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Double Inhibition of D-Glyceraldehyde-3-phosphate Dehydrogenase and Lactate Dehydrogenase

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The simultaneous action of ATP (partially uncompetitive inhibitor with respect to P_i) and quinaldate (purely non-competitive inhibitor with respect to P_i) on D-glyceraldehyde-3-phosphate dehydrogenase was analyzed kinetically. The interaction constant [as defined by Keleti and Fajszi (1971) Math. Biosci. *12* 197] of the two inhibitors for the D-glyceraldehyde-3-phosphate dehydrogenase-P_i complex is greater than 1, which means that the two inhibitors act antagonistically. The kinetic analysis of the double inhibition shows that there is no ATP-enzyme-quinaldate ternary complex, but a quaternary complex with P_i is formed. The interaction of the two inhibitors on the enzyme-P_i complex depends on substrate (P_i) concentration. The antagonistic effect of the two inhibitors becomes additive at low P_i concentrations (about 1 mM).

The simultaneous action of oxalate (purely uncompetitive inhibitor with respect to NAD) and quinaldate (partially mixed type inhibitor with respect to NAD) on lactate dehydrogenase was also analyzed. Oxalate and quinaldate act antagonistically on lactate dehydrogenase. However, at low NAD concentrations (about 0.06 mM) or at high quinaldate and low oxalate concentrations (around 7 and 1.7 mM, respectively) the antagonism turns into the simple summation of the effects of the two inhibitors.

Introduction

Many drugs exert their effect by influencing appropriate enzymes. Modern medicaments sometimes comprise two or more enzyme inhibitors acting simultaneously. Moreover, in the living cell several metabolites which may also behave as enzyme modifiers, act simultaneously on one and the same enzyme. Therefore, to study the effect of two or more modifiers on an enzyme is warranted.

The theoretical analysis of double inhibitions showed that modifiers, due to their interaction, may exert different kinetic effects on enzyme action depending on their types of inhibition. The study of these effects may elucidate the changes in steric structure caused by one or another inhibitor or by the substrate, the mecha-

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nism of action of inhibitors and some peculiar results of the modifier interactions (Keleti, Fajszi, 1971; Fajszi, 1976; Fajszi, Keleti, 1976).

D-glyceraldehyde-3-phosphate dehydrogenase is one of the key enzymes of the glycolytic pathway and, like other enzymes, it is affected *in vivo* by more than one modifier at the same time. The aim of this work is to examine the simultaneous effect of two inhibitors encountered in living cells, quinaldate and ATP*, on the activity of GAPD.

The inhibitory effect of ATP and quinaldate on GAPD, when added separately, has been analyzed in detail (Lien, Keleti, 1979). Theoretical consideration show that a triple faced enzyme inhibitor relation may exist if at least one of the inhibitors is of the partial type (Fajszi, Keleti, 1976). Since the inhibition of ATP is partially uncompetitive with respect to P_i, the latter was chosen as variable substrate.

With LDH quinaldate is a partially mixed type inhibitor in respect of NAD. Consequently, when we analyzed the simultaneous action of oxalate and quinaldate on LDH, NAD was used as variable substrate.

In this paper we study the interaction of the two inhibitors on GAPD and LDH and the change of their interaction as a function of substrate concentration.

Materials and methods

Four times recrystallized pig muscle GAPD was used (Előd i, Szörényi, 1956). Enzymic activity was measured spectrophotometrically in a standard assay mixture (Keleti, Batke, 1965), in 0.1 M glycine buffer, pH 8.5 or pH 7.5, at 25 °C. The molar specific activity of the enzyme preparations at pH 8.5 was 18 000 – 24 000 mole NADH/mole enzyme × min. The absorption of GAPD was taken to be $A^{1mg/ml}_{1cm,\ 280nm}$ = 1.0 (Fox, Dandliker, 1956) and its molecular weight = 145 000 (Előd i, 1958).

