Influence of Inorganic Phosphorus on Oxytetracycline Formation by
Streptomyces rimosus

WALTER A. ZYGMUNT

Department of Microbiology & Natural Products Research, Mead Johnson Research Center,
Mead Johnson & Company, Evansville, Indiana

Received for publication 25 October 1963

ABSTRACT

ZYGMUNT, WALTER A. (Mead Johnson Research Center, Mead Johnson & Co., Evansville, Ind.). Influence of inorganic phosphorus on oxytetracycline formation by Streptomyces rimosus. Appl. Microbiol. 12:195–196. 1964.—Production of oxytetracycline by Streptomyces rimosus in several chemically defined media containing graded concentrations of inorganic phosphorus was studied in shake flasks. Although high levels of inorganic phosphate have been reported to inhibit oxytetracycline formation, this study indicated that composition of the medium is an important factor in determining whether antibiotic production will be stimulated or inhibited by specific concentrations of inorganic phosphate.

The adverse effects of high concentrations of inorganic phosphate on chlortetracycline and oxytetracycline biosynthesis have been reported by others (Boretti et al., 1955; Prokofieva-Belgovskaya and Popova, 1959; Alikhanian et al., 1959). Since, under our experimental conditions, formation of oxytetracycline did not appear to be inhibited by concentrations of potassium phosphate in excess of those reported to be detrimental by others, it was of interest to study the role of media composition in determining the optimal concentration of phosphorus needed for maximal antibiotic production. In this report are summarized several observations on the relationship of media constituents to phosphate concentrations necessary for optimal oxytetracycline formation.

MATERIALS AND METHODS

Procedures used for all shake flask fermentations with Streptomyces rimosus (NRRL 2234), preparation of inocula, antibiotic assays, and determinations of growth were similar to those previously described (Zygmun, 1961). All fermentations were carried out at 27 C for 5 days with 50-ml volumes of medium in 250-ml Erlenmeyer flasks. Agitation was at 200 rev/min. Normally, duplicate flasks were pooled for analysis. Composition of an amino acid-containing basal medium follows (g per liter): amino acid, 1.0; glucose, 10.0 (autoclaved separately); NaCl, 5.0; MgSO4·7H2O, 1.0; CaCl2·2H2O, 0.4; (NH4)2HPO4, 0.2; FeSO4·7H2O, 0.02; and ZnSO4·7H2O, 0.01. Composition of an ammonium salt-dextrose medium follows (g per liter): (NH4)2SO4, 4.0; NH4Cl, 2.0; MgSO4·7H2O, 2.0; 

FIG. 1. Effect of phosphate concentration on antibiotic production by Streptomyces rimosus in several amino acid basal media.

ZnSO4·7H2O, 0.1; FeSO4·7H2O, 0.06; MnSO4·4H2O, 0.06; Co(NO3)2, 0.005; CaCO3, 7.5; oleic acid, 16.11; and dextrin, 25.0.

RESULTS AND DISCUSSION

Optimal concentrations of dipotassium phosphate necessary for maximal antibiotic production varied with the organic nitrogen source used in the medium (Fig. 1). With N-acetyl-DL-alanine, the most favorable phosphate level approximated 1 mg/ml as dipotassium phosphate. A fivefold increase in antibiotic formation was noted when the concentration of dipotassium phosphate in the medium was increased from 0.05 to 0.1 mg/ml. Antibiotic yields varied only slightly over a further 20-fold increase in phosphate concentrations (0.1 to 2.0 mg/ml).

In a medium containing β-alanine, antibiotic synthesis was increased twofold when the phosphate level was increased from 0.1 to 0.5 mg/ml. A striking end point for optimal antibiotic formation in a medium utilizing L-glutamic acid was noted at a phosphate level of 0.25 mg/ml. With L-glutamic acid, this level appeared to be slightly higher, and overall antibiotic formation appeared to be influenced to a lesser degree by phosphate. Antibiotic activities per unit of dry cell weight were calculated for all phosphate additions studied in N-acetyl-DL-alanine-, β-alanine-, and L-glutamic acid-containing media, and the results closely paralleled the stimulation or inhibition of antibiotic activity reported on a unit per volume of broth basis.
Previously (Zygmunt, 1961), it was shown that histidine was not utilized well by *S. rimosus* for antibiotic formation in a medium with a high phosphate content. In this study, antibiotic production was not stimulated by decreased phosphate levels.

Figure 2 represents the effect of graded concentrations of dipotassium phosphate on antibiotic synthesis in an ammonium salts-dextrin medium. Maximal antibiotic formation occurred when approximately 0.25 mg/ml of dipotassium phosphate was added. Concentrations above 0.5 mg/ml inhibited antibiotic formation. Thus, antibiotic production appears to be more adversely affected by high phosphate levels in a medium employing inorganic nitrogen than in those containing organic nitrogen as the principal nitrogen source.

In summary, whereas others have reported that specific levels of inorganic phosphate have a detrimental effect on tetracycline biosynthesis, these studies show that composition of the medium is an important factor in determining whether antibiotic production will be stimulated or inhibited by certain concentrations of inorganic phosphate. These differences in experimental results can in part be attributed to differences in the strains of *Streptomyces* species employed, to the cultural conditions used, and to the inherent capacities of the strains to produce tetracyclines.

**Literature Cited**


