Biochimica et Biophysica Acta, 523 (1978) 94—100

BBA 68377

INHIBITION OF PHOSPHOGLYCERATE KINASE BY SALICYLATES

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(Received July 18, 1977)

Summary

A kinetic analysis has been performed on the inhibition of the yeast phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) reaction by 2-hydroxybenzoate (salicylate) and two of its iododerivatives, 2-hydroxy-5-iodobenzoate and 2-hydroxy-3,5-diiodobenzoate.

The results give evidence that the salicylates mimic the nucleotide binding at the catalytic centre. The enzyme has an affinity for salicylate that dramatically increases for each iodine atom introduced to the benzene ring. Parabolic inhibition give evidence for two inhibitor binding sites per enzyme molecule. The two K_i values are 10 and 180 mM for salicylate, 0.60 and 13 mM for iodosalicylate and 0.064 and 0.70 mM for diiodosalicylate. The 2′-OH of the nucleotide substrate appears to be important for the catalytic events.

Introduction

Phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) catalyses the reversible transfer of a phosphoryl group from $MgATP^{2-}$ to 3-phospho-D-glycerate (3-*P*-glycerate). The substrates are capable of binding to the enzyme independently, and a rapid equilibrium random mechanism fits the available data [1—3].

The nucleotide binding domain in phosphotransferases, for example phosphoglycerate kinase, is very similar to the NAD^+ binding domain in dehydrogenases [4,5]. Salicylate appears to be a common inhibitor of many dehydrogenases (cf. ref. 6). In the case of alcohol dehydrogenase X-ray crystallographic and fluorimetric studies [6] have shown that salicylates bind to the adenosine binding site of the coenzyme binding domain in the enzyme. Thus it is reasonable to suggest that salicylate is a specific inhibitor of phosphotransferases also. For phosphoglycerate kinase the suggestion has been verified in the present work.

In the salicylate complexes of alcohol dehydrogenase a carboxyl oxygen in

the enzyme is hydrogen bonded to the 2-hydroxyl group of the inhibitor. The same carboxyl oxygen forms a hydrogen bond with the 2′-hydroxyl of the adenosine ribose moiety in the enzyme · NAD^+ complex [6]. In order to investigate the possible importance of the 2′-hydroxyl for the nucleotide binding and catalysis of phosphoglycerate kinase, some kinetic studies were in parallel performed with dATP and ATP.

Materials and Methods

Enzymes. Phosphoglycerate kinase was prepared from baker's yeast [7]; and the main electrophoretic component B was used. Glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12) from rabbit muscle was obtained from Boehringer Mannheim GmbH. Before use the crystals of this enzyme were collected by centrifugation (27 000 $\times g$, 20 min) and dissolved in 50 mM Tris · HCl (pH 7.8) containing 0.1 M NaCl.

Reagents. The barium salt of 3-*P*-glycerate, the sodium salts of equine muscle ATP and dATP and yeast NADH, were all purchased from Sigma Chemical Co. To determine the concentrations of the nucleotide · Mg^{2+} a dissociation constant of 0.08 mM was used (cf. ref. 8). Effects due to possible salicylate · Mg^{2+} complexes can be neglected. Mg^{2+} was introduced as analytical grade $MgCl_2$.

Salicylate and 2-hydroxy-5-iodobenzoate were from B.D.H. Chemicals (Poole) and EGA-Chemie (Heidenheim), respectively. The latter was recrystallized from acetone/water. 2-Hydroxy-3,5-diiodobenzoate was synthesized, washed with acetic acid and recrystallized from water (cf. ref. 6). Before use the solutions of these compounds were adjusted to pH 7.8 at 25°C with NaOH.

All solutions were made from analytical grade reagents and double distilled water, and were stored in acid-washed Duran glassware. The dithizone method was used to remove contaminating metal ions as described earlier [8].

3-*P*-Glycerate was liberated from its barium salt with Na_2SO_4 and neutralized by NaOH. The concentration was determined as described earlier [9].

Activity measurements. The activity of phosphoglycerate kinase was determined by the spectrophotometric method of Bücher [10], at conditions described earlier [8]. The initial velocity was expressed as $v = (dA_{366}/dt)_{t=0}$, in min^{-1}. About 0.2 μg of phosphoglycerate kinase was used per ml of the substrate.

The experiments were performed in 50 mM Tris · HCl (pH 7.8) at 25°C, containing 0.1 M NaCl. The assay mixture contained, unless stated otherwise, 1.0 mM ATP/1.0 mM Mg^{2+}/2.0 mM 3-*P*-glycerate/0.50 mM NADH.