Pig muscle lactate dehydrogenase was used after 4 recrystallizations (Jécsai, 1961). Enzymic activity was measured spectrophotometrically in standard assay mixture (Jécsai, 1961), in 0.1 M glycine buffer, pH 10.0, at 20 °C. The molar specific activity of the enzyme preparations was 8500 – 12 000 mole NADH/mole enzyme × min. The absorption of LDH was taken to be $A^{1\ mg/ml}_{1\ cm,\ 280\ nm}$ = 1.29 (Jécsai, 1961), and its molecular weight 140 000 (Jaenicke, Knof, 1968).

The molar extinction coefficient of NADH was taken to be $6.22 \times 10^3\ M^{-1}\ cm^{-1}$ (Horecker, Kornberg, 1948).

GAP was prepared according to Szewczuk et al. (1961), NAD (85% pure), ATP (90% pure) and Na-oxalate (95% pure) were Reanal preparations. Lactic acid (98% pure) was purchased from Roth or Riedel-de-Haen. Quinaldic acid was an Aldrich-Europe or Koch-Light preparation of 99% purity.

* *Abbreviations:* GAP, D-glyceraldehyde-3-phosphate; GAPD, D-glyceraldehyde-3-phosphate dehydrogenase (EC.1.2.1.12); LDH, lactate dehydrogenase (EC.1.1.1.27).

The measurements were carried out in an Opton PMQ II spectrophotometer equipped with a Servogor automatic recorder. Calculations were performed with a Hewlett–Packard 9810 A desk calculator and plotter using Gregory–Newton interpolation and linear regression by the least squares method.

The analysis of double inhibition was performed as described earlier (Keleti, Fajszi, 1971; Fajszi, 1976; Fajszi, Keleti, 1976).

Other conditions were as defined previously (Lien, Keleti, 1979).

Results and discussion

Initial velocity measurements of the GAPD reaction were performed in the presence of both inhibitors, ATP and quinaldate, in different concentrations. The experimental data are plotted according to Yonetani and Theorell (1964), (Fig. 1).

The straight lines in Fig. 1. A indicate that quinaldate is a pure type inhibitor of GAPD with respect to the substrate, P_i, if the latter is used in suboptimal concentration. On the other hand, the curves in Fig. 1. B indicate that ATP is a partial type inhibitor, in agreement with experiments in which the two inhibitors were used separately (Lien, Keleti, 1979).

The intercepts on the ordinate of Fig. 1. A are further plotted as a function of ATP concentration (Fig. 2). The resulting monotonously increasing hyperbola indicates that the interaction constant of the two inhibitors for the enzyme-substrate complex, β, is between 1 and ∞ (Keleti, Fajszi, 1971). This means that the binding of one inhibitor hinders the binding of the other, i.e. the two inhibitors act antagonistically.

Two plots are used to examine whether both ternary (ATP–GAPD-quinaldate) and quaternary complexes (ATP–GAPD-quinaldate) exist (Fajszi, 1976).

$$\Big|_{P_i}$$

Fig. 3 shows the double reciprocal plot of initial velocity in the presence of both inhibitors as a function of the concentration of the variable substrate. The parallel straight lines indicate that the dissociation constant of ATP from the ternary complex equals infinity.

Fig. 4 shows the plot $(1/[I_1])(1/v_{1,2}-1/v_2)$ *vs.* $1/[P_i]$. The straight lines parallel with each other and the abscissa again indicate that the dissociation constant of ATP from the ternary complex equals infinity.

Since these results suggest that a ternary complex containing both inhibitors does not exist in the given system, the two inhibitors can interact only at the quaternary complex containing the substrate.

These results are in good agreement with those obtained with the two inhibitors separately (Lien, Keleti, 1979), namely that ATP is uncompetitive inhibitor of GAPD with respect of P_i and therefore is unable to bind to the free enzyme. Consequently, a ternary complex with the two inhibitors not containing substrate, cannot be formed.

