MECHANISM OF INHIBITION OF THYMIDINE KINASE FROM *ESCHERICHIA COLI* BY CAFFEINE

Inger SANDLIE and Kjell KLEPPE
Department of Biochemistry, University of Bergen, Bergen, Norway

Received 17 December 1979

1. Introduction

The influence of caffeine on chromosome structure and DNA repair processes has been well documented. For a comprehensive review on caffeine, see [1]. Caffeine has also been noted to have an effect on the incorporation of thymidine into DNA both in eukaryotic and in prokaryotic cells [2–4]. In *Escherichia coli*, caffeine may affect both uptake, conversion of thymidine to dTTP and the DNA synthesis process itself [5]. Thymidine kinase (EC 2.7.1.21), an important enzyme in the conversion of thymidine to dTTP, was shown inhibited by caffeine [5]. Here we detail the mechanism of caffeine-induced inhibition of thymidine kinase from *E. coli*. Caffeine was found to be a competitive inhibitor of thymidine kinase, competing with the substrate ATP for its binding site.

2. Materials and methods

2.1. *Chemicals and enzymes*

All radioactively labelled compounds were obtained from The Radiochemical Centre, Amersham. Unlabelled nucleosides and nucleotides were from Sigma Chemical Co. Caffeine was a product of Koch Light Labs.

Thymidine kinase from *E. coli* was prepared from wild-type *E. coli* B or *E. coli* K-12, KMBL 1788 [6], essentially as in [7]. The specific activity of the purified enzyme was approximately the same as in [7].

2.2. *Assay for thymidine kinase*

The following assay mixture was routinely employed: 100 mM Tris–HCl (pH 7.8), 10 mM $MgCl_2$, 7 mM ATP, 2 mM β-mercaptoethanol and 1 μM [^{14}C]thymidine, spec. act. 0.1 Ci/mmol. When the purified enzyme was used, 0.3 mg/ml of bovine serum albumin was included. The reaction period was usually 60 min at 37°C. The reaction products were separated on polyethyleneimine-cellulose thin-layer plates as in [8]. The radioactive areas on the plates were cut out and counted in a toluene-based scintillation liquid.

3. Results

3.1. *Effect of caffeine on the time course of the reaction*

Figure 1 shows the time course for the thymidine kinase reaction using enzyme from the different purification steps and in the presence and absence of 10 mM caffeine. Linear kinetics were observed only for the most pure fractions, fraction V and VI. In all cases, however, caffeine inhibited the reaction. The inhibition increased linearly with increasing concentrations of caffeine up to 10 mM at which point an inhibition of 55% was observed. Caffeine at 20 mM gave 75% inhibition (results not shown).

3.2. *Influence of thymidine and ATP concentrations*

The effect of different concentrations of the substrates thymidine and ATP on the caffeine inhibition with the enzyme from *E. coli* were studied in detail. The result in the case of thymidine is presented in fig.2. Here the data were plotted according to [9] and two straight lines were obtained that intersect at the $1/s$ axis, indicating that caffeine is a non-competitive inhibitor with respect to thymidine. This is not surprising since caffeine is an adenine analogue and

