Differences between collagen hydroxylases and 2-oxoglutarate dehydrogenase in their inhibition by structural analogues of 2-oxoglutarate

Kari MAJAMAA,* Taina M. TURPEENNIEMI-HUJANEN,* Pirjo LATIPÄÄ,*
Volkmar GÜNZLER,† Hartmut M. HANAUSKE-ABEL,† Ilmo E. HASSINEN* and
Kari I. KIVIRIKKO*†

* Collagen Research Unit and Department of Medical Biochemistry, University of Oulu, SF-90220 Oulu, Finland, and † Prenatal Diagnosis Laboratory, Department of Pediatrics, Johannes Gutenberg University, D-6500 Mainz, Federal Republic of Germany

(Received 7 January 1985/5 March 1985; accepted 18 March 1985)

Inhibition of lysyl hydroxylase and prolyl 3-hydroxylase was studied with 23 selected aromatic and aliphatic structural analogues of 2-oxoglutarate and the results were compared with those previously reported for prolyl 4-hydroxylase. All the compounds inhibited the hydroxylases competitively with respect to 2-oxoglutarate and non-competitively with respect to Fe^{2+} and the peptide substrate. The inhibition patterns for the three collagen hydroxylases were basically similar, but certain differences in detail emerged. One systematic difference was that lysyl hydroxylase had a higher K_i for almost all the compounds than had the two prolyl hydroxylases. Another interesting difference was that pyridine-2,4-dicarboxylate was the most potent inhibitor of lysyl hydroxylase and prolyl 3-hydroxylase, with K_i values of $50\text{ }\mu\text{M}$ and $3\text{ }\mu\text{M}$ respectively, whereas pyridine-2,5-dicarboxylate was the most potent inhibitor of prolyl 4-hydroxylase. These and other data suggest that the three collagen hydroxylases have similar but not identical 2-oxoglutarate-binding sites. Pyridine-2,4-dicarboxylate and pyridine-2,5-dicarboxylate and their corresponding benzene derivatives were also found to inhibit 2-oxoglutarate dehydrogenase, but with this enzyme, unlike the collagen hydroxylases, no distinct difference in the K_i values was found between the corresponding pyridine and benzene derivatives. This demonstrates the importance of the metal ion for the binding of various compounds at the 2-oxoglutarate-binding site of the collagen hydroxylases. 2-Oxadipate was shown to replace 2-oxoglutarate in the lysyl hydroxylase and 2-oxoglutarate dehydrogenase reactions, as has previously been reported for prolyl 4-hydroxylase, whereas no other 2-oxo acid tested had any co-substrate activity. The 2-oxoglutarate-binding site of these enzymes is thus flexible to a certain degree, as it can accommodate molecules of different shapes and volumes. On the basis of the present data pyridine-2,5-dicarboxylate seems to be a quite specific inhibitor of prolyl 4-hydroxylase, the K_i for 2-oxoglutarate dehydrogenase being about 4000-fold higher.

Prolyl 4-hydroxylase (EC 1.14.11.2), prolyl 3-hydroxylase (EC 1.14.11.7) and lysyl hydroxylase (EC 1.14.11.4) catalyse the formation of 4-hydroxyproline, 3-hydroxyproline and hydroxylsine in collagens and other proteins with collagen-like amino acid sequences. All three enzymes hydroxylate proline or lysine residues in peptide linkages, and they all require Fe^{2+} , 2-oxoglutarate, O_2 and

ascorbate. 2-Oxoglutarate is stoichiometrically decarboxylated during hydroxylation, one atom of the O_2 molecule being incorporated into the succinate and the other being incorporated into the hydroxy group (for reviews see Cardinale & Udenfriend, 1974; Prockop *et al.*, 1976; Kivirikko & Myllylä, 1980).

The mechanisms of the prolyl 4-hydroxylase and lysyl hydroxylase reactions have been elucidated in extensive kinetic studies (Tuderman *et al.*, 1977; Myllylä *et al.*, 1977, 1978; Puistola *et al.*, 1980a,b).

† To whom correspondence should be addressed.

These and other data (de Jong *et al.*, 1982; Nietfeld *et al.*, 1982; Myllylä *et al.*, 1984) are consistent with an ordered binding of Fe^{2+} , 2-oxoglutarate, O_2 and the peptide substrate to the enzyme followed by an ordered release of the products. A stereochemical mechanism that agrees with the kinetic data and involves a pocket model for the active site has been suggested for prolyl 4-hydroxylase (Hanauske-Abel & Günzler, 1982).