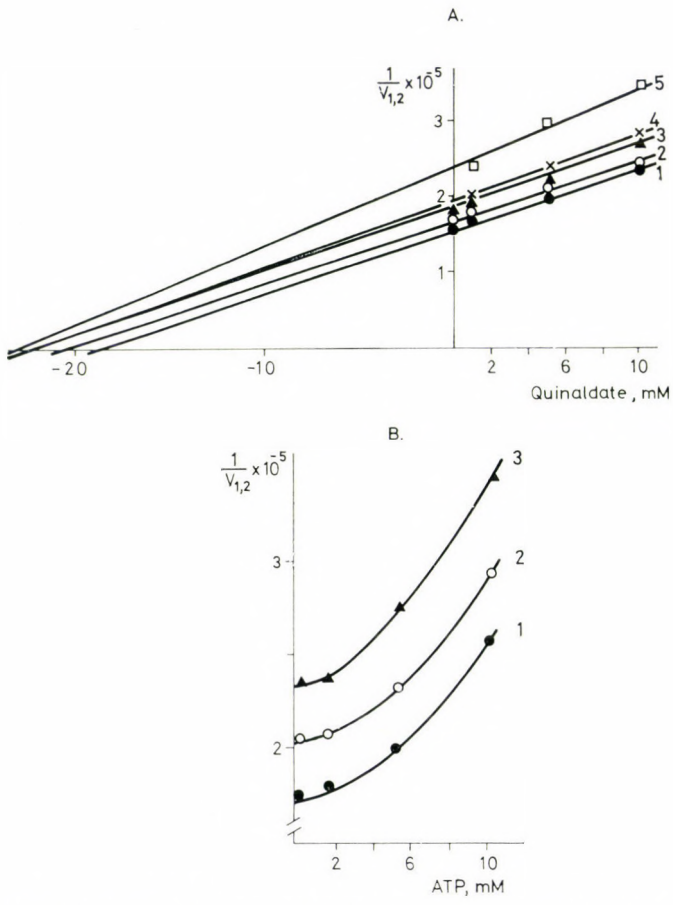

Fig. 1. Yonetani—Theorell plot of double inhibition of GAPD by quinaldate and ATP. The P_i concentration was 2 mM, the other substrates were employed at saturating concentration. [GAPD] = 6.9×10^{-6} mM. 0.1 M glycine buffer, pH 8.5, at 25 °C. $v_{1,2}$ = initial velocity in the presence of both inhibitors. A — $1/v_{1,2}$ *vs.* quinaldate concentration. ATP concentrations = 1: 0; 2: 1.5 mM; 3: 2.6 mM; 4: 5.3 mM; 5: 10.0 mM. B — $1/v_{1,2}$ *vs.* ATP concentration. Quinaldate concentrations = 1: 1.0 mM; 2: 5.0 mM; 3: 10.0 mM

We analyzed the interaction of ATP and quinaldate on GAPD as a function of P_i concentration (Fig. 5). At P_i concentrations higher than 1 mM, the two inhibitors act antagonistically, i.e. $v_0 v_{1,2}/v_1 v_2 > 1$. However, the antagonism of the two inhibitors turns into the simple summation of their effect ($v_0 v_{1,2}/v_1 v_2 = 1$) at the "characteristic substrate concentration" (cf. Fajszi, Keleti, 1976) which in the case of GAPD, is about 1 mM P_i (i.e. near physiological concentration). At this substrate concentration quinaldate and ATP act independently of each other, hence their effects are additive.

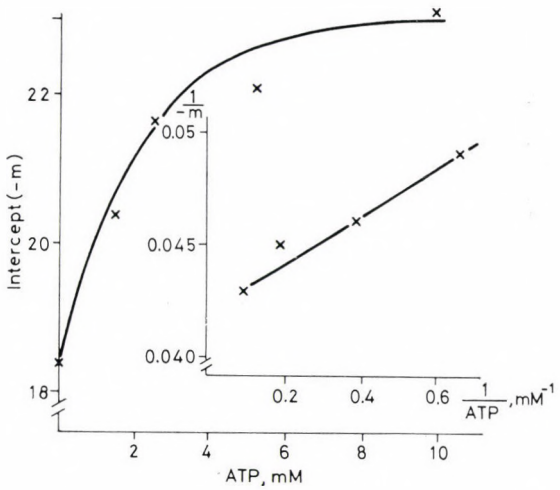

Fig. 2. Determination of interaction constant of the two inhibitors according to Keleti and Fajszi (1971). Intercepts in Fig. 1.A with the abscissa (−m) were plotted against the concentration of ATP at quinaldate concentrations: 0, 1, 2, 5 and 10 mM. Substrate (P_i) concentration was 2 mM. Other conditions as in Fig. 1. The straight line of the double reciprocal plot [ATP] *vs.* −m (insert) indicates that the curve in Fig. 2 is indeed a hyperbola

The double inhibition of LDH with NAD as variable substrate and oxalate and quinaldate as inhibitors was also studied. Novoa et al. (1959) found oxalate to be an uncompetitive inhibitor with respect to NAD. Kőnig et al. (1975) have shown that quinaldate is a mixed type inhibitor of LDH with respect to NAD.

