INFLUENCE OF 2,3-DIPHOSPHOGLYCERATE ON PHOSPHOFRUCTOKINASE OF HUMAN ERYTHROCYTES?

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Phosphofructokinase catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. Control of glycolysis is usually explained in terms of the properties of three enzymes: hexokinase, phosphofructokinase and pyruvate kinase. Recently Beutler [1] reported that phosphofructokinase (PFK) of human erythrocytes is inhibited by 2,3-DPG. It is known that the concentration of 2,3-DPG in erythrocytes is very high. In view of control of glycolysis the finding by Beutler is very important. However, our results are in disagreement with those of Beutler.

PFK from human erythrocytes was purified 20,000 times by employing column chromatography (DEAE-cellulose, Sepharose 6B) and ammonium sulphate precipitation (manuscript in preparation). The enzyme shows allosteric properties especially at about pH 7.0. The enzymatic activity of PFK can be influenced by various ligands such as ATP, ADP, P_i, cyclic AMP and 5'-AMP [2]. PFK activity was measured in the coupled aldolase test. No influence of 2,3-DPG (conc. in the cuvette 5 mM) on purified PFK could be demonstrated. This was also the case when the enzyme was tested at different pH-values and at different F6P and ATP concentrations. However, what then may be the reason that an inhibition by 2,3-DPG on PFK was found in hemolysate [1]? To investigate this problem it is necessary to realize what is happening in the incubation medium in the aldolase test when 2,3-DPG is added. When adding 5 mM Mg^{2+}, 2 mM ATP, 0.25 mM NADH and 5 mM 2,3-DPG to hemolysate, an oxidation of NADH is observed. The following sequence of reactions is involved:

$$\text{2,3-DPG} \longrightarrow \text{3-PG} \xrightarrow[\text{ATP ADP}]{Mg^{2+}} \text{1,3-DPG}$$

$$\xrightarrow[\text{NADH NAD}]{} \text{glyceraldehyde-3-P.}$$

When Mg^{2+} or ATP is omitted no reaction is observed. In our opinion the influence of 2,3-DPG on PFK in hemolysate can only be investigated when the reaction sequence as given above, is stopped. This is possible by adding 5 mM ADP to the reaction mixture; under these conditions the phosphoglycerate kinase reaction is inhibited.

5 mM ADP has little or no influence on PFK at pH 8.0. So only in this way can the influence of 2,3-DPG on PFK be investigated. Table 1 gives the influence of different media on PFK activity. Reaction medium I (without 2,3-DPG) gives 100% activity. In reaction medium II (with 2,3-DPG) the sequence of reactions as mentioned above is operating; when after 10–15 min incubation F6P is added, an apparent inhibition is observed (20–30% activity). However, when the same is carried out but now in the presence of ADP (reaction medium III) no inhibition is found at all. Without 2,3-DPG but in the presence of ADP (reaction medium IV) the same activity as in I and III is found. These results are in disagreement with those of Beutler.

References

[1] E. Beutler, Nature New Biology 232 (1971) 20.
[2] G.E.J. Staal, First Meeting European Division International Society Haematology, Milano (1971).

Table 1
The influence of different reaction media on PFK activity.

Reaction mixture	I	II	III	IV
	ATP	ATP	ATP	ATP
	Mg^{2+}, K^+	Mg^{2+}, K^+	Mg^{2+}, K^+	Mg^{2+}, K^+
	–	–	ADP	ADP
	NADH	NADH	NADH	NADH
	–	2,3-DPG	2,3-DPG	–
	Hemolysate	Hemolysate	Hemolysate	Hemolysate
	Auxiliary enzymes	Auxiliary enzymes	Auxiliary enzymes	Auxiliary enzymes
Activity (%)	100	20–30	100	100

The reactions were started by adding F6P (conc. in the cuvette 1 mM) after 10–15 min incubation at 25°. The oxidation of NADH was followed at 340 nm with a Perkin Elmer spectrophotometer model 124. Concentrations of the different compounds in the cuvette were: ATP = 2 mM, Mg^{2+} = 5 mM, K^+ = 6 mM, 2,3-DPG = 5 mM, ADP = 5 mM, NADH = 0.25 mM, 0.05 ml dialyzed auxiliary enzyme solution (aldolase, 10 mg/ml; triosephosphate isomerase, 2 mg/ml; glycerin-1-phosphate dehydrogenase, 2 mg/ml). Buffer 0.3 M Tris-HCl pH 8.0.