The structure and function of the 2-oxoglutarate-binding site of prolyl 4-hydroxylase has been studied by assaying the inhibitory potential of 24 selected aliphatic and aromatic compounds (Majamaa *et al.*, 1984). In accordance with the stereochemical model (Hanauske-Abel & Günzler, 1982), the data suggested that the 2-oxoglutarate-binding site can be divided into three distinct subsites (Majamaa *et al.*, 1984). Subsite I is probably a positively charged side chain of the enzyme that binds domain I, the C-5 carboxy group of the 2-oxoglutarate. This brings domain II, the C-1-C-2 moiety of the co-substrate, into the sterically correct position with respect to subsite II, two *cis*-positioned co-ordination sites of the enzyme-bound Fe^{2+} ion. Subsite III probably involves a hydrophobic binding site that interacts with domain III, the C-3-C-4 region of the 2-oxoglutarate. Chelation of the enzyme-bound iron by the C-1-C-2 moiety of the co-substrate is probably a crucial event for producing a decarboxylatable complex (Hanauske-Abel & Günzler, 1982; Majamaa *et al.*, 1984).

The three collagen hydroxylases have small but distinct differences in their Michaelis constants for 2-oxoglutarate, the highest value having been reported for lysyl hydroxylase (Kivirikko & Myllylä, 1980). This suggests that there may be slight structural differences in the 2-oxoglutarate-binding sites of the three enzymes. The purpose of the present work was to study the inhibition of lysyl hydroxylase and prolyl 3-hydroxylase by a number of compounds competing with 2-oxoglutarate and to compare these data with those previously reported for prolyl 4-hydroxylase (Majamaa *et al.*, 1984). Some of the aromatic compounds were also tested for their ability to inhibit 2-oxoglutarate dehydrogenase (EC 1.2.4.2), this latter enzyme differing distinctly from the collagen hydroxylases with respect to its other co-substrates and its reaction mechanism (Reed, 1974). These latter experiments studied in particular the importance of chelation of the enzyme-bound iron by the C-1-C-2 moiety of the 2-oxoglutarate for the reactions catalysed by the collagen hydroxylases. Special emphasis was laid on the question of whether collagen hydroxylases and 2-oxoglutarate dehydrogenase have such distinct differences at their 2-oxoglutarate-binding sites that would make it

possible to design specific inhibitors of collagen hydroxylases.

Experimental

Materials

Lysyl hydroxylase was purified from an ammonium sulphate fraction of chick-embryo extract by affinity chromatography on concanavalin A-agarose, affinity chromatography on collagen linked to agarose and gel filtration (Turpeenniemi-Hujanen *et al.*, 1980). Prolyl 3-hydroxylase was partially purified from an ammonium sulphate fraction of chick-embryo extract by affinity chromatography on collagen linked to agarose and gel filtration (Tryggvason *et al.*, 1979). 2-Oxoglutarate dehydrogenase was partially purified from pig heart as described previously (Sanadi, 1969). After ammonium acetate fractionation, the dialysed preparation was purified of small- M_r impurities by ultracentrifugation at 140000g for 4h.

The synthetic peptide substrate L-I, with the structure Ala-Arg-Gly-Ile-Lys-Gly-Ile-Arg-Gly-Phe-Ser-Gly (Kivirikko *et al.*, 1972), was purchased from the Protein Research Foundation (Minoh, Osaka, Japan), and the [^3H]lysine-labelled and [2,3- ^3H]proline-labelled procollagen substrates were prepared from freshly isolated chick-embryo tendon cells (Kivirikko & Myllylä, 1982). The [2,3- ^3H]proline-labelled procollagen was fully 4-hydroxylated with pure chick prolyl 4-hydroxylase before use (Kivirikko & Myllylä, 1982). 2-Oxo[1- ^{14}C]glutarate was purchased from New England Nuclear (Boston, MA, U.S.A.) and was adjusted to a specific radioactivity of 100000 d.p.m./0.1 μmol by mixing with the unlabelled compound (Fluka, Buchs, Switzerland).

The sources of the aliphatic and aromatic compounds tested as enzyme inhibitors were as reported previously (Majamaa *et al.*, 1984).

