Apparent equilibrium constant of the hypoxanthine guanine phosphoribosyltransferase-catalyzed IMP - GMP exchange

C. Salerno and A. Giacomello

Institute of Biological Chemistry and Institute of Rheumatology, University of Rome, I-00100 Rome (Italy), and Center of Molecular Biology of C.N.R., I-00100 Rome (Italy), 22 March 1982

Summary. Human hypoxanthine-guanine phosphoribosyltransferase catalyzes the reaction between IMP and guanine to form hypoxanthine and GMP in the presence of Mg^{2+} ions and pyrophosphate. The apparent equilibrium constant for this reaction has been determined.

Inosine 5′-monophosphate (IMP) is an intermediate in the formation of adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP), the purine nucleotide components of nucleic acid[1-4]. The synthesis of GMP from IMP involves a two-step reaction sequence. There is first an irreversible oxidation of IMP to xanthosine 5′-monophosphate (XMP) with nicotinamide adenine dinucleotide (NAD) as hydrogen acceptor. This reaction is inhibited by GMP. The 2nd reaction involves amination of XMP at the 2 position and requires ATP as an energy source. This 2nd reaction, too, seems to be irreversible. GMP may be converted to IMP through the irreversible reaction catalyzed by GMP reductase wich requires NADPH as hydrogen donor and is strongly inhibited by XMP. Recent studies[5] suggest that hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme of the purine salvage pathway, might also have a role in the conversion of IMP to GMP. In the present paper, the apparent equilibrium constant of the HGPRT-catalyzed IMP-GMP exchange is determined.

Materials. HGPRT was purified from human erythrocytes to apparent electrophoretic homogeneity as previously described[6]. IMP, GMP, hypoxanthine, and guanine were purchased from Boehringer AG (FRG). The commercial samples of hypoxanthine and guanine were at least 99% pure when assayed by the enzymatic spectrophotometric methods of Kalckar[7]. IMP and GMP were purified by QAE Sephadex A-25 (Pharmacia) column chromatography (bed dimension 1.5×18 cm). The eluant was 0.1 M Tris-HCl buffer, pH 8.3, with a linear gradient to 0.2 M NaCl. After purification, the nucleotides were at least 99% pure when assayed by enzymatic spectrophotometric methods[8,9]. All other reagents were high purity commercial samples from Merck AG and Boehringer AG (FRG).

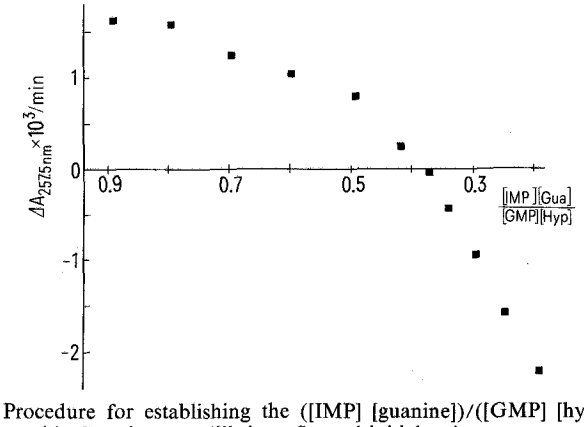
Methods. The apparent equilibrium constant was determined in Tris-HCl buffer using the method described by Segel[10]. Solutions containing known aliquots of IMP, GMP, hypoxanthine, guanine, Tris-HCl, $MgCl_2$, and pyrophosphate were incubated at different temperatures. HGPRT was added last to initiate the reaction. The initial rate of GMP formation or consumption was plotted against the initial ratio ([IMP] [guanine])/([GMP] [hypoxanthine]). The apparent equilibrium constant was given by the value of the intercept of the curve obtained as described above with the abscissa.

The variation of GMP concentration was followed spectrophotometrically at 257.5 nm as previously described[5]. The coefficient of variation for the assay was 6% when 15 measurements were performed at 37 °C and pH 7.4 under the same experimental conditions within 2 h. A Perkin-Elmer 2-wavelength spectrophotometer model 356 was employed. The noise was less than 0.0008 absorbance units and the total system drift was less than 0.001 absorbance units per h.

