Biochem. J. (1982) **205**, 503–510
Printed in Great Britain

Nucleoside exchange catalysed by the cytoplasmic 5′-nucleotidase

Yesehak WORKU and Andrew C. NEWBY*
University of Cambridge Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, U.K.

(*Received 12 March 1982/Accepted 14 May 1982*)

The inhibition of the cytoplasmic 5′-nucleotidase (EC 3.1.3.5) by its product, inosine, was studied with a partially purified preparation of the enzyme from rat liver. Inhibition of P_i production was found to be due to exchange of the inosine moiety between inosine and IMP. Exchange was not catalysed by reversal of the hydrolytic reaction, suggesting, instead, the mediation of an enzyme–phosphate intermediate. Two models for the catalytic mechanism are proposed and rate equations for the dependence of P_i production on inosine concentration are derived. The experimentally determined dependence was consistent with a mechanism in which hydrolysis of the enzyme–phosphate intermediate occurred only when it was unoccupied by inosine. This conclusion suggests that inosine analogues that cannot participate in exchange should inhibit the enzyme. Such inhibitors might be useful in defining the enzyme's physiological role or as pharmacological agents to decrease breakdown of purine nucleotides. The possibility that nucleoside exchange provides an alternative route for the phosphorylation of mutagenic or cytotoxic nucleoside analogues should also be considered.

The cytoplasmic 5′-nucleotidase (EC 3.1.3.5), as distinct from the cell-surface ecto-enzyme (Stanley *et al.*, 1982), has been shown to be the enzyme responsible for purine nucleotide catabolism through IMP (Itoh *et al.*, 1967, 1978; Van den Berghe *et al.*, 1977; Newby, 1980; Newby & Holmquist, 1981; Itoh, 1981). This process regulates the size of the nucleotide pool in response to energy depletion (Raivio *et al.*, 1969; Chapman & Atkinson, 1973; Chapman *et al.*, 1976) and may serve to transfer purines between cells (Murray, 1971). A further role for the enzyme in hydrolysing cytosolic AMP to adenosine has been suggested by experiments with intact rat polymorphonuclear leucocytes (Newby & Holmquist, 1981), with isolated perfused guinea-pig hearts (Schütz *et al.*, 1981) and with the purified enzyme from rat liver (Itoh, 1981). Adenosine formed by this pathway might act as a 'local hormone' (Arch & Newsholme, 1978; Fox & Kelley, 1978).

Selective inhibitors of the enzyme that could penetrate the intact cell would be useful in defining the enzyme's physiological role and perhaps even as therapeutic agents in disorders of purine metabolism (see, e.g., Seegmiller, 1973). We therefore studied in detail the previously reported inhibition of the enzyme by its product inosine (Itoh *et al.*, 1967). This led to the discovery that the apparent inhibition was due to exchange of the nucleoside moiety between inosine and IMP. The implications of this for the catalytic mechanism and for the design of nucleoside inhibitors were investigated.

* To whom correspondence should be addressed.

Materials and methods

Chemicals and radiochemicals

AMP and IMP were obtained from Boehringer Corp. (Lewes, E. Sussex, U.K.), alkaline phosphatase (type 1-S) and AMP deaminase (type IV) were from Sigma Chemical Co. (Poole, Dorset, U.K.), prepared thin layers of cellulose (CEL300) and silica (SIL G/UV_{254}) were from Camlab (Cambridge, U.K.) and phosphocellulose (P11) was from Whatman (Maidstone, Kent, U.K.). [2-^{3}H]-Adenosine (777GBq/mmol) and [2-^{3}H]AMP (714GBq/mmol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). [2-^{3}H]IMP (231GBq/mmol) was prepared from [2-^{3}H]AMP isotopically diluted and freeze-dried to remove ethanol. Batches of 9.25MBq of [2-^{3}H]-AMP were redissolved in 2ml of 50mM-ammonium acetate buffer, pH 6.5, and converted into IMP at

0306-3275/82/090503-08$01.50/1

37°C with 0.06 unit of AMP deaminase. The reaction was monitored spectrophotometrically at 265nm. The product was separated from the enzyme in the same buffer by filtration on an 18mm × 250mm column of Sephadex G-25 (Pharmacia, Hounslow, Middx., U.K.). Fractions containing radioactivity were then concentrated on a 1ml bed of AG1-X2 quaternary-amine anion-exchanger (Cl^- form) (Bio-Rad Laboratories, Watford, Herts., U.K.). IMP was eluted with several 0.5ml volumes of 50mM-HCl into tubes containing 0.5ml of aq. 50mM-NH_3. Fractions containing radioactivity were pooled and freeze-dried. Radiochemical purity of 97% was determined by t.l.c. on cellulose with the solvent system (isobutyric acid/1M-NH_3/0.1M-EDTA, 125:75:2, by vol.) of Krebs & Hems (1953). [2-^{3}H]Inosine (29.6GBq/mmol) was prepared from [2-^{3}H]adenosine as described previously (Newby, 1981). Rat liver plasma-membrane 5'-nucleotidase purified by the method of Bailyes *et al.* (1982) was generously given by Dr. R. Daw of this Department.