Methods

The reaction with lysyl hydroxylase was carried out in a final volume of 1.0 ml, which contained 0.2–2 μg of the enzyme, 0.5 mg of L-I/ml as substrate, 0.05 mM- FeSO_4 , 1.0 mM-ascorbate, 0.1 mg of catalase (Sigma Chemical Co., St. Louis, MO, U.S.A.)/ml, 0.1 mM-dithiothreitol, 1.0 mg of bovine serum albumin (Sigma Chemical Co.)/ml, 50 mM-Tris/HCl buffer adjusted to pH 7.8 at 25°C, and 0.1 mM-2-oxo[1- ^{14}C]glutarate (100000 d.p.m.) (Kivirikko & Myllylä, 1982). The peptide substrate was denatured by heating at 100°C for 10 min and quenched at 0°C immediately before addition to the incubation mixture. The samples were incubated at 37°C for 30 min, and the $^{14}\text{CO}_2$ formed was then trapped and its radioactivity counted (Kivirikko & Myllylä, 1982). The inhibitors to be

tested were added at two to four constant concentrations, and the concentration of either Fe^{2+} or 2-oxoglutarate was varied. All stock solutions of the inhibitors were carefully neutralized and buffered before addition to the incubation mixture. The K_i values were calculated from the secondary transforms of the primary plots (see Segel, 1975; Majamaa *et al.*, 1984), the value shown being the mean of those obtained when the Fe^{2+} or 2-oxoglutarate concentration was varied in four to eight experiments for each inhibitor.

Prolyl 3-hydroxylase activity was assayed by a method based on the release of $^3\text{H}_2\text{O}$ during 3-hydroxylation of biologically prepared [2,3- ^3H]proline-labelled polypeptide substrate, in which all the proline residues recognized by prolyl 4-hydroxylase had been converted into 4-hydroxyproline residues (Kivirikko & Myllylä, 1982). The reaction was carried out in a final volume of 1.5 ml with the additions mentioned above, except that [2,3- ^3H]proline-labelled protocollagen (1 000 000 d.p.m.) was used as the substrate, the concentration of unlabelled 2-oxoglutarate was 15 μM and the concentrations of ascorbate, catalase and bovine serum albumin were 2.0 mM, 0.2 mg/ml and 2.0 mg/ml respectively. The samples were incubated at 37°C for 30 min, after which 0.5 ml of 10% (w/v) trichloroacetic acid was added to stop the reaction. The whole incubation mixture was then distilled, and 1.6 ml of $^3\text{H}_2\text{O}$ was counted for radioactivity (Kivirikko & Myllylä, 1982). The plots of $1/v$ versus various inhibitor concentrations yielded a straight line with an x intercept equal to $-K_i(1+[S]/K_m)$, from which the K_i could be calculated once the parameters $[S]$ and the K_m for 2-oxoglutarate were known (Segel, 1975). This method was preferred in the case of prolyl 3-hydroxylase because of the difficulties encountered in preparing large amounts of this enzyme and its substrate.

The activity of 2-oxoglutarate dehydrogenase was assayed by measuring the formation of NADH spectrophotometrically at 340 nm minus 385 nm (Aminco DW-2) in 40 mM-arsenate buffer adjusted to pH 7.2 and containing 1.5 mM- MgCl_2 , 0.5 mM-2-mercaptoethanol, 0.4 mM-NAD $^+$ and 7.5 μM -CoA (Tubbs & Garland, 1969).

The ability of selected aliphatic 2-oxo acids to support the hydroxylation of a peptide substrate by replacing 2-oxoglutarate as the co-substrate was studied with a [^3H]lysine-labelled or a [2,3- ^3H]proline-labelled protocollagen (Kivirikko & Myllylä, 1982). Similar experiments were carried out with 2-oxoglutarate dehydrogenase, where the formation of NADH was measured in the presence of an aliphatic 2-oxo acid. The purity of the aliphatic 2-oxo acids was tested by h.p.l.c. as described previously (Majamaa *et al.*, 1984).

The lines of best fit for the primary plots and their secondary transforms were calculated by using the method of least squares (see Segel, 1975).