Results and discussion. The plot of the initial rate of GMP formation or consumption against the initial ratio ([IMP] [guanine])/([GMP] [hypoxanthine]) is shown in the figure. The value of the apparent equilibrium constant of the reaction

IMP + guanine $\overset{\text{HGPRT, PPi, } Mg^{2+}}{\rightleftharpoons}$ GMP-hypoxanthine

calculated as described under methods was $0.35 \leqslant Keq \leqslant 0.45$. The apparent equilibrium constant was not appreciably affected by temperature (from 30 °C to 70 °C), pH value (from 6.1 to 7.65), [Tris] (from 10^{-2} M to 10^{-1} M), [pyrophosphate] (from 10^{-4} M to 10^{-3} M), [$MgCl_2$] (from 10^{-3} M to 10^{-2} M), and enzyme concentration (from 0.01 to 0.1 ml of a standard HGPRT preparation in a final volume of 1 ml).



Procedure for establishing the ([IMP] [guanine])/([GMP] [hypoxanthine]) ratio at equilibrium. Several initial ratios are set up and the initial rate (v) of GMP change is measured. v is plotted against the initial ratio. When v = O, the ratio equals Keq. Incubation mixtures contained 0.1 M Tris-HCl, pH 7.4, 10^{-2} M $MgCl_2$, 10^{-3} M pyrophosphate, 0.1 ml of a standard hypoxanthine guanine phosphoribosyltransferase preparation, 1.9×10^{-5} M guanine, 2.95×10^{-5} M hypoxanthine, 3.83×10^{-5} M GMP, and IMP (from 1.2×10^{-5} M to 5.38×10^{-5} M) in a final volume of 1 ml. The incubation temperature was 37 ± 0.1 °C.

The stoichiometry and steady state kinetics of HGPRT-catalyzed IMP-GMP exchange have been recently studied[5]. 1 mole of hypoxanthine and GMP are formed for each mole of IMP and guanine consumed. 5-phosphoribosyl-1-pyrophosphate (PRPP) is a substrate of HGPRT, utilized in the salvage pathway to synthesise IMP and GMP from hypoxanthine and guanine respectively. However, during the HGPRT-catalyzed IMP-GMP exchange, no appreciable amount of PRPP is released in the incubation mixture[11] and the binary complex enzyme-PRPP does not appear to be formed[5]. GMP, IMP, and pyrophosphate bind to the free enzyme form while guanine and hypoxanthine bind to a different form that is made only in the presence of pyrophosphate and one of the nucleotides. A Theorell-Chance mechanism for guanine binding and hypoxanthine release has been postulated[5]. As a result, pyrophosphate is neither formed nor consumed during the IMP-GMP exchange. This explains why pyrophosphate concentration does not affect the apparent equilibrium constant. Magnesium is necessary for the reaction to occur since the

monomagnesium complexes of nucleotides and of pyrophosphate are the true substrates for the enzyme[12]. Mg-ion concentration and the pH-value does not affect the apparent equilibrium constant since the acid dissociation and Mg stability constants of IMP have almost the same values as those of GMP[11]. Taking into account that temperature, pH-value, magnesium, and pyrophosphate concentration do not appreciably affect the apparent equilibrium constant of the reaction, this value could be employed to discuss the role of the HGPRT-catalyzed IMP-GMP exchange within living cells.

The HGPRT-catalyzed IMP-GMP exchange is the 1st reaction of the interconversion pathway of these nucleotides so far described[2-4] which does not appear to be irreversible. Through this reaction, the reversible conversion of IMP to GMP does not depend on the availability of ATP, NAD, NADPH, and glutamine. Intracellular concentrations of IMP and GMP have been determined in different cells and vary both with the type of cell considered and with the composition of the medium in which cells are living[13-18]. The concentrations of the 2 nucleotides appear to be in most instances of the same order of magnitude. Hypoxanthine concentration has been determined in many intra- and extracellular fluids[19-21]. The concentration of guanine appears to be very low[19] and almost unassayable[22]. Although the HGPRT-catalyzed IMP-GMP exchange is reversible, the equilibrium favors the formation of hypoxanthine and GMP from IMP and guanine. It is possible that this reaction has an important role in determining and/or maintaining the cellular concentration of IMP, GMP, and their respective purine bases.