Partial purification of rat liver cytosolic 5'-nucleotidase

To improve the yield of enzyme while achieving adequate purity for kinetic experiments, the purification scheme described by Itoh (1980) was modified and truncated as follows. The initial extraction buffer was supplemented with 200mM-NaCl. In step 4 the enzyme precipitated between 30%- and 60%-saturated $(NH_4)_2SO_4$ was pooled and then dialysed against unmodified extraction buffer to remove $(NH_4)_2SO_4$. The overall yield was 16%, with a specific activity of 0.75 unit/mg of protein. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The enzyme was stored at −20°C in extraction buffer supplemented with 500mM-NaCl and 1mg of bovine serum albumin (batch AC 2864; a gift from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.)/ml. No contamination (limit of detection in each case was approx. 1%) was detected for acid phosphatase, alkaline phosphatase, Mg^{2+}-stimulated ATPase, myokinase, adenosine kinase, adenosine deaminase, AMP deaminase or nucleoside phosphorylase.

Enzyme assays

The assay conditions were based on those described by Itoh (1980). Incubations were performed at 37°C in 100μl containing 100mM-imidazole/HCl buffer, pH6.5, 50mM-$MgCl_2$, 500mM-NaCl, 0.1% bovine serum albumin and substrate at the concentrations stated in the text. The reaction was terminated by heating in a 100°C water bath for 3min, and denatured protein was removed by centrifugation at 3000*g* for 5min. P_i in the supernatant was determined colorimetrically (Itaya & Ui, 1966). The production of [^{3}H]inosine from [^{3}H]IMP (2–3kBq per incubation irrespective of substrate concentration) was determined with the use of the same assay conditions. After the mixture had been boiled, 0.5ml of 10mM-inosine was added, and the whole was then centrifuged. Supernatant (0.3ml) was loaded on to a 1ml bed of quaternary-amine anion-exchanger (Cl^- form), and [^{3}H]inosine was eluted with 3.7ml of 5mM carrier inosine. The eluate was collected in a scintillation vial, 10ml of scintillant was added and radioactivity was determined as described previously (Newby, 1981). Recovery of [^{3}H]inosine was >97%. Enzyme assays were linear from 5 to 45min and from 0.88 to 8.8μg of protein per assay.

Nucleoside exchange was determined with the same assay conditions with 3–15mM-[2-^{3}H]inosine (4–6kBq per assay). After the mixture had been boiled and centrifuged, 20μl samples of supernatant were subjected to silica-gel t.l.c. as described previously (Shimizu *et al.*, 1970; Newby, 1981). IMP was located by u.v. fluorescence and eluted and its radioactivity was determined as described previously (Newby, 1981).

For each enzyme assay the blank value was determined by boiling and subsequently processing mixtures of assay medium with enzyme before incubation at 37°C. However, in each case the same blank value was obtained when assay medium alone was so treated.

One unit of enzyme is defined throughout as the amount that converted 1μmol of substrate/min at 37°C at saturating concentrations of substrate.

Results

Kinetic constants of partially purified 5'-nucleotidase

In the presence of 50mM-Mg^{2+}, the plot of activity against IMP concentration (0.1–3mM) was hyperbolic, giving a K_m of 0.20 ± 0.01mM (mean ± S.D., $n = 4$). At 5mM-Mg^{2+} (Van den Berghe *et al.*, 1977) the plot was sigmoidal; $[S]_{0.5}$ was 0.5mM but the $V_{max.}$ was unaltered (results not shown). For AMP (0.5–60mM), even with 50mM-Mg^{2+}, the plot was sigmoidal, with $[S]_{0.5}$ 8.7 ± 0.2mM ($n = 3$). This preparation of enzyme was therefore similar to those previously characterized (Van den Berghe *et al.*, 1977; Itoh, 1980).