Results and discussion

Inhibition of lysyl hydroxylase and prolyl 3-hydroxylase by aliphatic and aromatic analogues of 2-oxoglutarate

The K_m of 2-oxoglutarate is about 20 μM in the prolyl 4-hydroxylase reaction (Myllylä *et al.*, 1977; Majamaa *et al.*, 1984) and about 100 μM in the lysyl hydroxylase reaction (Puistola *et al.*, 1980a). A K_m of 3 μM has been reported in the prolyl 3-hydroxylase reaction, on the basis of data in which the concentration of only 2-oxoglutarate was varied (Tryggvason *et al.*, 1979). Since the K_m values for this co-substrate were needed in order to interpret the data obtained here, the K_m was now determined for prolyl 3-hydroxylase by using initial-velocity data similar to those used in the cases of the two other hydroxylases, and was found to be about 5 μM .

Eleven aliphatic (Fig. 1) and 12 aromatic (Fig. 2) compounds known to inhibit prolyl 4-hydroxylase (Majamaa *et al.*, 1984) were tested as possible inhibitors of the two other 2-oxoglutarate dioxygenases involved in collagen biosynthesis. All these compounds were found to inhibit lysyl hydroxylase and prolyl 3-hydroxylase competitively with respect to 2-oxoglutarate and non-competitively with respect to Fe^{2+} and the peptide substrate (results not shown). The data suggest that the active site of each of the three enzymes can be divided into three subsites, as previously reported for prolyl 4-hydroxylase (Majamaa *et al.*, 1984). Every analogue with a carboxy group binding to subsite I was a more potent inhibitor than was the corresponding compound without this domain (Table 1). Similarly, every analogue with an intact domain II, the moiety chelating the enzyme-bound iron (see Figs. 1 and 2 for the chelating capacity), was a more potent inhibitor than the corresponding compound lacking the chelating capacity (Table 1). Subsite III appeared to have the weakest influence on the binding, but present and previous data demonstrate that a hydrophobic area between domains I and II of the analogues increases the inhibitory ability.

Lysyl hydroxylase was found to have a higher K_i for almost all the compounds than had the two prolyl hydroxylases (Table 1). This difference can probably be related in part to the high K_m for 2-oxoglutarate in the case of lysyl hydroxylase. Differences in the K_m values for this co-substrate are clearly not the only factor affecting the K_i values, however, as prolyl 3-hydroxylase has the lowest K_m for 2-oxoglutarate even though in many

No. of C atoms	R ¹	Compound
4	-CH ₂ -	Oxaloacetate
5	-CH ₂ CH ₂ -	2-Oxoglutarate
6	-CH ₂ CH ₂ CH ₂ -	2-Oxoadipate

No. of C atoms	R ²	Compound
3	H-	Pyruvate
4	CH ₃ -	2-Oxobutyrate
5	CH ₃ CH ₂ -	2-Oxovalerate

No. of C atoms	R ³	Compound
3	-	Malonate
4	-CH ₂ -	Succinate
5	-CH ₂ CH ₂ -	Glutarate
5	-CH ₂ C(O)-	3-Oxoglutarate
6	-CH ₂ CH ₂ CH ₂ -	Adipate

Laevulinate

Fig. 1. Structures of the aliphatic compounds

The Tables under the individual structural formulae show the total number of carbon atoms in the compound, the moiety represented by R¹, R² and R³ respectively, and the name of the compound. Laevulinate is given separately. The ability of the aliphatic 2-oxo carboxylic acids to chelate iron in the manner suggested for 2-oxoglutarate (Hanauske-Abel & Günzler, 1982) is indicated by arrows.

cases it had higher K_i values than prolyl 4-hydroxylase. An additional very distinct difference between the three hydroxylases was found in the optimal orientation of domain I with respect to domain II in the aromatic compounds (Table 1). With lysyl hydroxylase and prolyl 3-hydroxylase pyridine-2,4-dicarboxylate was definitely a more potent inhibitor than was pyridine-2,5-dicarboxyl-

ate, as is to be expected by analogy with the staggered conformation of 2-oxoglutarate. With prolyl 4-hydroxylase, however, pyridine-2,5-dicarboxylate was the more potent inhibitor. The optimal location of domain I at ring position 4 with lysyl hydroxylase also held for compounds with a disrupted domain II (pyridine-3,4-dicarboxylate compared with pyridine-3,5-dicarboxylate, and benzene-1,3-dicarboxylate compared with benzene-1,4-dicarboxylate in Table 1), whereas the pattern obtained with prolyl 3-hydroxylase was now closer to that reported for prolyl 4-hydroxylase (Table 1). These findings suggest a distinct difference between prolyl 4-hydroxylase and the two other hydroxylases either in the structure of the binding site on the enzyme or in the conformation of 2-oxoglutarate at this binding site.