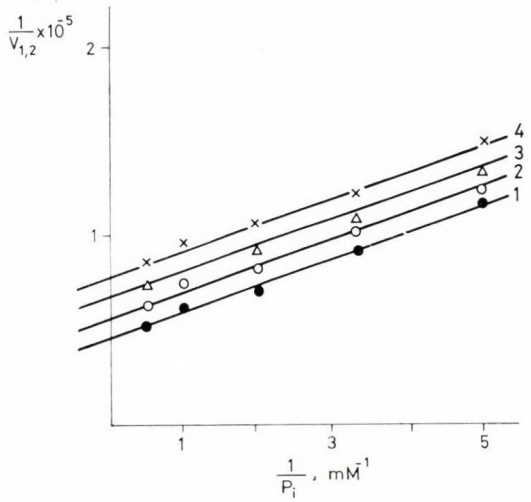

Fig. 3. Double reciprocal plot of $v_{1,2}$ against P_i concentrations. Initial velocities were measured with 1.5 mM ATP and the following quinaldate concentrations = 1: 0; 2: 1.0; 3: 3.0 4: 5.0 mM. Other conditions as in Fig. 1

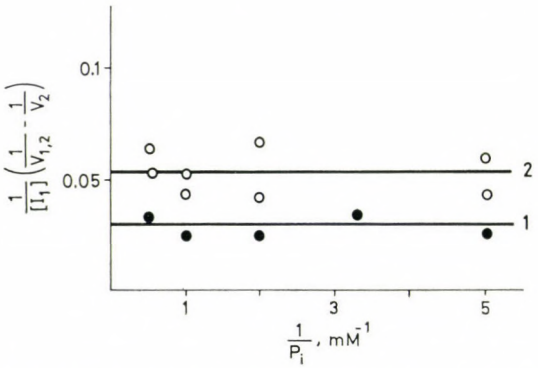

Fig. 4. Diagnostic test of GAPD-ATP-quinaldate complex. $[I_1]$ = concentration of quinaldate; v_2 = initial velocity of GAPD in the presence of ATP and $v_{1,2}$ = initial velocity in the presence of both quinaldate and ATP; 1: 3.3 mM quinaldate + 8.0 mM ATP; 2: 5.0 mM quinaldate + 5.0 mM ATP. Other conditions as in Fig. 1

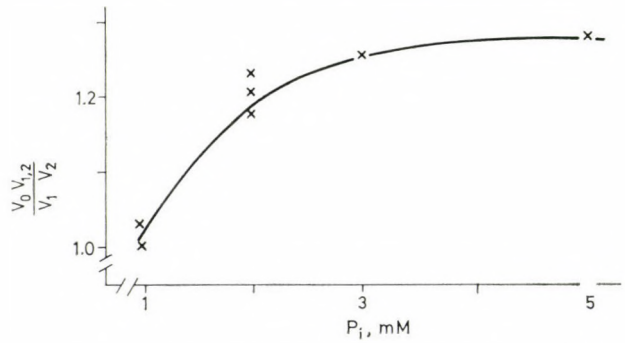

Fig. 5. Effect of substrate concentration on the interaction of two inhibitors on GAPD. Initial velocities of GAPD were measured in the presence of 2.5 mM quinaldate (v_1), 5.0 mM ATP (v_2), and both ($v_{1,2}$) as well as in the absence of inhibitors (v_0). The concentration of GAPD was 6.9×10^{-6} mM. The measurements were carried out in 0.1 M glycine buffer, pH 7.5 at 25 °C

Our experiments agreed with these results. Furthermore, we proved that oxalate is a purely uncompetitive whereas quinaldate is a partially mixed type inhibitor.