Address correspondence to: Kjell KLEPPE

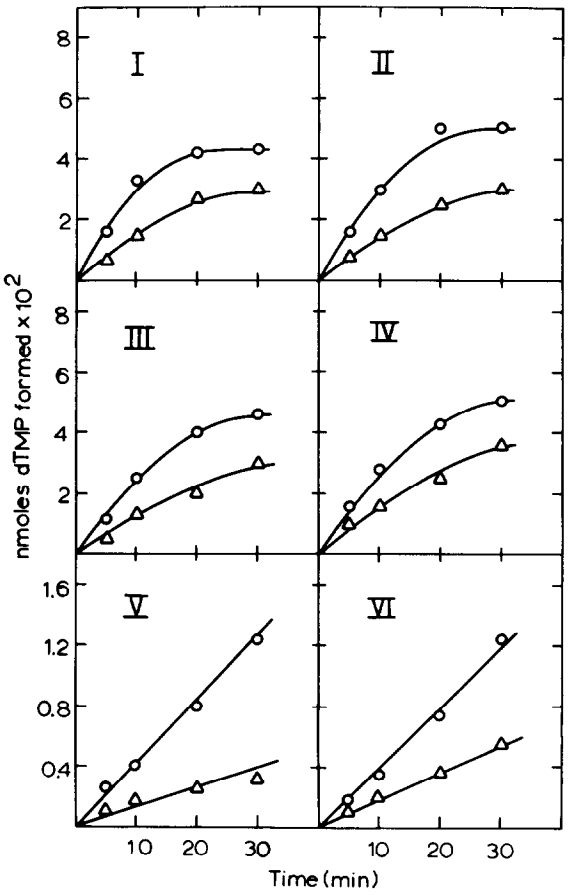

Fig.1. Time course of the thymidine kinase catalyzed reaction in the presence (△——△) and absence (○——○) of 10 mM caffeine, using enzyme from the 6 purification steps: step I, crude extract; step II, supernatant from heat-treatment; step III, supernatant from the streptomycin sulfate precipitation; step IV, first ammonium sulfate treatment; V, second ammonium sulfate treatment; step VI, DEAE-cellulose fraction. For more detials see [7].

would be expected to compete with ATP. Some evidence for the latter view is shown in fig.3. In agreement with the data in [7] the saturation curves for ATP were found to be hyperbolic when the data were plotted in a double-reciprocal manner. The results clearly show that the caffeine-induced inhibition can be abolished by increasing the concentration of ATP in the assay mixture.

3.3. *Effect of activators*

Thymidine kinase from *E. coli* is known to be activated by various deoxynucleoside diphosphates and deoxynucleoside triphosphates such as dCDP, dCTP

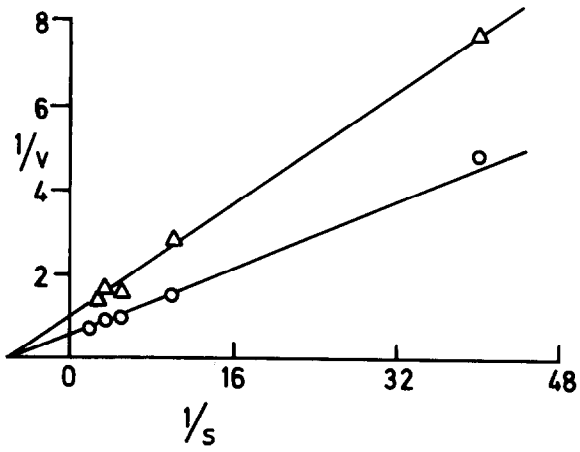

Fig.2. Effect of thymidine concentration on the rate of dTMP formation in the presence (△——△) and absence (○——○) of 10 mM caffeine using the purified enzyme from *E. coli*. v = nmol dTMP formed/h. s = mM thymidine.

and dATP. The strongest activators dCDP and dCTP cannot be used as phosphate donors by the enzyme whereas dATP can substitute completely for ATP [10]. With increasing concentrations of dCTP the specific activity of the enzyme increased up to 5-fold. High concentrations of dCTP did not, however, abolish the caffeine-mediated inhibition of the enzyme whereas high concentrations of dATP did, due to the fact that it can substitute for ATP (results not shown).

The influence of varying ATP concentrations in the presence of dCTP and caffeine is shown in fig.3B. Here, as expected, straight lines were obtained when the data were plotted in a double-reciprocal manner. The lines intersected at the $1/v$ axis suggesting that caffeine is a competitive inhibitor of ATP for the enzyme. The caffeine inhibition observed could also be due to an effect of caffeine on the affinity of the enzyme for ATP. The inhibitor could combine with the enzyme at a second site, not identical wholly or in part to the substrate binding site for ATP, with the result that the rate of formation of products becomes slower, but is not stopped. Kinetically such a mechanism is indistinguishable from true competitive inhibition [11]. Since thymidine kinase from *E. coli* is an allosteric enzyme, caffeine might resemble the effector, bind partially to this site and hence influence the catalytic activity of the enzyme. To rule out the latter possibility the activity was measured at varying caffeine concentrations and 3