Results

Overall inhibition by salicylate, iodosalicylate and diiodosalicylate. Inhibition of phosphoglycerate kinase at various concentrations of salicylate and two of its iodo derivatives are shown in Fig. 1. The curves show that diiodosalicylate is a stronger inhibitor than monoiodosalicylate, which is a stronger inhibitor than salicylate. Solubility properties limit the use of higher inhibitor concentrations.

Inhibition by salicylate and diiodosalicylate at varied $MgATP^{2-}$ and 3-P-

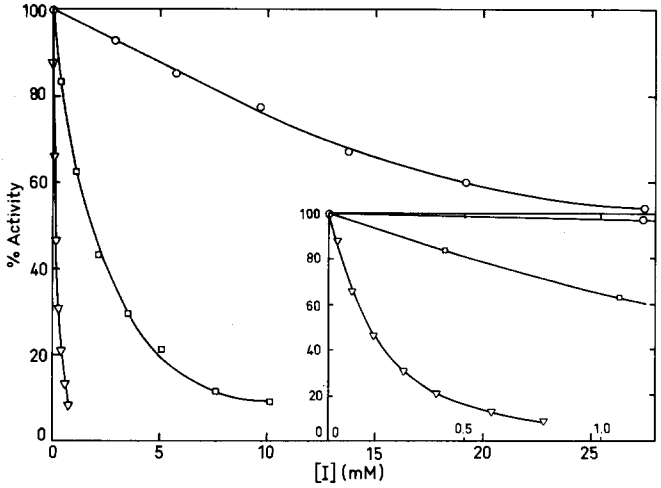

Fig. 1. Effects of the inhibitor concentration on the activity of phosphoglycerate kinase. ○———○, salicylate; □———□, 2-hydroxy-5-iodobenzoate; ▽———▽, 2-hydroxy-3,5-diiodobenzoate. The inset figure is a separate enlargement, showing the activity at inhibitor concentrations up to about 1 mM.

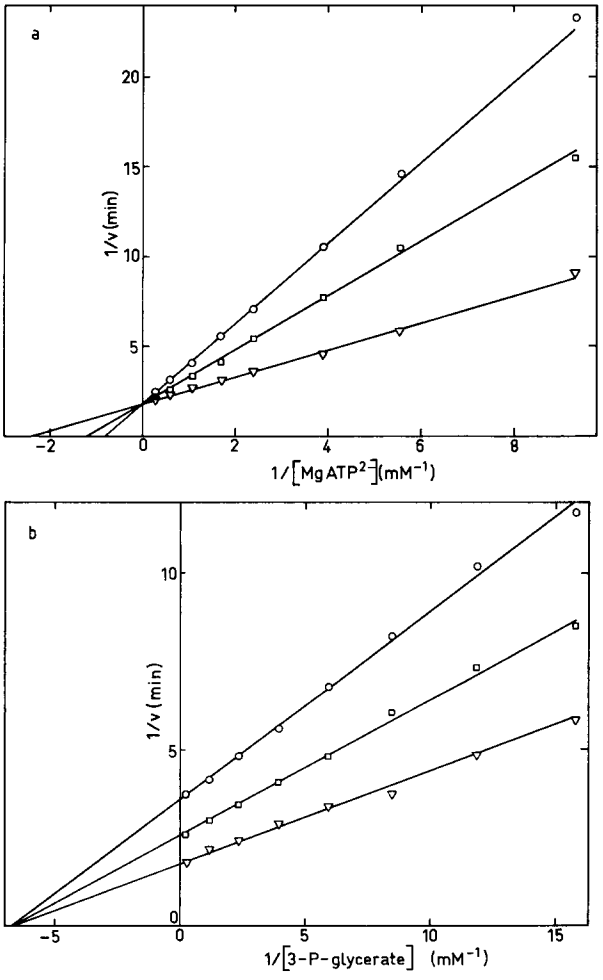

Fig. 2. Effects of the salicylate concentration on the kinetics of (a) $MgATP^{2-}$: with ▽———▽, 0 mM; □———□, 10.8 mM; ○———○, 20.3 mM salicylate; the 3-*P*-glycerate concentration was 2.0 mM. (b) 3-*P*-glycerate: with ▽———▽, 0 mM; □———□, 10.9 mM; ○———○, 22.3 mM salicylate; the total Mg^{2+} and ATP concentrations were 1 mM.

glycerate concentrations. Fig. 2 shows that the salicylate ion is a competitive inhibitor of $MgATP^{2-}$ and a non-competitive inhibitor of 3-*P*-glycerate. At corresponding conditions exactly the same kinetic patterns are obtained with the diiodosalicylate ion (Fig. 3).