1 Wyngaarden, J.B., and Kelley, W.N., Gout and Hyperuricemia. Grune and Stratton, New York 1976.
2 Magasanik, B., Moyed, H.S., and Gehring, L.G., J. biol. Chem. *226* (1957) 339.
3 Abrams, R., and Bentley, M., Archs Biochem. Biophys. *79* (1959) 91.
4 Magasanik, B., Meth. Enzymol. *6* (1963) 106.
5 Salerno, C., and Giacomello, A., J. biol. Chem. *254* (1979) 10232.
6 Giacomello, A., and Salerno, C., J. biol. Chem. *253* (1978) 6038.
7 Kalckar, H.M., J. biol. Chem. *167* (1947) 429.
8 Salerno, C., Giacomello, A., and Messina, E., Experientia *35* (1979) 1016.
9 Grassl, M., in: Methods of Enzymatic Analysis, vol. 4, p. 2161. Ed. H.V. Bergmeyer. Academic Press, New York 1974.
10 Segel, I.H., Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. John Wiley, New York 1975.
11 Giacomello, A., and Salerno, C., in: Purine Metabolism in Man-III, vol. 122B, p. 93. Eds A. Rapado, R.W.E. Watts and H.M.M. De Bruyn. Plenum Press, New York 1980.
12 Salerno, C., and Giacomello, A., J. biol. Chem. *256* (1981) 3671.
13 Dean, B.M., Perrett, D., and Sensi, M., Biochem. biophys. Res. Commun. *77* (1978) 147.
14 Dean, B.M., and Perrett, D., Biochim. biophys. Acta *437* (1976) 1.
15 Brenton, D.P., Astrin, K.H., Cruikshank, M.K., and Seegmiller, J.E., Biochem. Med. *17* (1977) 231.
16 Mandel, P., Wintzerith, M., Klein-Pete, N., and Mandel, L., Nature *198* (1963) 1000.
17 Tseveleva, A., and Libinzon, R.E., Biokhimiya *27* (1962) 305.
18 Mandel, P., in: Progress in Nucleic Acid Research and Molecular Biology, vol. 3, p. 299. Eds J.N. Davidson and W.E. Cohn. Academic Press, New York 1964.
19 Murray, A.W., A. Rev. Biochem. *40* (1971) 811.
20 Parker, R., Snedded, W., and Watts, R.W.E., Biochem. J. *116* (1970) 317.
21 DeLapp, N.W., and Fisher, J.R., Biochim. biophys. Acta *269* (1972) 505.
22 Nees, S., Gerbes, A.L., Willershausen-Zönnchen, B., and Gerlach, E., in: Purine Metabolism in Man-III, vol. 122B, p. 25. Eds A. Rapado, R.W.E. Watts and H.M.M. De Bruyn. Plenum Press, New York 1980.

Effect of an ecdysteroid on DNA synthesis, DNA polymerase α and thymidine kinase activities of *Drosophila melanogaster* cells in vitro

Nicole Munsch and Denise Cade-Treyer

Institut de Recherches Scientifiques sur le Cancer, Laboratoire d'Immunochimie, B.P. 8, F-94802 Villejuif Cedex (France), and Laboratoire de Zoologie, Université Paris VI, 7 Quai Saint-Bernard, F-75230 Paris Cedex 05 (France), 23 October 1981

Summary. 10^{-7}M 20-OH-ecdysone treatment of a diploid *Drosophila* clone results in an inhibition of 60% of the DNA synthesis from 18 h of treatment on. After 48 h of hormone treatment the thymidine kinase activity is 70% inhibited; concomitantly a 60–70% lowering of the acid-soluble specific activity is observed. In the meantime the DNA polymerase α activity is reduced.

Cell proliferation and differentiation in vivo result in a complex balance between distinct but interrelated controls. Interactions between cells and diffusible factors such as hormones or nutrients are one of these controls. Despite numerous investigations, how and why cells start growing, or differentiate, are not cleary understood. In this perspective our purpose was to study the mechanism of action of an insect steroid hormone, 20-OH-ecdysone, on in vitro cultures of a clonal *Drosophila melanogaster* cell line. It has already been shown that ecdysteroid hormone treatment causes morphological transformation of *Drosophila* cell lines in vitro, and also agglutination[1], amplification of specific protein synthesis[2-4] and enzymatic induction[5,6]. The inhibition of cell proliferation of 20-OH-ecdysone treated *Drosophila* cell lines has been observed earlier[7,8].

To obtain further insight into the mechanism of inhibition of cell growth and cell proliferation by 20-OH-ecdysone we have studied the effect of this hormone on DNA synthesis and enzymes involved in DNA replication, such as DNA polymerase α and thymidine kinase, of a *Drosophila melanogaster* diploid cell clone.

Material and methods. The diploid (XX haplo IV) 5284 clone derived from the Kc cell line[9] of *Drosophila melanogaster* was grown in D22 medium supplemented with 10% fetal calf serum, at 23 °C. The cells were incubated for various times with 20-hydroxyecdysone (Rhoto, Osaka, Japan) added 2 h after plating. Tritiated thymidine [(^{3}H) TdR] (23 Ci/mM) from the Radiochemical Centre (Amersham, England) was added to control and treated cells at a final concentration of 0.5 μCi/ml 1 h before the end of the incubation.