Colorimetric Determination of Inorganic Phosphorus in the Presence of Glucose-1-phosphate and Adenosine Triphosphate

THERE are many occasions in biochemical work in which inorganic phosphorus must be determined in the presence of some acid-labile phosphorus compounds such as glucose-1-phosphate or adenosine triphosphate. In Allen's method[1], for example, molybdenum blue colour is developed in 0·5 *N* perchloric acid. Owing to high acidity of the medium, glucose-1-phosphate or adenosine triphosphate is more or less hydrolysed during colour development and interferes with the determination.

During their studies on the mechanism of action of amylophosphorylase, Whelan and Bailey[2] found that hydrolysis of glucose-1-phosphate could be reduced to one-seventh as compared with Allen's original method by halving the specified amount of the reducing agent in colour development. It was interesting to re-examine their experiments for two reasons: (1) Although there are some reports[3,4] on the catalytic effect of molybdate on the acid hydrolysis of some acid-labile phosphorus compounds, there is no report pertaining to the effect of the amidol reagent. (2) Moreover, one of us[5] developed, several years ago, a modification of Allen's method, in which sulphuric acid was used in place of perchloric acid and the amount of the amidol reagent was halved. It was, at that time, not known that the amount of the amidol reagent might have some effect on the hydrolysis of glucose-1-phosphate. The amount of the amidol reagent was halved only because half the original amount was sufficient for most determinations and excess might better be avoided.

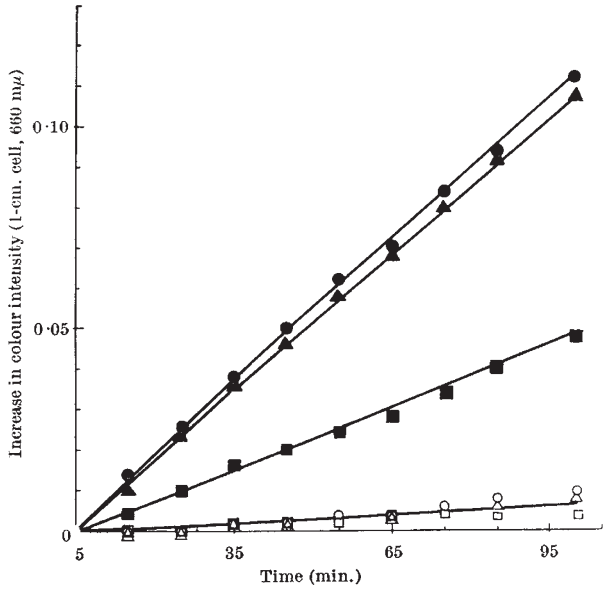

Fig. 1. Increase in intensity of molybdenum blue colour due to hydrolysis of glucose-1-phosphate. Circles, Allen's method—4·8 per cent perchloric acid, 0·08 per cent amidol, 1·6 per cent sodium bisulphite and 0·33 per cent ammonium molybdate. Triangles, Whelan and Bailey's modification—4·8 per cent perchloric acid, 0·04 per cent amidol, 0·8 per cent sodium bisulphite and 0·33 per cent ammonium molybdate. Squares, Nakamura's modification—1·5 per cent sulphuric acid, 0·04 per cent amidol, 0·8 per cent sodium bisulphite and 0·33 per cent ammonium molybdate. The reaction mixtures contained, in addition, 0·07 μmole of potassium dihydrogen phosphate and 1·14 μmoles of glucose-1-phosphate per ml. of the final solution. Open signs, colour developed at 18° C.; black signs, at 33° C. The colour intensity at 5 min. after colour development was arbitrarily taken as zero

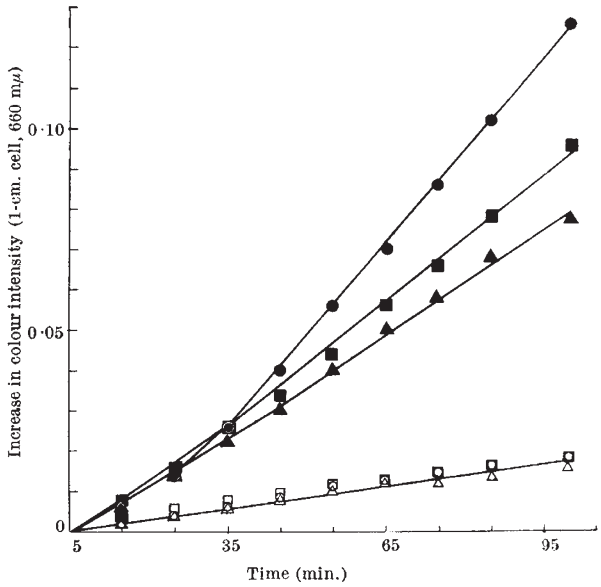

Fig. 2. Increase in intensity of molybdenum blue colour due to hydrolysis of adenosine triphosphate. The reaction mixtures contained 0·63 μmole of adenosine triphosphate per ml. in place of glucose-1-phosphate. Other additions and conditions are the same as in Fig. 1

Typical results of determinations of inorganic phosphorus by the three methods mentioned above in the presence of glucose-1-phosphate and adenosine triphosphate are shown in Figs. 1 and 2, respectively. In these figures, increase in colour intensity is plotted against time of colour development, the absorbancy at 5 min. after colour development being arbitrarily taken as zero. The amount of the amidol reagent has indeed some effect on the hydrolysis of glucose-1-phosphate and adenosine triphosphate when colour is developed at 33° C.; but the temperature at which colour is developed is a far more important factor. At 18° C., very little hydrolysis of glucose-1-phosphate or adenosine triphosphate occurs irrespective of the amount of the amidol reagent and the nature of the acid used. Although the acidity of the medium is approximately the same and hydrolysis of adenosine triphosphate is also the same in these three methods, the hydrolysis of glucose-1-phosphate at 33° is much smaller in sulphuric acid medium than in perchloric acid media.

Fructose-1,6-diphosphate and phosphoryl(enol)-pyruvate interfere less seriously with the determination of inorganic phosphorus by these methods.

Details will be published later (*Bull. Agric. Chem. Soc. Japan*).

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