Inhibition of the enzyme by inosine

With 3mM-[2-^{3}H]IMP as substrate the effect of inosine was assessed both on the initial rate of P_i release and on the production of [2-^{3}H]inosine (Fig. 1). As reported previously (Itoh *et al.*, 1967), inosine inhibited P_i release. However, [^{3}H]inosine production was not inhibited (Fig. 1). This contradiction could be explained by exchange of the inosine moiety

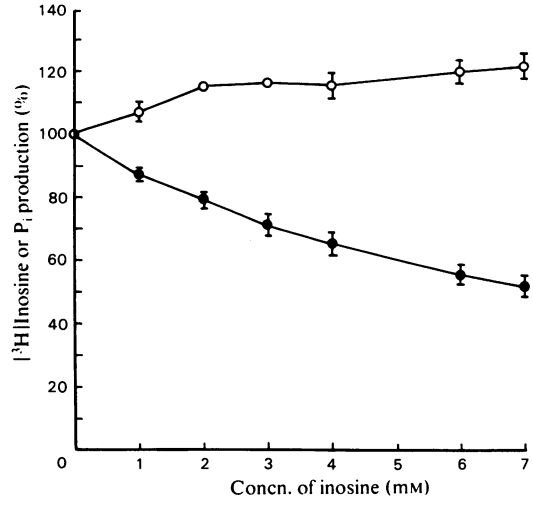


Fig. 1. *Effect of inosine on P_i release and [3H]inosine formation by cytosolic 5'-nucleotidase*

Cytosolic 5'-nucleotidase (2.2–3.0 munits) was incubated in triplicate with 3mM-[3H]IMP and inosine at the concentrations indicated. P_i release (●) and [3H]inosine production (○) were determined and expressed as percentages of their values in the absence of added inosine. For full experimental details see the text. Values are means ± S.E.M. for six (●) or three (○) experiments.

between IMP and inosine (Scheme 1). In this model, the hydrolysis of IMP is mediated by an enzyme–phosphate intermediate. The presence of added inosine would promote the resynthesis of IMP. However, if the second equilibrium were rapid compared with IMP synthesis, isotope dilution of inosine would result. Hence the rate of [3H]inosine production would not decrease. An alternative model for nucleoside exchange could be based on the reversal of the hydrolytic reaction. However, when the enzyme was incubated with 3mM-[3H]inosine and 3mM-P_i the rate of IMP synthesis was undetectable (0.06 ± 0.16% of IMP 5'-nucleotidase activity, $n = 3$).

Measurement of [3H]IMP synthesis from [3H]-inosine

To demonstrate nucleoside exchange in the reverse direction, the enzyme was incubated with 3mM-IMP in the presence or in the absence of [3H]inosine. In each case the rate of P_i release was measured (Table 1). In the presence of [3H]inosine, synthesis of [3H]IMP was observed. The rate of IMP synthesis accounted for the diminished rate of P_i production (Table 1).

$$E + [^3H]IMP \rightleftharpoons E\text{–}P_i \cdot [^3H]I \rightleftharpoons E\text{–}P_i + [^3H]I \quad (+ I)$$

Scheme 1. *Proposed mechanism of nucleoside exchange and isotope dilution of inosine catalysed by cytosolic 5'-nucleotidase (E)*

Catalytic mechanism

Two simple kinetic mechanisms are proposed (Scheme 2). A common feature is the presence of enzyme–phosphate intermediate, the hydrolysis of which is irreversible. Mechanism 1 assumes that the enzyme–phosphate intermediate undergoes hydrolysis only when unoccupied by inosine. In mechanism 2 hydrolysis is independent of occupation by inosine.

Rate equations for the release of P_i were derived by using Alberty's (1953) approach.

Mechanism 1 in Scheme 2 yielded (see the Appendix):

$$v_{P_i} = \frac{V_{max.}}{1 + \frac{K_1[I]}{[S]} + K_2[I] + \frac{K_m}{[S]}} \quad (1)$$

whereas mechanism 2 yielded:

$$v_{P_i} = \frac{V_{max.}(1 + K'[I])}{1 + \frac{K'_1[I]}{[S]} + K'_2[I] + \frac{K'_m}{[S]}} \quad (2)$$

where [I] is the concentration of inosine, [S] is the concentration of IMP, K' is $k_{-3}/(k_{+3} + k_{+4})$ and K_1, K_2, and K'_1 and K'_2 values are complex constants.

At any fixed concentration of IMP these equations may be simplified. For clarity we also consider $[S] \gg K_m$, i.e. saturating concentrations of IMP.