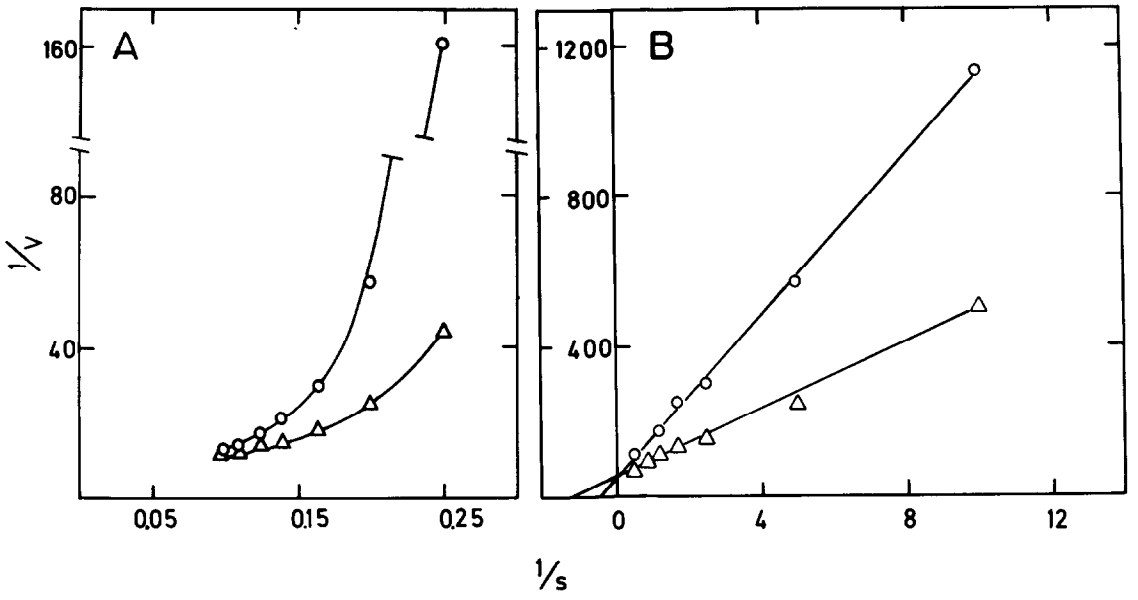

Fig.3. Effect of ATP concentration on the rate of dTMP formation in the presence (o——o) and absence (Δ——Δ) of 10 mM caffeine. The purified enzyme from *E. coli* was employed. v = nmol dTMP formed/h. s = mM ATP. (A) The assay mixture was the one routinely employed. (B) The assay mixture contained 5.6 mM dCTP in addition to the usual ingredients.

different concentrations of ATP. When the data were plotted as $1/v$ versus concentration of caffeine, shown in fig.4, 3 straight lines were obtained which intersected at 1 point. This clearly proves that caffeine competes with ATP for the substrate binding site of the enzyme [11,12]. The intersection point of the 3 lines corresponds to $-K_i$ for caffeine. This was estimated to be 5.7 mM.

3.4. *Influence of caffeine analogues*

Caffeine analogues such as theophylline and adenine were also tested. At 10 mM these inhibited the reaction by 30% and 43%, respectively. Under the same conditions the caffeine-induced inhibition was 55%.

4. Discussion

These results show clearly that caffeine inhibits thymidine kinase and furthermore that caffeine is a competitive inhibitor of ATP. Thymidine kinase from *E. coli* is an allosteric enzyme whose activity is regulated by various nucleoside diphosphates and triphosphates, the most active being dCTP [10]. Both nucleotide activators and inhibitors are known to cause dimerization of the enzyme [13]. Since different concentrations of dCTP had little effect on the degree of inhibition caused by caffeine it is unlikely that caffeine binds to the activator site to any extent. The fact that a strict competitive inhibition pattern with ATP was observed suggests strongly that caffeine binds to the same site as ATP on the enzyme.