Inhibition of 2-oxoglutarate dehydrogenase by selected aromatic analogues of 2-oxoglutarate

The effective inhibition of collagen hydroxylases by pyridine derivatives as compared with inhibition by benzene derivatives lends strong support for the hypothesis that an intact iron-chelating group is a critical domain for the binding of 2-oxoglutarate analogues to the active sites of collagen hydroxylases (Hanauske-Abel & Günzler, 1982). 2-Oxoglutarate dehydrogenase catalyses the decarboxylation of 2-oxoglutarate by a mechanism that is distinctly different from that involved in the collagen hydroxylase reactions in that its mechanism does not involve a metal ion (Reed, 1974). The above hypothesis therefore predicts that with 2-oxoglutarate dehydrogenase there should be no marked differences between the two groups of compounds. Therefore the metal-chelating pyridine-2,4-dicarboxylate and pyridine-2,5-dicarboxylate were compared as possible inhibitors with the corresponding non-chelating benzene derivatives benzene-1,3-dicarboxylate and benzene-1,4-dicarboxylate (see the legend to Fig. 2 for differences in nomenclature).

All four compounds were competitive inhibitors with respect to 2-oxoglutarate. Unlike in the cases of the three collagen hydroxylases, the pyridine derivatives were not significantly more potent inhibitors of 2-oxoglutarate dehydrogenase than were the benzene derivatives (Table 2). As with lysyl hydroxylase and prolyl 3-hydroxylase, pyridine-2,4-dicarboxylate was a more potent inhibitor than was pyridine-2,5-dicarboxylate (Table 2).

Replacement of 2-oxoglutarate by aliphatic 2-oxo acids in the decarboxylation reactions catalysed by lysyl hydroxylase, prolyl 3-hydroxylase and 2-oxoglutarate dehydrogenase

Aliphatic compounds with an intact domain II are theoretically prone to decarboxylation in the

Substituents at ring carbons					Compound
R ²	R ³	R ⁴	R ⁵	R ⁶	
CO ₂ H	CO ₂ H	H	H	H	Pyridine-2,3-dicarboxylate
CO ₂ H	H	CO ₂ H	H	H	Pyridine-2,4-dicarboxylate
CO ₂ H	H	H	CO ₂ H	H	Pyridine-2,5-dicarboxylate
H	CO ₂ H	CO ₂ H	H	H	Pyridine-3,4-dicarboxylate
H	CO ₂ H	H	CO ₂ H	H	Pyridine-3,5-dicarboxylate
CO ₂ H	H	H	H	H	Pyridine-2-carboxylate
H	CO ₂ H	H	H	H	Pyridine-3-carboxylate
H	H	CO ₂ H	H	H	Pyridine-4-carboxylate

Substituents at ring carbons					Compound
R ²	R ³	R ⁴	R ⁵	R ⁶	
CO ₂ H	H	H	H	H	Benzene-1,2-dicarboxylate
H	CO ₂ H	H	H	H	Benzene-1,3-dicarboxylate
H	H	CO ₂ H	H	H	Benzene-1,4-dicarboxylate
H	H	H	H	H	Benzoate

Fig. 2. Structures of the aromatic compounds

The Tables under the individual structural formulae show the substituents at the ring carbon atoms. The arrows indicate the ability of pyridine-2-carboxylate and its derivatives to chelate iron in the manner suggested for 2-oxoglutarate. The numbering of the benzene derivatives differs from that of the pyridine derivatives because of the absence of the heteroatom in the ring.