Mechanism 1 yields:

$$v_{P_i} = \frac{V_{max.}}{1 + \left(\frac{K_1}{[S]} + K_2\right)[I]} \quad (3)$$

Mechanism 2 yields:

$$v_{P_i} = \frac{V_{max.}(1 + K'[I])}{1 + \left(\frac{K'_1}{[S]} + K'_2\right)[I]} \quad (4)$$

Eqn. (3) predicts a linear dependence of $1/v$ on [I], whereas eqn. (4) predicts a linear relation at low concentrations but negative deviation at higher concentrations. In fact, at limiting concentrations of

Table 1. *Comparison of inhibition by inosine of cytoplasmic 5'-nucleotidase and other phosphomonoesterases with nucleoside exchange*

Cytoplasmic 5'-nucleotidase or purified rat liver plasma-membrane 5'-nucleotidase (sp. activity 246 units/mg) or calf intestine alkaline phosphatase was incubated in triplicate for 30min at 37°C with 3mM-IMP. The initial rate of P_i release was for the cytoplasmic enzyme 2.2–3.0nmol/min, for the plasma-membrane enzyme 2.9 or 4.0nmol/min and for alkaline phosphatase 4.1 or 5.7nmol/min. The rate of P_i release was also assessed in the presence of inosine at the concentrations indicated and the percentage inhibition was calculated. Inosine was supplemented in each case by 4–6kBq of [2-^{3}H]inosine. Formation of [^{3}H]IMP by nucleoside exchange was determined by t.l.c. and expressed as a percentage of control IMP 5'-nucleotidase activity. The ratio of nucleoside exchange to inhibition of P_i release was computed for each experiment. Values shown are means ± average error for three observations or fewer and means ± S.E.M. for five observations, as shown in parentheses.

Enzyme	Concn. of inosine (mM)	Inhibition of P_i release (% of IMP 5'-nucleotidase activity)	Nucleoside exchange (% of IMP 5'-nucleotidase activity)	Nucleoside exchange/ inhibition of P_i release ratio
Cytoplasmic 5'-nucleotidase	3 (3)	32 ± 2	34 ± 3	1.06 ± 0.13
	6 (5)	49 ± 1	53 ± 2	1.07 ± 0.04
	10 (3)	63 ± 2	67 ± 5	1.05 ± 0.06
	15 (3)	72 ± 2	73 ± 11	1.01 ± 0.14
Plasma-membrane 5'-nucleotidase	6 (2)	−0.6 ± 0.5	−0.05 ± 0.03	—
Calf intestine alkaline phosphatase	6 (2)	0.1 ± 0.1	−0.03 ± 0.01	—

Mechanism 1

$$E + IMP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E\cdot IMP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E\text{–}P_i\cdot I \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E\text{–}P_i + I \xrightarrow{k_{+4}} E + P_i$$

Mechanism 2

$$E + IMP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E\cdot IMP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E\text{–}P_i\cdot I \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E\text{–}P_i + I \xrightarrow{k_{+4}} E + P_i$$

$$E\text{–}P_i\cdot I \xrightarrow{k_{+4}} E\cdot I + P_i$$

Scheme 2. *Two mechanisms for the production of P_i from IMP catalysed by cytosolic 5'-nucleotidase (E)*

inosine eqn. (3) predicts complete inhibition, whereas eqn. (4) predicts a residual activity independent of inosine concentration.

Fig. 2 shows the relation of $V_{max.}/v$ plotted versus [I] obtained at 3mM-IMP. No deviation from linearity was seen up to 70mM-inosine, at which concentration 93% inhibition was achieved. Mechanism 2 was therefore eliminated (see also the Discussion section). The slope of the plot of $V_{max.}/v$ versus [I] yields $(K_1/[S]) + K_2$. Its value was $0.147 \pm 0.008\,mM^{-1}$ ($n = 5$). From eqn. (3) it can be seen that the inosine concentration giving 50% inhibition (K_i) is the reciprocal of this value, i.e. 6.8 ± 0.4mM.

Kinetic constant of the exchange reaction

In Table 1 we demonstrated that the rate of nucleoside exchange at several inosine concentrations was equal to the inhibition of P_i release. Hence we can write:

$$v_{exchange} = V_{max.} - v_{P_i} \quad (5)$$

From eqn. (3):

$$v_{exchange} = \frac{V_{max.}[I]}{\dfrac{1}{(K_1/[S] + K_2)} + [I]} \quad (6)$$

Therefore the inhibitor constant (6.8 ± 0.4mM) obtained for inosine on P_i release is the K_m (inosine) for the exchange reaction at 3mM-IMP.

