THE EFFECT OF 2,3-DPG ON RED CELL PHOSPHOFRUCTOKINASE

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We have previously reported that erythrocyte phosphofructokinase (PFK) was inhibited by 2,3-DPG [1,2]. In a recent letter, Staal and Koster [3] wrote that our results were in error. In the meanwhile, the prior observations of Busch and Boie [4], indicating that hemolysate PFK is inhibited by 2,3-DPG have come to our attention. Our findings regarding inhibition of PFK by 2,3-DPG have also now been confirmed by Ponce et al. [5], using partially purified enzyme and by Tarui [6], using purified enzyme. It is quite clear that 2,3-DPG does inhibit PFK.

Staal and Koster suggested that our findings were due to hydrolysis of 2,3-DPG by 2,3-DPG phosphatase to form 3-PGA which then resulted in oxidation of NADH through the phosphoglycerate kinase and glyceraldehyde phosphate dehydrogenase steps. Their interpretations are erroneous in several respects:

1. The 2,3-DPG phosphatase reaction occurs at such a slow rate that we have been unable to measure it using the proposed reaction scheme. The activity of this enzyme is of the order of 12 milliunits per gram hemoglobin [7]; this is 1/100th of the rate of the phosphofructokinase reaction with which they propose it interferes.

2. The magnesium and ATP dependent reaction to which Dr. Staal and Koster refer is observed only when commercial 2,3-DPG preparations contaminated with 3-phosphoglyceric acid (3-PGA) are used. Such contamination unfortunately seems to be present very frequently. For example, enzymatic assays of several lots of 2,3-DPG in our laboratory recently revealed that Sigma Lot No. 1100–0830 contained 4.35% 3-PGA while Boehringer Lot No. 7470416 contained 3.69% and lot 7332316 4.55% 3-PGA on a molar basis. Not all commercial 2,3-DPG is as heavily contaminated with 3-PGA. Boehringer Lot No. 7200215 was found to contain very little (.031%) 3-PGA, and lot 7412117 only 0.07% PGA. We have been able to prepare 2,3-DPG which contains <0.01% 3-PGA by absorbing contaminated commercial 2,3-DPG on a Dowex 1-formate column and eluting the 3-PGA with 2 N ammonium formate–formic acid buffer, pH 3.0 [8]. The 2,3-DPG can be eluted with 5 N ammonium formate, the ammonium ion removed on a Dowex 50 column and the formic acid extracted with ether. It is best to avoid lyophilization of such purified 2,3-DPG because there is a tendency for some of it to decompose to 3-PGA in the process. Using these pure preparations, the ATP and magnesium dependent rate to which Doctors Staal and Koster refer is not found. But inhibition of PFK occurs nonetheless. It is clear that studies of 2,3-DPG inhibition of PFK in crude hemolysates must be carried out using 2,3-DPG which is quite free of 3-PGA.

The inhibition by 2,3-DPG of PFK which we have observed in crude hemolysates, and others have observed with purified enzyme, is clearly not due to the side reaction proposed by Staal and Koster. Why then, are they unable to find an inhibitory effect? It is axiomatic that it is essential to use the same techniques in attempting to reproduce another investigator's findings. The inability of Staal and Koster to demonstrate inhibition of PFK by 2,3-DPG can be attributed to two factors. First of all, the inhibition of PFK by 2,3-DPG is competitive with fructose 6-phosphate (F6-P) Although Staal and Koster state that they have varied the concentration of F6-P, the concentration given in their table is too high for meaningful inhibi-

tion to be observed. Indeed, using this concentration we were not able to detect significant degrees of inhibition of PFK by 2,3-DPG [2]. Secondly, the concentration of some of the reagents they used are significantly different from those which we reported. Of special importance is the fact that they employ a 300 mM Tris–HCl buffer, six times the concentration which we use [9]. We find that this concentration of Tris–chloride is inhibitory to the enzyme in our assay system, and that the inhibition of PFK which is readily observed at low F6-P levels at a 50 mM Tris–HCl concentration is much less apparent at 300 mM Tris–HCl. For example, using our assay system with a F6-P concentration of 100 μM and 50 mM Tris buffer, 5.38 U of PFK/gm Hb were found without 2,3-DPG and only 0.44 U with 5 mM 2,3-DPG (92% inhibition). When the same hemolysate was assayed with 300 mM Tris buffer the values obtained were 3.45 U and 2.83 U respectively (only 18% inhibition). It is likely that the bonds between 2,3-DPG and PFK are disrupted by high ionic strength; NaCl has a similar effect as Tris–HCl.

In conclusion, there appears to be no question that PFK is inhibited by 2,3-DPG. Staal and Koster have been misled by the use of impure commercial preparations of 2,3-DPG and by inadvertent use of assay conditions which are unsuitable for the demonstration of this inhibitory effect.

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