catalytic cycle of the 2-oxoglutarate dioxygenases and 2-oxoglutarate dehydrogenase. Nevertheless no formation of hydroxy[³H]lysine was found in experiments with [³H]lysine-labelled protocollagen when oxaloacetate, 2-oxovalerate, 2-oxobutyrate or 3-oxoglutarate was used to replace 2-oxoglutarate (results not shown). A similar result was previously reported when these compounds were tested for their ability to replace 2-oxoglutarate in the prolyl 4-hydroxylase reaction (Majamaa *et al.*, 1984), and was also obtained here when these compounds were used in the prolyl 3-hydroxylase and 2-oxoglutarate dehydrogenase reactions (results not shown). 2-Oxadipate was found to support the hydroxylation of lysine residues by lysyl hydroxylase and the formation of NADH by 2-oxoglutarate dehydrogenase, however (Fig. 3). These data agree with those reported for 2-oxoadipate in the prolyl 4-hydroxylase reaction

(Majamaa *et al.*, 1984), except in that the maximal velocity of this enzyme reaction was the same with 2-oxoadipate and with 2-oxoglutarate, whereas the maximal velocities of the lysyl hydroxylase and 2-oxoglutarate dehydrogenase reactions were lower with 2-oxoadipate. Prolyl 3-hydroxylase did not catalyse any hydroxylation with 2-oxoadipate, but, owing to the lower degree of purification of this enzyme compared with the other two collagen hydroxylases, a very low reaction rate cannot be completely excluded. The ability of two of the collagen hydroxylases and 2-oxoglutarate dehydrogenase to utilize 2-oxoadipate but not oxaloacetate point to the requirement of a certain minimum length of the co-substrate and also to a considerable protein flexibility at their 2-oxoglutarate-binding sites, as this site can decarboxylate a distinctly longer molecule than the ordinary co-substrate.

Table 1. Inhibition constants for the aliphatic and aromatic compounds tested in the reactions of the three collagen hydroxylases
Previously reported values for prolyl 4-hydroxylase (Majamaa *et al.*, 1984) are shown for comparison.

Compound	K_i (mM)		
	Lysyl hydroxylase	Prolyl 3-hydroxylase	Prolyl 4-hydroxylase
Oxaloacetate	0.5	0.5	0.1
2-Oxoadipate	4.8	1.0	0.9
Pyruvate	20	4.2	15
2-Oxobutyrate	> 20	9.9	7.6
2-Oxovalerate	10	> 15	9.4
Malonate	10	7.4	5.9
Succinate	10	0.8	0.4
Glutarate	15	3.6	4.2
3-Oxoglutarate	8.6	2.8	0.8
Adipate	15	6.0	> 15
Laevulinate	> 20	8.0	> 15
Pyridine-2,3-dicarboxylate	1.0	0.7	0.21
Pyridine-2,4-dicarboxylate	0.05	0.003	0.002
Pyridine-2,5-dicarboxylate	0.15	0.015	0.0008
Pyridine-3,4-dicarboxylate	2.8	2.0	2.2
Pyridine-3,5-dicarboxylate	4.0	0.5	0.9
Pyridine-2-carboxylate	0.1	0.2	0.025
Pyridine-3-carboxylate	1.7	0.5	0.5
Pyridine-4-carboxylate	17	0.3	0.9
Benzene-1,2-dicarboxylate	> 20	1.3	> 10
Benzene-1,3-dicarboxylate	1.8	0.5	5.8
Benzene-1,4-dicarboxylate	4.3	0.5	3.3
Benzoate	4.0	3.1	9.9

Fig. 3. Reaction velocity in lysyl hydroxylase (a) and 2-oxoglutarate dehydrogenase reaction (b) with 2-oxoglutarate or 2-oxoadipate as the co-substrate
Lysyl hydroxylase was incubated with the [³H]lysine-labelled protocollagen and various concentrations of either 2-oxoglutarate (○) or 2-oxoadipate (●). Hydroxyl[³H]lysine was assayed as described previously (Kivirikko & Myllylä, 1982). 2-Oxoglutarate dehydrogenase was incubated under the conditions described in the Experimental section and the formation of NADH was measured in the presence of various concentrations of either 2-oxoglutarate (○) or 2-oxoadipate (●).