Specificity

The ability of other nucleosides to inhibit P_i release was assessed. At 3mM-IMP, the inhibition caused by 5mM-guanosine was 8 ± 1%. For adeno-

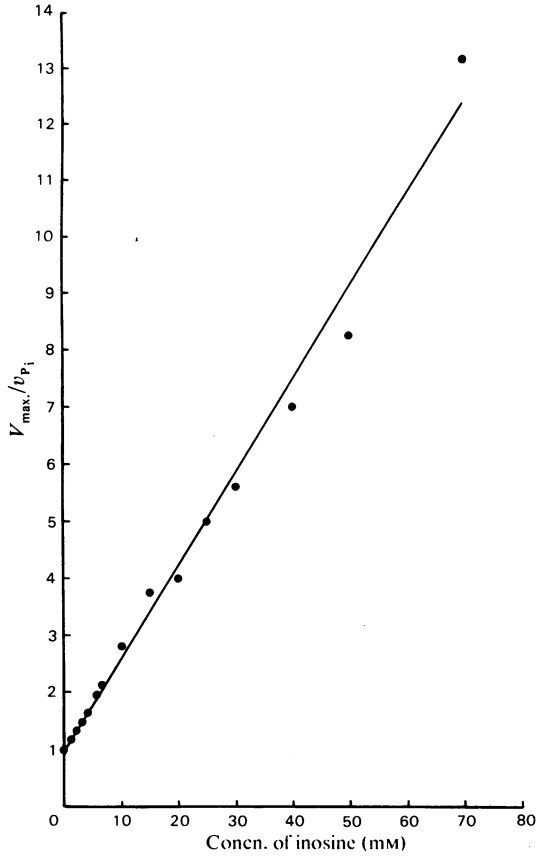


Fig. 2. *Dependence of the initial rate of P_i production by cytosolic 5′-nucleotidase on the concentration of inosine*
Cytosolic 5′-nucleotidase (2.4–2.7 munits) was incubated in triplicate with 3mM-IMP and inosine at the concentrations shown. P_i release was determined. For full experimental details see the text. To normalize the results from the three experiments performed $V_{max.}/v_{P_i}$ was plotted against [I]. Values are means.

sine, cytosine, thymidine and uridine the inhibition was less than 0.2%.

Inhibition of P_i release and synthesis of IMP from [^{3}H]inosine were investigated with purified rat liver plasma-membrane 5′-nucleotidase and calf intestinal alkaline phosphatase. Amounts of each enzyme that showed approximately the same IMP 5′-nucleotidase activity at their respective pH optima were incubated with 3mM-IMP with or without 6mM-[^{3}H]inosine. The inhibition of P_i production and the rate of [^{3}H]IMP formation was assessed (Table 1). Only the cytosolic 5′-nucleotidase showed significant inhibition or nucleoside exchange under these conditions.

Discussion

The discovery of nucleoside exchange catalysed by the cytosolic 5′-nucleotidase has several important implications. Firstly, any kinetic mechanism proposed for the enzyme must explain the phenomenon. Mechanism 1 predicted the dependence of P_i release on inosine concentration. Mechanism 2 yielded an equation of the same form at low concentrations, but predicted deviation at higher ones. Since we observed isotope dilution of [^{3}H]-inosine derived from [^{3}H]IMP and unlabelled inosine (Fig. 1), it is clear that equilibration of inosine with the enzyme was fast compared with the hydrolysis of the enzyme–phosphate intermediate E–P_i or compared with the exchange reaction. Hence one can argue that K' in eqn. (2) is approximately equal to (k_{-3}/k_{+3}), i.e. K_a for inosine binding to the enzyme. K_m for the exchange reaction can, by similar logic, be argued to be approximately equal to K_d. Thus $K'K_m \simeq 1$. This means that significant deviations should have been observed at inosine concentrations in the region of the K_m for exchange. Since we observed no deviation even at 10 times that concentration, mechanism 2 was eliminated.

This conclusion has an important consequence for the effect of nucleoside inhibitors on the reaction. If an analogue of inosine cannot participate in the exchange, mechanism 1 predicts that it should completely block IMP hydrolysis. Mechanism 2 would predict no inhibition. Since we have eliminated mechanism 2, it is worthwhile proceeding in an attempt to find nucleoside inhibitors of the enzyme. These would be much more likely to enter intact cells than nucleotide analogues of the substrate. The differences from plasma-membrane 5′-nucleotidase and from alkaline phosphatase suggest that such inhibitors might be selective.

