Immunological characterization of lysyl hydroxylase, an enzyme of collagen synthesis

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Antibodies to pure lysyl hydroxylase from whole chick embryos were prepared in rabbits and used for immunological characterization of this enzyme of collagen biosynthesis. In double immunodiffusion a single precipitation line was seen between the antiserum and crude or pure chick-embryo lysyl hydroxylase. The antiserum effectively inhibited chick-embryo lysyl hydroxylase activity, whether measured with the biologically prepared protocollagen substrate or a synthetic peptide consisting of only 12 amino acids. This suggests that the antigenic determinant was located near the active site of the enzyme molecule. Essentially identical amounts of the antiserum were required for 40% inhibition of the same amount of lysyl hydroxylase activity units from different chick-embryo tissues synthesizing various genetically distinct collagen types. In double immunodiffusion a single precipitation line of complete identity was found between the antiserum and the purified enzyme from whole chick embryos and the crude enzymes from chick-embryo tendon, cartilage and kidneys. These results do not support the hypothesis that lysyl hydroxylase has collagen-type-specific or tissue-specific isoenzymes with markedly different specific activities or immunological properties. The antibodies to chick-embryo lysyl hydroxylase showed a considerable degree of species specificity when examined either by activity-inhibition assay or by double immunodiffusion. Nevertheless, a distinct, although weak, cross-reactivity was found between the chick-embryo enzyme and those from all mammalian tissues tested. The antiserum showed no cross-reactivity against prolyl 3-hydroxylase, hydroxylysyl galactosyl-transferase or galactosylhydroxylysyl glucosyltransferase in activity-inhibition assays, whereas a distinct cross-reactivity was found against prolyl 4-hydroxylase. Furthermore, antiserum to pure prolyl 4-hydroxylase inhibited lysyl hydroxylase activity. These findings suggest that there are structural similarities between these two enzymes, possibly close to or at their active sites.

Lysyl hydroxylase (peptidyl-lysine, 2-oxoglutarate:oxygen 5-oxidoreductase, EC 1.14.11.4) catalyses the formation of hydroxylysine in collagen by the hydroxylation of certain lysyl residues in peptide linkages (for a recent review, see Kivirikko & Myllylä, 1980). The enzyme has been isolated from chick embryos as a homogeneous protein (Turpeenniemi-Hujanen *et al.*, 1980) and found to be a glycoprotein, having a molecular weight of about 200 000 in gel filtration and consisting of only one type of subunit, with molecular weight 85 000 (Ryhänen, 1976; Turpeenniemi *et al.*, 1977; Turpeenniemi-Hujanen *et al.*, 1980).

The hydroxy groups of hydroxylysyl residues serve as sites of attachment for carbohydrate units, in the form of either the monosaccharide galactose

or the disaccharide glucosylgalactose (see Kivirikko & Myllylä, 1979), and they are essential for the stability of the intermolecular collagen cross-links (see Bornstein & Traub, 1979; Eyre, 1980). There is a wide variation in the extent lysyl hydroxylation between genetically distinct collagen types, the highest extent being found in type IV collagen from basement membranes and the lowest in type I collagen, and even within the same collagen type from different tissues (see Bornstein & Traub, 1979; Prockop *et al.*, 1979*a,b*; Kivirikko & Myllylä, 1980). The deficiency in lysyl hydroxylase activity present in the type VI variant of the Ehlers–Danlos syndrome leads to hydroxylysine deficiency, which likewise shows a wide variation in extent between various collagen types and tissues (Pinnell *et al.*,

1972; Krane, 1980). The mutant lysyl hydroxylase from fibroblasts of a patient with this disease has been reported to differ from the enzyme in normal fibroblasts in that its residual activity is preferentially directed towards type IV collagen (Risteli *et al.*, 1980). All these observations suggest that lysyl hydroxylase might have collagen-type-specific or tissue-specific isoenzymes, but there are no direct data to support this possibility.

In the work presented here antiserum was prepared in rabbits against pure lysyl hydroxylase from whole chick embryos, and inhibition by this antiserum was tested in a reaction mixture with different substrates. Special emphasis was laid on the question of whether there are immunologically different enzyme types in various species and different chick-embryo tissues. Experiments were also performed to ascertain whether the chick-embryo enzymes catalysing other intracellular post-translational modifications in collagen biosynthesis show any cross-reactivity with the antiserum to chick lysyl hydroxylase.