Table 2. Inhibition constants for selected aromatic compounds used in the 2-oxoglutarate dehydrogenase reaction

Compound	K_i (mM)
Pyridine-2,4-dicarboxylate	0.4
Pyridine-2,5-dicarboxylate	3.0
Benzene-1,3-dicarboxylate	0.5
Benzene-1,4-dicarboxylate	1.1

Significance of the data for design of inhibitors for collagen hydroxylases

Pyridine-2,4-dicarboxylate and pyridine-2,5-dicarboxylate seem promising experimental tools for the inhibition either of all three collagen hydroxylases or only of prolyl 4-hydroxylase, depending on the concentrations of these compounds used, and the latter would seem especially attractive for the selective inhibition of prolyl 4-hydroxylase. The K_i for this enzyme is only $0.8\mu\text{M}$, whereas the K_i values for prolyl 3-hydroxylase, lysyl hydroxylase and 2-oxoglutarate dehydrogenase are about 20-fold, 200-fold and 4000-fold higher respectively. The low membrane permeability of pyridine-2,5-dicarboxylate will probably make this compound unsuitable for inhibition of the prolyl 4-hydroxylase reaction, and hence for inhibition of the accumulation of extracellular collagen fibres in cultured cells or *in vivo*, but further alterations in the structure might help to overcome this problem. The high specificity of this compound with respect to prolyl 4-hydroxylase suggests that attempts to modify its structure are advisable.

This work was supported in part by grants from the Medical Research Council of the Academy of Finland, the E. Galois Foundation and Deutsche Forschungsgemeinschaft (Grant Ha 1293/1-1). We gratefully acknowledge the expert technical assistance of Mrs. Maija Seppänen, Miss Helmi Konola and Mrs. Raija Pietilä.

References

- Cardinale, G. & Udenfriend, S. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol.* **41**, 245–300
- de Jong, L., Albracht, S. P. J. & Kemp, A. (1982) *Biochim. Biophys. Acta* **704**, 326–332
- Hanauske-Abel, H. M. & Günzler, V. (1982) *J. Theor. Biol.* **94**, 421–455
- Kivirikko, K. I. & Myllylä, R. (1980) in *The Enzymology of Post-translational Modification of Proteins* (Freedman, R. B. & Hawkins, H. C., eds.), pp. 53–104, Academic Press, London
- Kivirikko, K. I. & Myllylä, R. (1982) *Methods Enzymol.* **82A**, 245–304
- Kivirikko, K. I., Shudo, K., Sakakibara, S. & Prockop, D. J. (1972) *Biochemistry* **11**, 122–129
- Majamaa, K., Hanauske-Abel, H. M., Günzler, V. & Kivirikko, K. I. (1984) *Eur. J. Biochem.* **138**, 239–245
- Myllylä, R., Tuderman, L. & Kivirikko, K. I. (1977) *Eur. J. Biochem.* **80**, 349–357
- Myllylä, R., Kuutti-Savolainen, E.-R. & Kivirikko, K. I. (1978) *Biochem. Biophys. Res. Commun.* **83**, 441–448
- Myllylä, R., Majamaa, K., Günzler, V., Hanauske-Abel, H. M. & Kivirikko, K. I. (1984) *J. Biol. Chem.* **259**, 5403–5405
- Nietfeld, J. J., de Jong, L. & Kemp, A. (1982) *Biochim. Biophys. Acta* **704**, 321–325
- Prockop, D. J., Berg, R. A., Kivirikko, K. I. & Uitto, J. (1976) in *Biochemistry of Collagen* (Ramachandran, G. N. & Reddi, A. H., eds.), pp. 163–273, Plenum Press, New York
- Puistola, U., Turpeenniemi-Hujanen, T. M., Myllylä, R. & Kivirikko, K. I. (1980a) *Biochim. Biophys. Acta* **611**, 40–50
- Puistola, U., Turpeenniemi-Hujanen, T. M., Myllylä, R. & Kivirikko, K. I. (1980b) *Biochim. Biophys. Acta* **611**, 51–60
- Reed, L. J. (1974) *Acc. Chem. Res.* **7**, 40–46
- Sanadi, D. R. (1969) *Methods Enzymol.* **13**, 52–55
- Segel, I. H. (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, 1st edn., pp. 1–957, John Wiley and Sons, New York
- Tryggvason, K., Majamaa, K., Risteli, J. & Kivirikko, K. I. (1979) *Biochem. J.* **183**, 303–307
- Tubbs, P. K. & Garland, P. B. (1969) *Methods Enzymol.* **13**, 535–551
- Tuderman, L., Myllylä, R. & Kivirikko, K. I. (1977) *Eur. J. Biochem.* **80**, 341–348
- Turpeenniemi-Hujanen, T. M., Puistola, U. & Kivirikko, K. I. (1980) *Biochem. J.* **189**, 247–253