Inosine 5'-Monophosphate Dehydrogenase of *Escherichia coli* K12: the Nature of the Inhibition by Guanosine 5'-Monophosphate

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When IMP is the variable substrate, IMP dehydrogenase (EC 1.2.1.14) gives non-linear Lineweaver–Burk plots in the presence of GMP. At 0.1 mM-GMP the Hill coefficient $n = 1.15$ and at 0.2 mM-GMP $n = 1.34$. In the absence of GMP $n = 1.0$. The fact that n exceeds 1.0 in the presence of GMP indicates that the enzyme possesses regulatory (allosteric) properties. The inhibition by GMP is competitive with respect to IMP and non-competitive with respect to NAD^+ .

IMP occurs at a branch point in the biosynthesis of purine nucleotides, and the enzyme IMP dehydrogenase (EC 1.2.1.14) catalyses the first reaction in the conversion of IMP into GMP. IMP dehydrogenase is subject to feedback inhibition by GMP (Mager & Magasanik, 1960), but it is not clear whether the enzyme from *Escherichia coli* is inhibited at a specific regulatory site or whether GMP competes with IMP for the active site. Powell *et al.* (1969) have purified the IMP dehydrogenase of *E. coli* and shown that GMP is a competitive inhibitor with respect to IMP. Evidence that GMP acts at the IMP-binding site, and is a normal competitive inhibitor with respect to IMP, has been reported for the enzyme from *Aerobacter aerogenes* (Hampton & Nomura, 1967). Co-operativity was not observed for the enzyme from either *E. coli* or *A. aerogenes*.

Buzzee & Levin (1968) have shown that IMP dehydrogenase from *Salmonella typhimurium* possesses a regulatory binding site for GMP and that GMP induces co-operativity between the IMP-binding sites (Hill coefficient $n = 1.3$ at 0.2 mM-GMP). The partially purified enzyme from *Bacillus subtilis* has been investigated by Ishii & Shiio (1968). They demonstrated that GMP is a competitive inhibitor with respect to IMP and that double-reciprocal plots of $1/v$ against $1/[\text{IMP}]$ curve upwards in the presence of GMP (Hill coefficient $n > 1.2$). These findings suggest that the IMP dehydrogenases from *S. typhimurium* and from *B. subtilis* are allosteric enzymes.

The subunit structure of IMP dehydrogenase from any of these sources has not been firmly established, although polymeric arrangements have been proposed (Brox & Hampton, 1970; Hannon & Levin, 1971).

The results reported below suggest that the IMP dehydrogenase of *E. coli* possesses regulatory properties similar to those of the enzymes from *B. subtilis* and from *S. typhimurium*.

Materials and methods

GSH and NAD^+ were obtained from Boehringer Corporation (London) Ltd., London W.5, U.K., and Tris was from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. All other chemicals were supplied by BDH Chemicals Ltd., Poole, Dorset, U.K.

IMP dehydrogenase was partially purified by the method of Powell *et al.* (1969) from a cell-free extract of the *E. coli guaA* strain PL 1068 (Lambden & Drabble, 1973). The mutant, which lacks XMP aminase activity and therefore requires guanine for growth, was cultured in defined minimal medium under conditions of guanine starvation (7 $\mu\text{g}/\text{ml}$ final concentration). This procedure results in a 40-fold increase in the production of IMP dehydrogenase relative to the basal value in the wild-type strain of *E. coli*.

The assay system for IMP dehydrogenase was based on the method of Magasanik *et al.* (1957). The reactions were followed continuously by measuring the increase in absorbance at 340 nm due to the production of NADH. Reactions were carried out in 10 mm silica cuvettes at 25°C in a Unicam SP.8000 recording spectrophotometer. NAD^+ or IMP was omitted from the reference cuvette. One unit of enzyme activity is defined as the amount of enzyme required to cause a change of 1.0 E_{340} unit/min at 25°C.

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin (fraction V) as the standard.

Results and discussion

Inhibition of IMP dehydrogenase by GMP at various concentrations of IMP. The experiment was carried out in the presence of two concentrations of GMP (0.1 mM and 0.2 mM). The assay procedure and results are presented in Fig. 1. The double-reciprocal plot of

Fig. 1. Inhibition of IMP dehydrogenase by GMP at various concentrations of IMP

The reaction mixture contained: IMP, 0.01–0.20 mM; NAD⁺, 1.25 mM; Tris–HCl buffer, pH 8.0, 50 mM; KCl, 33 mM; GSH, 5 mM; enzyme fraction, 2 µg of protein. The enzyme assay procedure is described in the text under 'Materials and methods'. ○, Control (no GMP); △, 0.1 mM-GMP; □, 0.2 mM-GMP. K_m (IMP) is 0.028 mM.

$1/v$ against $1/[IMP]$ shows a linear relationship in the absence of GMP. However, in the presence of GMP a non-linear relationship is obtained and the lines curve upwards. These data, when replotted according to the Hill equation (Atkinson *et al.*, 1965), give a Hill coefficient $n = 1.0$ in the absence of GMP, whereas in the presence of 0.1 mM-GMP and 0.2 mM-GMP $n = 1.15$ and 1.34 respectively. The Hill coefficient n is considered to be a function of the number of substrate-binding sites on the enzyme molecule and of the degree of interaction between these sites (Atkinson *et al.*, 1965). Values for the Hill coefficient greater than 1.0 indicate that the enzyme possesses regulatory or allosteric properties. GMP inhibits the enzyme competitively with respect to IMP.

Inhibition of IMP dehydrogenase by GMP at various concentrations of NAD⁺. Two concentrations of GMP were used (0.1 mM and 0.2 mM) at NAD⁺ concentrations ranging from 0.1 mM to 2.0 mM. Double-reciprocal plots of $1/v$ against $1/[NAD^+]$ showed linear relationships both in the absence and in the presence of GMP. The inhibition by GMP appeared to be non-competitive with respect to NAD⁺, and K_m (NAD⁺) was 0.83 mM.

These kinetics for partially purified IMP dehydrogenase of *E. coli* suggest that the enzyme possesses allosteric properties characteristic of regulatory enzymes. Similar kinetics have been obtained from studies of the IMP dehydrogenases from *S. typhimurium* (Buzzee & Levin, 1968) and from *B. subtilis* (Ishii & Shiio, 1968). In particular, co-operativity is not seen in the absence of the feedback inhibitor GMP.

The enzyme from *E. coli* has been extensively purified by Powell *et al.* (1969). The enzyme was competitively inhibited by GMP with respect to IMP, but there was no evidence of co-operative interactions. This apparent contradiction with our present results cannot be explained reasonably, particularly as similar enzyme purification procedures were used. However, the enzyme used in our study was the fraction precipitated by between 50% and 60% (v/v) acetone, whereas the enzyme isolated by Powell *et al.* (1969) was precipitated at 50% (v/v) acetone. The enzyme fraction used for the kinetic analysis by Powell *et al.* (1969) was also more extensively purified (the IMP dehydrogenase preparation was free of any major non-enzymic protein contaminant) than the enzyme fraction used in our study.

At this stage it is not possible to state whether the IMP dehydrogenase from *E. coli* is or is not an allosteric enzyme. It is worth noting, however, that IMP dehydrogenase is the first enzyme after a metabolic branch point, and as such is likely to play an important role in the regulation of GMP synthesis.

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