Fig. 6 shows that the two inhibitors interact on LDH in the same way as on GAPD: lowering the concentration of the substrate the antagonism between the two inhibitors turns into additivity. At high quinaldate concentration (7 mM) and low oxalate concentration (1.7 mM) practically the simple summation of the effects of the two inhibitors can be demonstrated independently of substrate concentration.

These results are consistent with the theoretical predictions that the antagonism or synergy of two inhibitors may turn into additivity or *vice versa* depen-

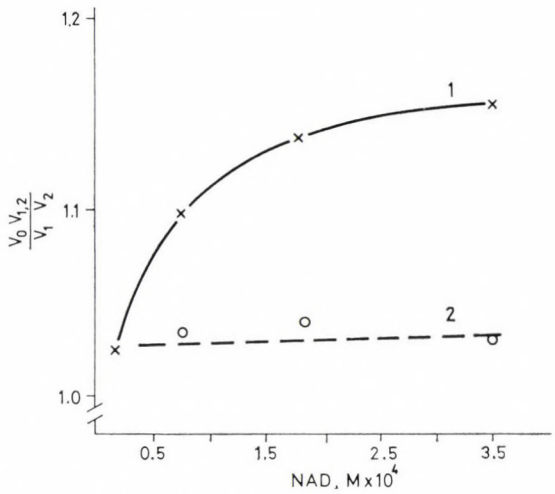

Fig. 6. Effect of substrate concentration on the interaction of two inhibitors on LDH. Initial velocities of LDH were measured in the presence of: 1: 6.8×10^{-3} M oxalate and/or 3.5×10^{-3} M quinaldate, 2: 1.7×10^{-3} M oxalate and/or 7.0×10^{-3} M quinaldate. The concentration of LDH was 8.6×10^{-6} mM. The measurements were carried out in 0.1 M glycine buffer, pH 10.0 at 20 °C

ding on substrate concentration (Fajszi, Keleti, 1976). Moreover, our data confirm that this phenomenon may be observed if at least one of the inhibitors is a partial type inhibitor; in fact two different dehydrogenases showed this effect.

References

Elődi, P. (1958) Acta Physiol. Acad. Sci. Hung. *13* 199—206
Elődi, P., Szörényi, E. T. (1956) Acta Physiol. Acad. Sci. Hung. *9* 339—350
Fajszi, Cs. (1976) in Mathem. Models of Metabol. Regul. (Keleti, T., Lakatos, S. ed.) Symp. Biol. Hung. Vol. 18. Akadémiai Kiadó, Budapest, pp. 77—103
Fajszi, Cs., Keleti, T. (1976) in Mathem. Models of Metabol. Regul. (Keleti, T., Lakatos, S. ed.) Symp. Biol. Hung. Vol. 18. Akadémiai Kiadó, Budapest, pp. 105—119
Fox, J. B., Dandliker, W. B. (1956) J. Biol. Chem. *221* 1005—1017
Horecker, B. L., Kornberg, A. (1948) J. Biol. Chem. *175* 385—390
Jaenicke, R., Knof, S. (1968) Eur. J. Biochem. *4* 157—163
Jécsai, G. (1961) Acta Physiol. Acad. Sci. Hung. *20* 339—345
Keleti, T., Batke, J. (1965) Acta Physiol. Acad. Sci. Hung. *28* 195—207
Keleti, T., Fajszi, Cs. (1971) Mathem. Biosci. *12* 197—215
Kőnig, T., Kocsis, B., Pocskay, E. (1975) Acta Biochim. Biophys. Acad. Sci. Hung. *10* 171—176
Lien, L. V., Keleti, T. (1979) Acta Biochim. Biophys. Acad. Sci. Hung. *14* 1—9
Novoa, W. B., Winer, A. D., Glaid, A. J., Schwert, G. W. (1959) J. Biol. Chem. *234* 1143—1148
Szewczuk, A., Wolny, E., Wolny, M., Baranowski, T. (1961) Acta Biochim. Polon. *8* 201—207
Yonetani, T., Theorell, H. (1964) Arch. Biochem. Biophys. *106* 243—251