria spontaneously release DNA (Borenstein & Ephrati-Elizur, 1969). The bacterial DNA shed can be taken up by other bacteria (Borenstein & Ephrati-Elizur, 1969) as well as by cells of plants or animals (Stroun *et al.*, 1970; Stroun & Anker, 1971a,b; Stroun, 1971). In the present paper we report the release of nucleic acids by living frog auricles, which can be maintained for more than 2 days in Ringer solution.

Extracted auricles were placed for various times in sterile Ringer solution containing [^3H]thymidine. From the auricles and the Ringer solution DNA was extracted by the Marmur and the Schmidt-Tannhauser techniques. The amount of DNA shed after 24h can represent over 10% of the total DNA of the auricles. This DNA can be hybridized *in vitro* with frog auricle [^3H]RNA and sediments after a CsCl-density-gradient centrifugation at the level of frog DNA. Sterility tests have shown that the nucleic acids found in Ringer solution cannot come from bacteria. They are not produced by organelles or organisms such as mycoplasma, since no pellet was found after centrifugation of the Ringer solution at 3×10^4g for 4h. The nucleic acids shed cannot come from the wounded cells, since the cut cells resulting from the ablation of the ventricles represent only 0.2% of the total cells. Moreover auricles that have been cut in three do not release more nucleic acids than whole auricles.

The specific radioactivity of the released DNA is 2-3 times that of the DNA extracted from the auricles, indicating that it has been recently synthesized. Moreover the auricles are composed of non-dividing cells. It therefore seems that the released DNA is metabolic DNA.

These results suggest that nucleic acids may circulate from cell to cell in certain conditions. Indeed the DNA released represent more than the totality of the nucleic acids contained by the cells on the surface of the explants. The possibility of finding some circulating DNA completely *in vivo* is being investigated.

Borenstein, S. & Ephrati-Elizur, E. (1969) *J. Mol. Biol.* **45**, 137