Estimation of the inhibitor constants. The graphs representing the slopes, the vertical intercept (for non-competitive inhibition) or the apparent K_m value (for competitive inhibition) versus the concentration of the inhibitor, salicylate or diiodosalicylate ion ([I]), are non-linear (cf. Figs. 4a and 4b). So are also the experimental data of Fig. 1 when presented as the reciprocal of the activity versus the inhibitor concentration for all three inhibitors (cf. Fig. 4c). The

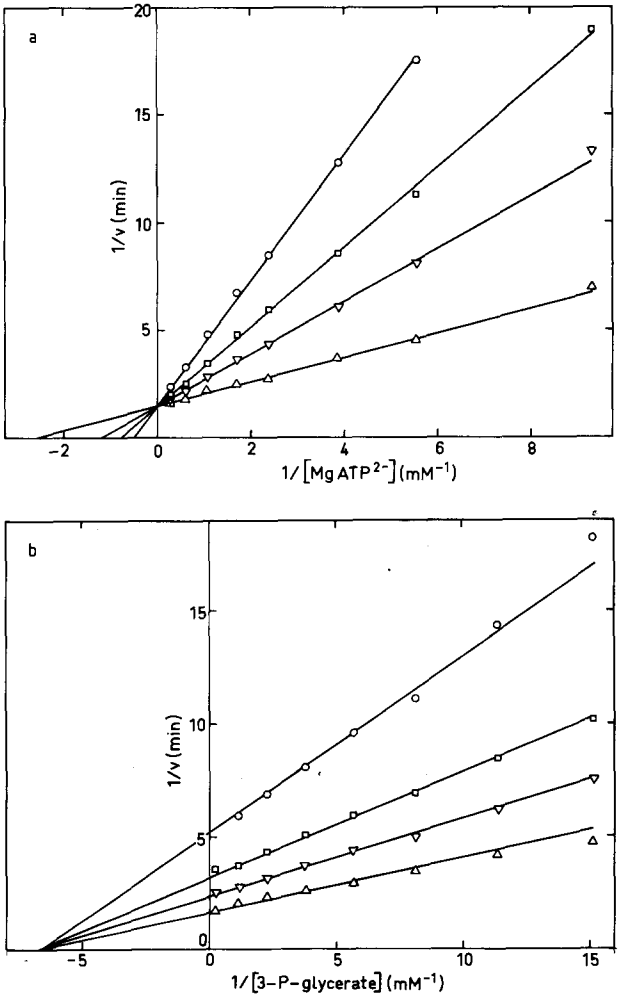

Fig. 3. Effects of the 2-hydroxy-3,5-diiodobenzoate concentration on the kinetics of (a) $MgATP^{2-}$: with △———△, 0 mM; ▽———▽, 0.073 mM; □———□, 0.14 mM; ○———○, 0.22 mM 2-hydroxy-3,5-diiodobenzoate; the 3-*P*-glycerate concentration was 2.0 mM. (b) 3-*P*-glycerate: with △———△, 0 mM; ▽———▽, 0.073 mM; □———□, 0.14 mM; ○———○, 0.28 mM 2-hydroxy-3,5-diiodobenzoate; the total Mg^{2+} and ATP concentrations were 1 mM.

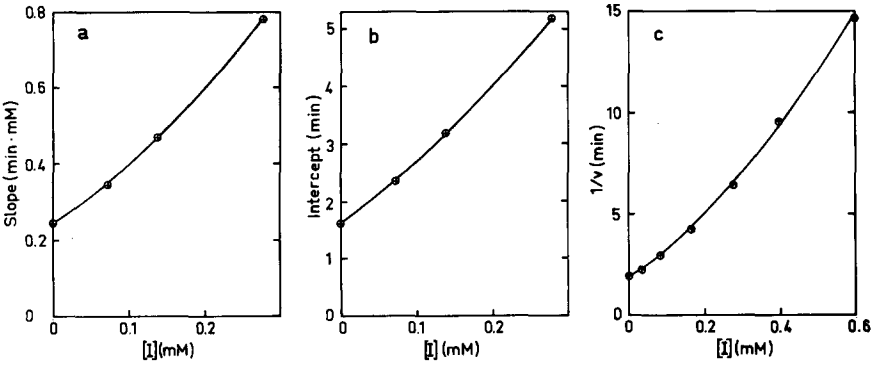

Fig. 4. Replotted kinetic data for estimation of K_i and K_i'. a and b data from Fig. 3b; and c data from Fig. 1. I means 2-hydroxy-3,5-diiodobenzoate. The curves are drawn after fitting the data to parabolas.

method of least squares, utilized for fitting the experimental points, showed that the inhibition kinetics with all the three inhibitors can be represented by parabolas. The correlation coefficient was 0.99 or higher. These results indicate two inhibitor binding sites in the enzyme molecule [11].