It has been noted (Van den Berghe *et al.*, 1977; Newby & Holmquist, 1981) that IMP hydrolysis during energy depletion is only transient. It is pertinent to ask whether nucleoside exchange could contribute to the physiological regulation of IMP concentration. The concentration of inosine (0.5mM) measured in polymorphonuclear leucocytes 10min after inhibition with 2-deoxyglucose (Newby & Holmquist, 1981) was well below our measured inhibitor constant. This argues that nucleoside inhibition would be relatively insignificant under these conditions. However, our data are based on the enzyme in the maximally activated state with respect to allosteric effectors. Investigation of nucleoside exchange under other conditions of activation may be valuable.

Another possibility, worthy of further investigation, is that nucleoside exchange catalysed by the soluble 5′-nucleotidase might provide an alternative

route for the phosphorylation of nucleoside analogues. Thus analogues that are not substrates for the recognized nucleoside kinases (Carson *et al.*, 1977; Miller *et al.*, 1979) might be incorporated into nucleotides and exert mutagenic or cytocidal actions.

We thank Dr. J. P. Luzio, Dr. H. B. F. Dixon and Dr. P. Leadlay for critical reviews of the manuscript. Y. W. holds a World Health Organisation Fellowship from the University of Addis Abeba, Ethiopia. This work was supported by a project grant from the Medical Research Council.

References

Alberty, R. A. (1953) *J. Am. Chem. Soc.* **75**, 1928–1932
Arch. J. R. S. & Newsholme, E. A. (1978) *Essays Biochem.* **14**, 82–123
Bailyes, E. M., Newby, A. C., Siddle, K. & Luzio, J. P. (1982) *Biochem. J.* **203**, 245–251
Carson, D. A., Kaye, J. & Seegmiller, J. E. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5677–5681
Chapman, A. G. & Atkinson, D. E. (1973) *J. Biol. Chem.* **248**, 8309–8312
Chapman, A. G., Miller, A. L. & Atkinson, D. E. (1976) *Cancer Res.* **36**, 1144–1150
Fox, I. H. & Kelley, W. N. (1978) *Annu. Rev. Biochem.* **47**, 655–686
Itaya, K. & Ui, M. (1966) *Clin. Chim. Acta* **14**, 361–366
Itoh, R. (1980) *Biochim. Biophys. Acta* **657**, 402–410
Itoh, R. (1981) *Biochim. Biophys. Acta* **659**, 31–37
Itoh, R., Mitsui, A. & Tsuschima, K. (1967) *Biochim. Biophys. Acta* **146**, 151–159
Itoh, R., Usami, C., Nishino, T. & Tsushima, K. (1978) *Biochim. Biophys. Acta* **526**, 154–162
Krebs, H. A. & Hems, R. (1953) *Biochim. Biophys. Acta* **12**, 172–180
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Miller, R. L., Adamczyk, D. L., Miller, W. H., Koszalka, G. W., Rideout, J. L., Beacham, L. M., Chao, E. Y., Haggerty, J. J., Krenitzky, T. A. & Elion, G. B. (1979) *J. Biol. Chem.* **254**, 2346–2349
Murray, A. W. (1971) *Annu. Rev. Biochem.* **40**, 811–826
Newby, A. C. (1980) *Biochem. J.* **186**, 907–918
Newby, A. C. (1981) *Biochem. Pharmacol.* **30**, 2611–2615
Newby, A. C. & Holmquist, C. A. (1981) *Biochem. J.* **200**, 399–403
Raivio, K. O., Kekomäki, M. P. & Mäenpaä, P. H. (1969) *Biochem. Pharmacol.* **18**, 2615–2624
Schütz, W., Schrader, J. & Gerlach, E. (1981) *Am. J. Physiol.* **240**, H963–H970
Seegmiller, J. (1973) in *Duncans Diseases of Metabolism* (Bondy, P. K. & Rosenberg, L. E., eds.), 7th edn., pp. 655–774, W. B. Saunders, Philadelphia
Shimizu, H., Creveling, C. R. & Daly, J. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **65**, 1033–1040
Stanley, K. K., Newby, A. C. & Luzio, J. P. (1982) *Trends Biochem. Sci.* **7**, 145–147
Van den Berghe, G., van Pottelsberghe, C. & Hers, H.-G. (1977) *Biochem. J.* **162**, 611–616

