Beryllium and Alkaline Phosphatase

RECENTLY, two papers^{1,2} have appeared in which it has been shown that beryllium inhibits alkaline phosphatase by competition with magnesium. However, as inhibitions of more than 60 per cent were rarely attained, the results are of qualitative interest only. The authors were presumably aware of the insolubility of beryllium phosphate, since their alkaline phosphatase preparations were dialysed "to remove all traces of inorganic phosphate"². But the inhibitor, beryllium, was added to the substrates (B. glycerophosphate or disodium phenyl phosphate) before the enzyme. These substrates undoubtedly contain inorganic phosphate in concentrations comparable to those of the inhibitor.

Alkaline phosphatase has been prepared from rabbit kidney by a slight modification of the method of Abul-Fadl *et al.*³ and dialysed until the concentration of inorganic phosphorus was 2 μ gm./ml. ($6.6 \times 10^{-5} M$). This enzyme solution (90 Armstrong-King units/mgm. protein nitrogen) was diluted 125 times for use, giving a final phosphorus concentration of $5.3 \times 10^{-7} M$. The enzyme in carbonate/bicarbonate buffer was incubated with beryllium sulphate, the substrate added and the activity determined by the method of King⁴. Table 1 shows the inhibition of the unactivated and magnesium-activated enzyme.

Table 1

Unactivated enzyme		Activated enzyme, $1.0 \times 10^{-3} M$ magnesium	
Concentration of beryllium during incubation with enzyme (M)	Inhibition (per cent)	Concentration of beryllium during incubation with enzyme (M)	Inhibition (per cent)
5.2×10^{-6}	100	1.7×10^{-5}	95
1.3×10^{-6}	79	1.7×10^{-6}	70
3.3×10^{-7}	66	3.3×10^{-7}	30
8.1×10^{-8}	43	1.7×10^{-7}	15
2.0×10^{-8}	24		

An experiment was also designed to find out whether the reversal of inhibition by magnesium was immediate. In Table 2 the results of such an experiment are given. The enzyme was incubated for 10 min. with either beryllium or magnesium, followed by magnesium or beryllium respectively, for various periods of time.

Table 2. Beryllium concentration $3.3 \times 10^{-7} M$. Magnesium concentration $1.3 \times 10^{-3} M$. All percentage inhibition values based on activity of fully activated enzyme

No.	Addition for first 10-min. incubation	Further addition	Inhibition (per cent)
1 2 3 4	Beryllium	Nil	80
		Magnesium for 2 min.	30
		" " 10 "	26
		" " 30 "	34
			Mean 30
5 6 7 8	Magnesium	Nil	0
		Beryllium for 2 min:	35
		" " 10 "	37
		" " 30 "	38
			Mean 36.5

This demonstrates that the reversal of beryllium inhibition is immediate. Grier¹ states that if the enzyme is incubated for long periods with beryllium, the inhibition becomes difficult to reverse. Preliminary experiments indicate that this is probably also an artefact, since the control inhibition experiments were carried out in the presence of substrate (containing inorganic phosphate). Further work is in progress and will be reported later.

Beryllium is thus an extremely potent inhibitor of alkaline phosphatase (50 per cent inhibition concentration for the unactivated enzyme is $1.2 \times 10^{-7} M$, and for the magnesium-activated enzyme, $6.6 \times 10^{-7} M$). Addition of magnesium (ratio of concentrations Mg:Be $\approx 40,000:1$) reduces inhibition from 80 to 30 per cent. This reversal of inhibition is immediate. The hypothesis that beryllium may compete for the magnesium in magnesium-activated enzymes may help to provide an explanation of the toxic properties of beryllium.

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¹ Grier, R. S., Hood, M. B., and Hoagland, M. B., *J. Biol. Chem.*, **180**, 289 (1949).

² Klemperer, F. W., Miller, J. M., and Hill, C. T., *J. Biol. Chem.*, **180**, 281 (1949).

³ Abul-Fadl, M. A. M., King, E. J., Roche, J., and Nguyen-van Thoai, *Biochem. J.*, **44**, 428 (1949).

⁴ King, E. J., "Microanalysis in Medical Biochemistry" (London: Churchill, 1947).

Crystallization of Congo Red

ACCORDING to Robinson and Mills¹, "in general, the cotton substantive dyes cannot be recrystallized from water". But some of the benzidine disazo dyes may with difficulty be recrystallized from alcohol-water mixtures².

It has been confirmed that the potassium salt of Congo Red will crystallize as needles from various ethyl alcohol-water mixtures, but only with difficulty and generally in company with much amorphous material. As a result of an accidental observation during a trial of various salting-out procedures, however, the potassium salt of Congo Red has been found to be precipitated in crystalline form by salting out with potassium acetate at about 95° C. in the following way.

A 2 per cent solution of Congo Red (B.D.H. indicator) in water is heated on a water bath to 95° C., and 20 per cent by volume of 40 per cent wt./vol. potassium acetate solution (adjusted to slightly pink to phenol phthalein) is added slowly with vigorous stirring. The crystalline precipitate which forms immediately is filtered off hot on a glass filter, redissolved in water to make a 2 per cent solution and the crystallization carried out four more times. The