For determination of the inhibitor constants the estimated coefficients of the parabolic equations were identified with the corresponding ones obtained from the following rate equation for a parabolic inhibition:

$$v = \frac{V}{1 + \frac{K_B}{[B]} + \left(1 + \frac{[I]}{K_i} + \frac{[I]^2}{K_i K_i'}\right)\left(\frac{K_A K_B}{[A][B]} + \frac{K_A}{[A]}\right)} \quad (1)$$

The symbols represent: A, $MgATP^{2-}$; B, 3-*P*-glycerate; V, the initial reaction velocity at saturating conditions of both the substrates; K_i and K_i', inhibitor constants; and K_A and K_B, the Michaelis constants.

The calculated inhibitor constants are presented in Table I. The various types of plot give agreeing values of K_i and K_i' showing that the phosphoglycerate kinase reaction, inhibited by salicylates, can be described by Eqn. 1.

Kinetic parameters of $MgdATP^{2-}$ related to those of $MgATP^{2-}$. The initial velocity was studied at various concentrations of $MgATP^{2-}$, and $MgdATP^{2-}$,

TABLE I

K_i AND K_i', OBTAINED AFTER REPLOTTING DATA PRESENTED IN FIGS. 1—3 (cf. Fig. 4).

Variable substrate	Type of plot used for estimation	Salicylate		Iodosalicylate		Diiodosalicylate	
		K_i (mM)	K_i' (M)	K_i (mM)	K_i' (mM)	K_i(μM)	K_i' (mM)
$MgATP^{2-}$	Slope=f([I])	11	0.21			75	0.58
	$K_{m,app}$=f([I])	11	0.31			73	0.65
3-*P*-Glycerate	Slope=f([I])	9	0.13			65	0.59
	Intercept=f([I])	10	0.09			60	0.81
—	1/v=f([I])	11	0.18	0.60	13	49	0.87
Mean ± S.D.		10 ± 1	0.18 ± 0.08	0.60	13	64 ± 10	0.7 ± 0.1

respectively. Lineweaver-Burk plots show that K_m is 0.40 mM for $MgATP^{2-}$ and 3.45 mM for $MgdATP^{2-}$. V with $MgdATP^{2-}$ as a substrate is 1/4 of the V value obtained with $MgATP^{2-}$ (cf. also ref. 12).

Discussion

The inhibition patterns for salicylate and its diiododerivative are typical in the case the inhibitor binds to the catalytic $MgATP^{2-}$ site. The structural similarity of the monoiododerivative and the fact that the inhibited reaction for all the three salicylates can be described by the same equation indicate analogous binding of this inhibitor to the enzyme. For each iodine atom incorporated into the phenyl ring, the extent of inhibition increases drastically.

From X-ray diffraction data it was obvious that salicylate binding to alcohol dehydrogenase resembles the binding of the adenosine residue of NAD^+ [6]. The phenyl ring overlaps the position of its adenine part. C-5 and C-3 in the phenyl ring corresponding to N-15 and N-3 in the nucleotide residue [6]. The hydroxyl group of the salicylate ion forms a hydrogen bond with a carboxyl oxygen in the enzyme. In the corresponding enzyme · NAD^+ complex the same oxygen is hydrogen bonded to the 2′-OH of the adenosine ribose moiety [6]. For phosphoglycerate kinase the kinetic constants of $MgATP^{2-}$ and $MgdATP^{2-}$ indicate that the 2′-OH in the ribose moiety is important for the binding of the nucleotide substrate to the enzyme. Its interaction with the protein appears to cause a proper orientation of groups in the enzyme-substrate complex that are actively involved in the catalytic reaction (cf. refs. 13 and 14). If it is a carboxyl group that interacts with the 2′-OH of $MgATP^{2-}$ is still an open question, however (see ref. 14). Modification of a carboxyl group in yeast phosphoglycerate kinase inhibits the enzyme [15,16]. On the other hand, recent studies (Schierbeck, B. and Larsson-Raźnikiewicz, M., unpublished) have shown that the most reactive carboxyl group is not involved in $MgATP^{2-}$ binding to the catalytic centre (see ref. 19).