APPENDIX

Derivation of rate equations

Mechanism 1

$$E + IMP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E\cdot IMP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E\text{–}P_i\cdot I \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E\text{–}P_i + I \xrightarrow{k_{+4}} E + P_i$$

Symbols

$x_1 = [E\cdot IMP]$
$x_2 = [E\text{–}P_i\cdot I]$
$x_3 = [E\text{–}P_i]$
$[S] = [IMP]$
$[I] = [\text{inosine}]$
$[E]_t$ = total enzyme concentration
$[E]_f$ = free enzyme concentration
k_{+1}, k_{+2}, k_{+3}, k_{+4}, k_{-1}, k_{-2} and k_{-3} are rate constants.

By steady-state considerations

$$\dot{x}_1 = -(k_{+2}+k_{-1})x_1 + k_{-2}x_2 + k_{+1}[S]([E]_t - x_1 - x_2 - x_3)$$
$$\dot{x}_2 = k_{+2}x_1 - (k_{+3}+k_{-2})x_2 + k_{-3}x_3$$
$$\dot{x}_3 = k_{+3}x_2 - (k_{-3}[I] + k_{+4})x_3$$
$$[E]_f = [E]_t - x_1 - x_2 - x_3$$
$$v_{P_i} = k_{+4}x_3$$

x_3 can be calculated by using Cramer's rule (Trench & Kolman, 1972):

$$x_3 = \frac{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & (k_{+1}[S]-k_{-2}) & k_{+1}[S][E]_t \\ k_{+2} & -(k_{+3}+k_{-2}) & 0 \\ 0 & k_{+3} & 0 \end{vmatrix}}{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & (k_{+1}[S]-k_{-2}) & k_{+1}[S] \\ k_{+2} & -(k_{+3}+k_{-2}) & k_{-3} \\ 0 & k_{+3} & -(k_{-3}[I]+k_{+4}) \end{vmatrix}}$$

On expansion:

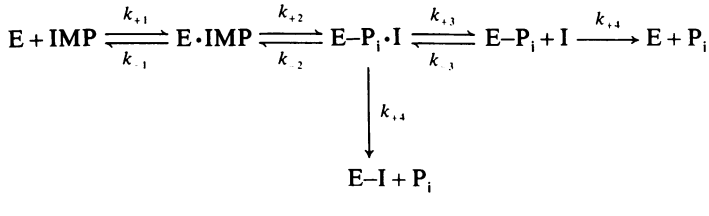
$$v_{P_i} = \frac{\dfrac{k_{+1}k_{+2}k_{+3}k_{+4}[E]_t}{k_{+1}k_{+2}k_{+3}+k_{+1}k_{+4}k_{-2}+k_{+1}k_{+2}k_{+4}+k_{+1}k_{+3}k_{+4}-k_{+1}k_{+3}k_{-3}}}{1+\dfrac{k_{-1}k_{-2}k_{-3}+k_{-1}k_{-3}k_{+3}+k_{+2}k_{-2}k_{-3}+k_{+2}k_{+3}k_{-3}}{k_{+1}k_{+2}k_{+3}+k_{+1}k_{+4}k_{-2}+k_{+1}k_{+2}k_{+4}+k_{+1}k_{+3}k_{+4}-k_{+1}k_{+3}k_{-3}}\cdot\dfrac{[I]}{[S]}}$$

$$+\frac{k_{+1}k_{+2}k_{-3}+k_{+1}k_{-2}k_{-3}+k_{+1}k_{+3}k_{-3}}{k_{+1}k_{+2}k_{+3}+k_{+1}k_{+4}k_{-2}+k_{+1}k_{+2}k_{+4}+k_{+1}k_{+3}k_{+4}-k_{+1}k_{+3}k_{-3}}[I]$$

$$+\frac{k_{+4}k_{-1}k_{-2}+k_{+4}k_{-2}^2+k_{+3}k_{+4}k_{-1}+k_{+3}k_{+4}k_{-2}-k_{+3}k_{-1}k_{-3}-k_{+3}k_{-2}k_{-3}}{k_{+1}k_{+2}k_{+3}+k_{+1}k_{+4}k_{-2}+k_{+1}k_{+2}k_{+4}+k_{+1}k_{+3}k_{+4}-k_{+1}k_{+3}k_{-3}}\cdot\frac{1}{[S]}$$

