Effect of bivalent metal ions on the spectrum of the interaction product of glutathione and fluoropyruvate

Fluoropyruvate inhibits the respiration of mitochondrial preparations[1,2] probably because of its reaction with thiol compounds[3,4]. The products of this interaction have the general formula $RCH_2SCH_2.CO.COOH$ and show well defined absorption peaks in the ultraviolet. Those derived from α or β aminothiols (cysteine, etc.) show peaks between 295 and 300 mμ (Type II spectrum); other thiols lacking the free amino group (glutathione, etc.) show a much weaker peak between 265 and 275 mμ (Type I spectrum)[3]. A conversion of Type I spectrum to Type II takes place in conc. borate solution[5]. As borate is known to combine with the enol form of certain α-keto acids[6] (shifting the keto–enol equilibrium in favour of the enol), its effect on the spectrum of Type I has been tentatively attributed to the enolization of the α-carbonyl with the formation of a conjugated double bond ($RCH_2SCH{:}C(OH)COOH$). Since some bivalent metal ions like Zn^{++} and Cd^{++} are known to enhance the enolization of pyruvate[7] and acetylacetone[8], it was thought interesting to compare their effect and that of borate on the compound between glutathione and fluoropyruvate.

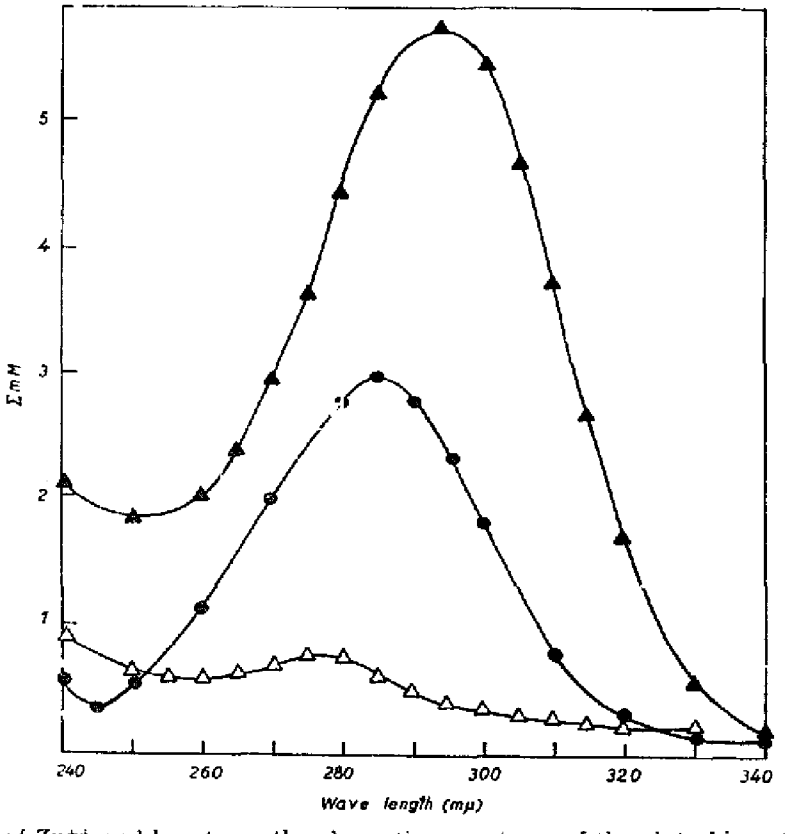

Fig. 1. Effect of Zn^{++} and borate on the absorption spectrum of the glutathione-fluoropyruvate compound. The latter was prepared by adding 0.2 ml 0.1 M fluoropyruvate to a 10^{-4} M solution of reduced glutathione, in 0.1 M tris (hydroxymethyl)aminomethane or 0.5 M borate buffer of pH 8. The reaction mixture was incubated at 37° for 15 min and cooled to room temperature. △, in tris (hydroxymethyl)aminomethane buffer; ▲, in tris (hydroxymethyl)aminomethane buffer with $2 \cdot 10^{-3}$ M $ZnSO_4$ added 5 min before the measurements; ●, in borate buffer.

On addition of Zn^{++} to this compound the absorbance in the ultraviolet increased immediately; the maximum effect was reached when the ratio of Zn^{++} concentration to that of the glutathione–fluorpyruvate compound was 10 to 1. The spectrum of the zinc enolate, which is presumably formed, is compared with the spectrum of the borate complex in Fig. 1. It can be seen that the bathochromic and hyperchromic shift occurring is larger in the case of the metal enolate. Among the various bivalent ions tested, Zn^{++} and Ni^{++} were the most effective (Table I). The effect of Zn^{++} on the spectrum was reversed by substances which form stable complexes with this ion. The chelating agents ethylendiaminetetraacetic acid, histidine, adenosine tri- or diphosphate were effective when employed in a 5- to 10-fold excess relative to the concentration of the Zn^{++}. Adenosine monophosphate and inorganic orthophosphate are ineffective even when added in a 100-fold excess.

TABLE I

EFFECT OF BIVALENT METALS ON THE LIGHT ABSORPTION OF THE GLUTATHIONE–FLUOROPYRUVATE COMPOUND

Metals ions were added to the compound in a concentration of $3.6 \cdot 10^{-4}$ *M*. (Zn^{++} produced at this concentration approximately half of the maximum absorbancy change attainable.)

Additions	$\Delta A_{295\ m\mu}$
None	—
Zn^{++}	0.278
Cd^{++}	0.031
Ni^{++}	0.327
Co^{++}	0.100
Cu^{++}	0.067
Mn^{++}	0.015
Mg^{++}, Ca^{++}, Sr^{++}, Ba^{++}	No effect

* Difference in the absorbance before and after addition of the metal ion.

In contrast to the glutathione–fluoropyruvate compound the spectrum of the compound with cysteine showed no immediate change upon the addition of Zn^{++} or other bivalent ions. These ions, however, accelerated the rate of decay of the absorbance at 300 mμ, which is otherwise relatively slow[3].

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Israel Institute for Biological Research, Ness-Ziona (Israel) Y. AVI-DOR

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