Regulation of human placental deoxyguanosine kinase by nucleotides

Yasukazu Yamada, Haruko Goto and Nobuaki Ogasawara

Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03, Japan

Received 28 April 1983

The activity of deoxyguanosine kinase purified from human placenta was regulated by various nucleotides. dTTP, an activator, only increased the V_{max} value of the enzyme. The feedback inhibition by dGTP, dGDP and dGMP were competitive with respect to deoxyguanosine. Both the activation by dTTP and the inhibition by dGTP were reversible.

Deoxyguanosine kinase *Human placenta* *Regulation* *dTTP* *dGTP*

1. INTRODUCTION

As immunodeficiency is associated with dGTP accumulation [1–3], due to a lack of purine nucleoside phosphorylase activity, the phosphorylation of deoxyguanosine is considered important. In mammalian cells, cytosomal deoxycytidine kinase (EC 2.7.1.74) and mitochondrial deoxyguanosine kinase were capable of phosphorylating deoxyguanosine [4–10]. Deoxyguanosine kinase was partially purified from various mammalian tissues [7–10]. These studies showed that the phosphorylation of deoxyguanosine by deoxyguanosine kinase differed in K_m value for deoxyguanosine, optimal pH and effects of nucleotides from that by deoxycytidine kinase. Deoxyguanosine kinase activity is stimulated by dTTP [7,10] and inhibited by dGTP, dGDP and dGMP [7–10]. Here, we report regulation of human placental deoxyguanosine kinase by: nucleotides; activator, dTTP; and inhibitors, dGTP, dGDP and dGMP.

2. MATERIALS AND METHODS

2.1. *Materials*

[8-^{3}H]Deoxyguanosine (1.7 Ci/mmol) was from Amersham (Bucks). Nucleosides, nucleotides, phosphoenolpyruvate and pyruvate kinase were from Sigma (St Louis MO) and Boehringer (Mannheim). Other reagents were commercial preparation of the highest purity available.

2.2. *Enzyme assay and protein determination*

Deoxyguanosine kinase activity was assayed at pH 7.4 (50 mM Tris–HCl) with 10 μM [8-^{3}H]-deoxyguanosine, 10 mM ATP and 10 mM $MgCl_2$, as in [10]. One unit of enzyme activity is defined as the amount catalyzing the phoshorylation of 1 nmol deoxyguanosine for 1 min. Specific activity was defined as units . min^{-1} . mg $protein^{-1}$. Protein concentration was determined by the Lowry method [11] using bovine serum albumin as a standard.

2.3. *Purification of enzyme*

Human placenta (300 g) stored at −70°C was homogenized in 900 ml 50 mM Tris–HCl buffer (pH 8.0) contained 1 mM dithiothreitol. The homogenate was centrifuged at 20000 × *g* for 20 min. Deoxyguanosine kinase was purified from the supernatant by ammonium sulfate fractionation (0–55%), affinity chromatography on AMR–Sepharose 4B, ion-exchange chromatography on DEAE-cellulose (Whatman, DE-52), gel filtration on Sephadex G-75, affinity chro-

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

matography on blue–Sepharose Cl-6B, then DE-52 column chromatography again, as in [10]. To stabilize the enzyme activity, bovine serum albumin (0.2 mg/ml) was added to the final enzyme preparation, and the preparation was stored at −70°C.

3. RESULTS AND DISCUSSION

The specific activity of the final enzyme preparation was 10.3 $nmol.min^{-1}.mg\ protein^{-1}$ at pH 6.0. When 10 mM ATP was used as a phosphate donor, the pH optimum was 6.0, but shifted to pH 6.8 on the addition of 1 mM dTTP [10]. At physiological pH, the activity was stimulated 2-fold by 0.2 mM dTTP and 3–4-fold by the optimal concentration (fig.1A). Also by addition of CTP, dUTP and UTP (1 mM), the activity was stimulated 2.3-, 2.2- and 1.7-fold, respectively [10]. By the activation of dTTP only the V_{max} value of the enzyme was increased; the K_m value for deoxyguanosine was not affected (fig.1B). In the absence of dTTP, enzyme has 2 app. K_m-values for $MgATP^{2-}$, 0.13 and 2.2 mM [10]. However, on addition of dTTP, the low-affinity K_m value for $MgATP^{2-}$ of 2.2 mM disappeared (fig.1C).

In contrast, deoxyguanosine kinase activity was inhibited by the addition of a low concentration of dGTP and dGDP; app. K_i-values of dGTP and dGDP were 1.0 and 2. μM, respectively (fig.2A). The activity was also inhibited by higher concentrations of dGMP, GTP and dATP [10]. An app. K_i-value of dGMP was 0.33 mM. dGTP appeared to be a competitive inhibitor with respect to deoxyguanosine (fig.2B). Furthermore, at >0.5 mM $MgATP^{2-}$, the inhibition by dGTP became competitive with respect to $MgATP^{2-}$ (fig.2C). The same type of inhibition was observed by the addition of appropriate concentrations of dGDP or dGMP.

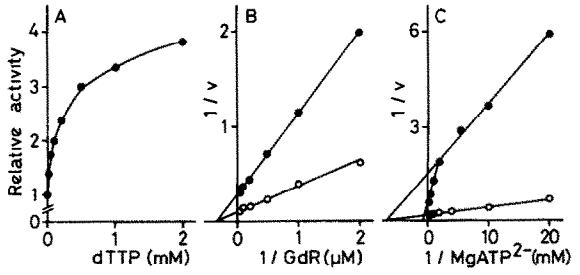

Fig.1. (A) Effects of [dTTP] on deoxyguanosine kinase activity. (B) Double reciprocal plots for various deoxyguanosine concentrations: (●—●) none; (○—○) plus 1.0 mM dTTP. (C) Double reciprocal plots for various $MgATP^{2-}$ concentrations: (●—●) none; (○—○) plus 1.0 mM dTTP.