$$v_{P_i} = \frac{V_{max.}}{1+\dfrac{K_1[I]}{[S]}+K_2[I]+\dfrac{K_m}{[S]}}$$

Mechanism 2



By steady-state considerations

$$\dot{x}_1 = -(k_{+2}+k_{-1})x_1 + k_{-2}x_2 + k_{+1}[S]([E]_t - x_1 - x_2 - x_3)$$
$$\dot{x}_2 = k_{+2}x_1 - (k_{+3}+k_{+4}+k_{-2})x_2 + k_{-3}x_3$$
$$\dot{x}_3 = k_{+3}x_2 - (k_{-3}[I]+k_{+4})x_3$$
$$[E]_f = [E]_t - x_1 - x_2 - x_3$$
$$v_{P_i} = k_{+4}(x_2+x_3)$$

x_2 and x_3 can be calculated by using Cramer's rule:

$$x_2 = \frac{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & k_{+1}[S][E]_t & k_{+1}[S] \\ k_{+2} & 0 & k_{-3} \\ 0 & 0 & -(k_{-3}[I]+k_{+4}) \end{vmatrix}}{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & (k_{+1}[S]-k_{-2}) & k_{+1}[S] \\ k_{+2} & -(k_{+3}+k_{+4}+k_{-2}) & k_{-3} \\ 0 & k_{+3} & -(k_{-3}[I]+k_{+4}) \end{vmatrix}}$$

$$x_3 = \frac{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & (k_{+1}[S]-k_{-2}) & k_{+1}[S][E]_t \\ k_{+2} & -(k_{+3}+k_{+4}+k_{-2}) & 0 \\ 0 & k_{+3} & 0 \end{vmatrix}}{\begin{vmatrix} (k_{+1}[S]+k_{+2}+k_{-1}) & (k_{+1}[S]-k_{-2}) & k_{+1}[S] \\ k_{+2} & -(k_{+3}+k_{+4}+k_{-2}) & k_{-3} \\ 0 & k_{+3} & -(k_{-3}[I]+k_{+4}) \end{vmatrix}}$$

$$v_{P_i} = \frac{\dfrac{k_{+2}[E]_t}{(k_{+2}k_{+3}+k_{+4}k_{-2}+k_{+3}k_{+4}+k_{+3}k_{-3}+k_{+2}k_{+4}+k_{+4}^2)(1+k_{+3}/k_{+4})}\left[1+\left(\dfrac{k_{-3}}{k_{+3}+k_{+4}}\right)[I]\right]}{1+\dfrac{k_{-1}k_{-2}k_{-3}+k_{+2}k_{-2}k_{-3}+k_{+3}k_{-1}k_{-3}+k_{+2}k_{+3}k_{-3}-k_{+2}k_{-2}k_{-3}+k_{+2}k_{+4}k_{-3}+k_{+4}k_{-1}k_{-3}}{k_{+1}(k_{+2}k_{+3}+k_{+4}k_{-2}+k_{+3}k_{+4}+k_{+3}k_{-3}+k_{+2}k_{+4}+k_{+4}^2)}\cdot\dfrac{[I]}{[S]}}$$

$$+\frac{k_{+1}k_{-2}k_{-3}+k_{+1}k_{+3}k_{-3}+k_{+1}k_{+2}k_{-3}+k_{+1}k_{+4}k_{-3}}{k_{+1}(k_{+2}k_{+3}+k_{+4}k_{-2}+k_{+3}k_{+4}+k_{+3}k_{-3}+k_{+2}k_{+4}+k_{+4}^2)}[I]$$

$$+\frac{k_{+4}k_{-1}k_{-2}+k_{+2}k_{+4}k_{-2}+k_{+3}k_{+4}k_{-1}+k_{+2}k_{+3}k_{+4}-k_{+3}k_{-1}k_{-3}-k_{+2}k_{+3}k_{-3}-k_{+2}k_{+4}k_{-2}+k_{-1}k_{+4}^2+k_{+2}k_{+4}^2}{k_{+1}(k_{+2}k_{+3}+k_{+4}k_{-2}+k_{+3}k_{+4}+k_{+3}k_{-3}+k_{+2}k_{+4}+k_{+4}^2)}\cdot\frac{1}{[S]}$$

$$v_{P_i} = \frac{V_{max.}(1+K'[I])}{1+\dfrac{K'_1[I]}{[S]}+K'_2[I]+\dfrac{K'_m}{[S]}}$$

Reference

Trench, W. F. & Kolman, B. (1972) *Multivariable Calculus with Linear Algebra and Series*, p. 82, Academic Press, London and New York