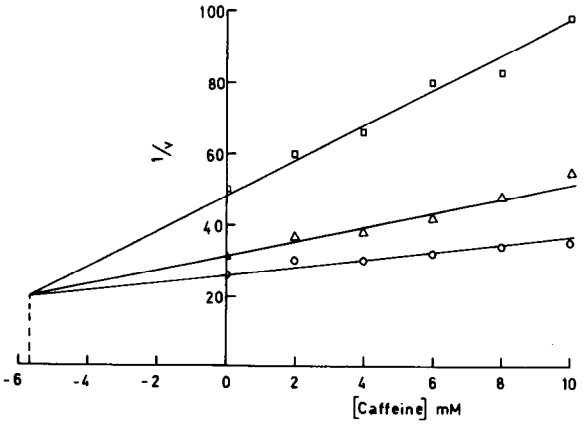

Fig.4. The rate of the thymidine kinase reaction measured at different caffeine concentrations and 3 different ATP concentrations: 0.4 mM (□——□); 0.8 mM (Δ——Δ); 2 mM (o——o). The purified enzyme from *E. coli* was employed. v = nmol dTMP formed/h.

Several enzymes from *E. coli* are known to be inhibited by caffeine such as purine nucleoside phosphorylase [14], DNA polymerase I (nuclease activity only) [6], *uvrAuvrBuvrC* endonuclease [15,16]. In the latter case the K_i obtained was approximately twice that found here. With regard to enzymes from eukaryotic cells, caffeine has been shown to inhibit cAMP phosphodiesterase [17], glycogen phosphodiesterase [17], glycogen phosphorylase from skeletal muscle [18] and human erythrocyte phosphatidylinositol kinase [19]. Our preliminary results show that the thymidine kinase from human fibroblast cells is also inhibited by caffeine. The mechanism of inhibition could be similar to that of the enzyme from *E. coli*.

Acknowledgements

This study was supported in parts from the Norwegian Research Council for Science and Humanities and the Norwegian Cancer Society.

References

[1] Kihlman, B. A. ed (1977) Caffeine and Chromosomes, Elsevier/North-Holland, Amsterdam, New York.
[2] Lehmann, A. R. and Kirk-Bell, S. (1974) Mut. Res. 26, 73–83.
[3] Lieb, M. (1961) Z. Vererbungsl. 92, 416–429.
[4] Grigg, G. W. (1968) Mol. Gen. Genet. 102, 316–336.
[5] Sandlie, I., Solberg, K. and Kleppe, K. (1980) submitted.
[6] Solberg, K. A., Øvrebø, S., Kleppe, R. K. and Kleppe, K. (1978) Mut. Res. 51, 1–10.
[7] Okazaki, R. and Kornberg, A. (1964) J. Biol. Chem. 239, 269–274.
[8] Beck, C. F., Ingraham, J. L., Neuhard, J. and Thomassen, E. (1972) J. Bacteriol. 110, 219–228.
[9] Lineweaver, H. and Burk, D. (1934) J. Am. Chem. Soc. 56, 658–666.
[10] Okazaki, R. and Kornberg, A. (1964) J. Biol. Chem. 239, 275–284.
[11] Worcel, A., Goldman, D. S. and Cleland, W. W. (1965) J. Biol. Chem. 240, 3399–3407.
[12] Dixon, M. (1953) Biochem. J. 55, 170–174.
[13] Iwatsuki, N. and Okazaki, R. (1967) J. Mol. Biol. 29, 139–154.
[14] Koch, A. L. and Lamont, W. A. (1956) J. Biol. Chem. 219, 189–201.
[15] Seeberg, E., Nissen-Meyer, J. and Strike, P. (1976) Nature 263, 523–526.
[16] Braun, A., Hopper, P. and Grossman, L. (1975) Molecular mechanisms for repair of DNA, pt A (Hanawalt, P. C. and Setlow, R. B. eds) p. 183–190 Plenum, London, New York.
[17] Butcher, R. W. and Sutherland, E. W. (1962) J. Biol. Chem. 237, 1244–1250.
[18] Kihlman, B. and Overgaard-Hansen, K. (1955) Exp. Cell Res. 8, 252–255.
[19] Buckley, J. T. (1977) Biochim. Biophys. Acta 498, 1–9.