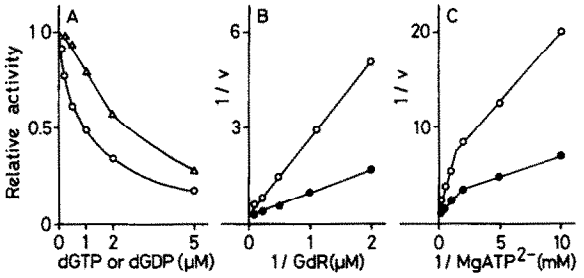

Fig.2. (A) Effects of dGTP or dGDP concentration on deoxyguanosine kinase activity: (○—○) dGTP; (△—△) dGDP. (B) Double reciprocal plots for various deoxyguanosine concentrations: (●—●) none; (○—○) plus 0.2 μM dGTP. (C) Double reciprocal plots for various $MgATP^{2-}$ concentrations: (●—●) none; (○—○) plus 0.2 μM dGTP.

Combination studies were also carried out in the presence of activator and inhibitor (fig.3). The addition of dTTP could reverse the inhibition due to dGTP. The activities stimulated by dTTP were

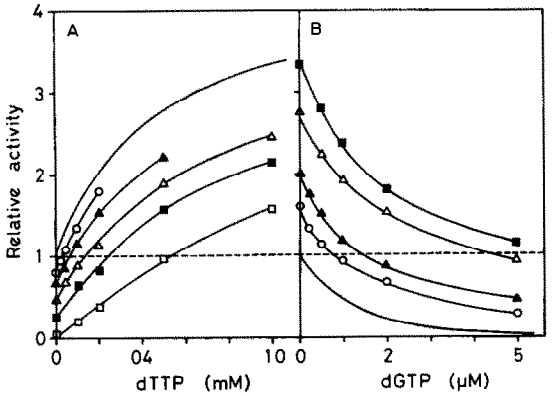

Fig.3. Combination effects of activator and inhibitor. Enzyme activity was assayed in the presence of various concentrations of inhibitor and activator. (A) Activation by dTTP: (——) no dGTP; (○—○) plus 0.2 μM dGTP; (▲—▲) 0.5 μM dGTP; (△—△) 1.0 μM dGTP; (■—■) 2.0 μM dGTP; (□—□) 5.0 μM dGTP. (B) Inhibition by dGTP: (——) no dTTP; (○—○) plus 0.1 mM dTTP; (▲—▲) 0.2 mM dTTP; (△—△) 0.5 mM dTTP; (■—■) 1.0 mM dTTP.

also decreased by further addition of dGTP. Thus, both the activation and inhibition were reversible. However, as the activity was strongly inhibited by low [dGTP], to reverse the inhibition by 1 μM dGTP, 125 μM dTTP was necessary.

Deoxyguanosine kinase seems to be dimer consisting of 2 identical subunits of M_r 29000 [10]. The regulation of the enzyme activity might be caused by the structural change of the enzyme. Therefore, we carried out sucrose density gradient analysis in the presence of activator (1 mM dTTP) or inhibitor (20 μM dGTP). However, the enzyme activity sedimented at the same position in the absence and presence of effectors. Thus, activation and inhibition were not caused by the association and dissociation of enzyme subunits.

Deoxyguanosine kinase purified from human placenta was regulated by various nucleotides. It is of interest that the activity is stimulated by dTTP, CTP, dUTP and UTP. However, the activation was observed at higher physiological concentrations of these nucleotides [10]. In contrast, the activity was strongly inhibited by a low concentration of dGTP or dGDP at both optimal pH and physiological pH. At physiological concentration of dGTP, the inhibition was readily observed. If deoxyguanosine were phosphorylated mostly by deoxyguanosine kinase, and not by deoxycytidine kinase, dGTP would not accumulate in T-lymphoblast in purine nucleoside phosphorylase deficiency.

ACKNOWLEDGEMENTS

We wish to thank Dr Mitsuoki Asai and Dr Shunji Goto for providing the human placenta. This work was partly supported by the grant from the Ogyah-Kenkin.

REFERENCES

[1] Cohen, A., Gudas, L.J., Ammann, A.J., Staal, G.E.J. and Martin, D.W. jr (1978) J. Clin. Invest. 61, 1405–1409.
[2] Chan, T.-S. (1978) Cell 14, 523–530.
[3] Gudas, L.J., Ullman, B., Cohen, A. and Martin, D.W. jr (1978) Cell 14, 531–538.
[4] Momparler, R.L. and Fischer, G.A. (1968) J. Biol. Chem. 243, 4298–4304.
[5] Durham, J.P. and Ives, D.H. (1970) J. Biol. Chem. 245, 2276–2284.
[6] Krenitsky, T.A., Tuttle, J.V., Koszalka, G.W., Chen, I.S., Beacham, L.M. iii, Rideout, J.L. and Elion, G.B. (1976) J. Biol. Chem. 251, 4055–4061.
[7] Gower, W.R. jr, Carr, M.C. and Ives, D.H. (1979) J. Biol. Chem. 254, 2180–2183.
[8] Green, F.J. and Lewis, R.A. (1979) Biochem. J. 183, 547–553.
[9] Barker, J. and Lewis, R.A. (1981) Biochim. Biophys. Acta 658, 111–123.
[10] Yamada, Y., Goto, H. and Ogasawara, N. (1982) Biochim. Biophys. Acta 709, 265–272.
[11] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.