Multiple nucleotide binding sites exist in phosphoglycerate kinase [17—20] though the enzyme appears to have only one active centre [19]. The two salicylate binding sites appearing in our studies support earlier suggestion that salicylate can be used for studing nucelotide binding sites in proteins.

Sodium salicylate and aspirin (acetylsalicylic acid) are two common analgesic and anti-inflammatory drugs. In the body aspirin is hydrolysed to salicylic acid fairly rapidly [21]. This compound appears to cause metabolic effects [21]. The pharmacologically used concentrations of the drug are lower than the ones used in the present work. It is evident, however, that an overdose in a reversible way might affect the carbohydrate metabolism via the phosphoglycerate kinase reaction. Diiodosalicylate, which is such a strong inhibitor of the enzyme, is used as a nutritional iodine source for example to promote growth of hogs, cattle and poultry [22].

Acknowledgements

We want to thank Professor M. Zeppezauer for introducing us the problem and giving us the salicylate derivatives; Professor I. Lindqvist and Professor C.-I.

Brändén for generous support and helpful discussions; Professor B. Plapp for valuable comments during the preparation of the manuscript; Miss E. Knöppel for preparation of the enzyme; and Sven and Ebba-Christina Hagberg's foundation and the Swedish Council for Forestry and Agricultural Research for financial support.

References

1 Larsson-Raźnikiewicz, M. (1967) Biochim. Biophys. Acta 132, 33—40
2 Larsson-Raźnikiewicz, M. and Arvidsson, L. (1971) Eur. J. Biochem. 22, 506—512
3 Larsson-Raźnikiewicz, M. and Schierbeck, B. (1974) Biochem. Biophys. Res. Commun. 57, 627—634
4 Bryant, T.N., Watson, H.C. and Wendell, P.L. (1974) Nature 247, 14—17
5 Blake, C.C.F. and Evans, P.R. (1974) J. Mol. Biol. 84, 585—601
6 Einarsson, R., Eklund, H., Zeppezauer, E., Boiwe, T. and Brändén, C.-I. (1974) Eur. J. Biochem. 49, 41—47
7 Arvidsson, L., Schierbeck, B. and Larsson-Raźnikiewicz, M. (1976) Acta Chem. Scand. B 30, 228—234
8 Larsson-Raźnikiewicz, M. (1964) Biochim. Biophys. Acta 85, 60—68
9 Larsson-Raźnikiewicz, M. (1972) Eur. J. Biochem. 30, 579—583
10 Bücher, Th. (1947) Biochim. Biophys. Acta 1, 292—314
11 Cleland, W.W. (1970) in The Enzymes (Boyer, P.D., ed.), Vol. II, pp. 1—65, Academic Press, New York
12 Krietsch, W.K.G. and Bücher, Th. (1970) Eur. J. Biochem. 17, 568—580
13 Suhadolnik, R.J., Lennon, M.B., Uematsu, T., Monahan, J.E. and Baur, R. (1977) J. Biol. Chem. 252, 4125—4133
14 Pai, E.F., Sachsenheimer, W., Schirmer, R.H. and Schultz, G.E. (1977) J. Mol. Biol. 114, 37—45
15 Brevet, A., Roustan, C., Desvages, G., Pradel, L.-A. and van Thoai, N. (1973) Eur. J. Biochem. 39, 141—147
16 Brake, A.J. and Weber, B.H. (1974) J. Biol. Chem. 249, 5452—5457
17 Larsson-Raźnikiewicz, M. (1973) Arch. Biochem. Biophys. 158, 754—762
18 Larsson-Raźnikiewicz, M. and Schierbeck, B. (1977) Biochim. Biophys. Acta 481, 283—287
19 Larsson-Raźnikiewicz, M. and Schierbeck, B. (1977) Biochem. Soc. Trans. 5, 770—771
20 Tanswell, P., Westhead, E.W. and Williams, R.J.P. (1974) FEBS Lett. 48, 60—63
21 Goth. A. (1972) Medical Pharmacology, 6th edn., pp. 316—321, The C.V. Mosby Company, Saint Louis
22 The Merck Index (Windholz, M., Budavari, S., Stroumtsos, L.Y. and Noether Fertig, M., eds.), 9th edn. (1976) No. 3174, Merck and Co., Inc., Rahway, N.J.