Experimental

Materials

[¹⁴C]Lysine and [¹⁴C]proline were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). UDP-D-[¹⁴C]glucose, UDP-D-[¹⁴C]-galactose and 2-oxo[1-¹⁴C]glutarate were from New England Nuclear Corp. (Boston, MA, U.S.A.). 2-Oxo[1-¹⁴C]glutarate was adjusted to a specific radioactivity of 100 000 d.p.m./0.1 μmol by mixing with the unlabelled compound. Gelatinized insoluble collagen was prepared from rat skin (Myllylä *et al.*, 1975) and [¹⁴C]lysine- and [¹⁴C]proline-labelled procollagen substrates were prepared from freshly isolated chick-embryo tendon cells (Risteli & Kivirikko, 1976; Risteli *et al.*, 1976). The synthetic peptide substrates L-I (Ala-Arg-Gly-Ile-Lys-Gly-Ile-Arg-Gly-Phe-Ser-Gly) and (Pro-Pro-Gly)₁₀-9H₂O were purchased from the Protein Research Foundation (Minoh, Osaka, Japan).

Lysyl hydroxylase was purified to homogeneity from whole chick embryos by a procedure involving fractionation with (NH₄)₂SO₄, affinity chromatography on concanavalin A-agarose, affinity chromatography on collagen linked to agarose and gel filtration (Turpeenniemi-Hujanen *et al.*, 1980). Prolyl 4-hydroxylase was purified to homogeneity from whole chick embryos (Tuderman *et al.*, 1975) and from foetal human tissues (Kuutti *et al.*, 1975), and antiserum against pure chick prolyl 4-hydroxylase was prepared in rabbits as described by Kuutti *et al.* (1975). Prolyl 3-hydroxylase was highly purified (Tryggvason *et al.*, 1979).

Preparation of antiserum against pure chick-embryo lysyl hydroxylase

The isolated enzyme pools (70 μg each) were dialysed exhaustively against 0.15 M-NaCl, mixed with an equal volume of complete Freund's adjuvant and injected intradermally at various sites in the backs of two rabbits. Crude *Bordetella pertussis* vaccine (0.5 ml) was injected subcutaneously at separate sites. The rabbits were then injected with a further 70 μg of lysyl hydroxylase mixed with incomplete Freund's adjuvant, as above, 2, 4 and 8 weeks later. The rabbits were bled 2 weeks after the last injections and the sera stored at -20°C until used.

Preparation of tissue extracts

The tissues were homogenized in a cold (0°C) solution consisting of 0.2 M-NaCl, 0.1 M-glycine, 30 μM-dithiothreitol, 0.1% (w/v) Triton X-100 and 0.05 M-Tris/HCl buffer adjusted to pH 7.5 at 4°C. The 13-day-old whole chick embryos and *post-partum* human placentas were homogenized in the proportions 1 g of tissue/1 ml of solution by using a Waring blender at full speed for two bursts of 30 s with a 1 min interval. All the separate tissues from chick embryos were homogenized in the proportions 1 g of tissue/10 ml of solution with a Teflon/glass homogenizer for 80 s. The kidneys, tendons and cartilage from a 60-day-old rabbit, skin of a 60-day-old mouse, skin, kidney and cartilage of a 90-day-old rat, and skin of a newborn pig were homogenized in the proportions 1 g of tissue/4 ml of solution four times with an Ultra-Turrax homogenizer for 15 s each time. The homogenates were incubated for 30 min at 4°C and then centrifuged at 15 000 g for 30 min at 4°C. The supernatants were used as the crude enzyme preparations for immunological studies.

Preparation of the immunoglobulin fraction from the antiserum

For this, 5 ml of the antiserum was precipitated with (NH₄)₂SO₄ (0–40% saturation). The (NH₄)₂SO₄-precipitate fraction was passed through a DEAE-cellulose column as described by Livingston (1974). The immunoglobulin-G fraction of the antiserum was then used for further characterization of the antibodies.

Assays for enzyme activities

Lysyl hydroxylase activity was measured with [¹⁴C]lysine-labelled procollagen substrate, as described previously (Kivirikko & Prockop, 1972; Puistola *et al.*, 1980a). In some experiments the activity was determined with the synthetic peptide substrate L-I (Kivirikko *et al.*, 1972; Puistola *et al.*, 1980a), this assay being based on the measurement

of the $^{14}\text{CO}_2$ formed during the hydroxylation-coupled decarboxylation of 2-oxo[1- ^{14}C]glutarate (see Kivirikko & Myllylä, 1980).

The activities of hydroxylysyl galactosyltransferase and galactosylhydroxylysyl glucosyltransferase were measured with gelatinized insoluble rat skin collagen as a substrate. These assays are based on the transfer of [^{14}C]galactose and [^{14}C]glucose from their radioactively labelled UDP-derivatives to hydroxylysyl residues and galactosylhydroxylysyl residues respectively (Myllylä *et al.*, 1975, 1976). The assays of prolyl 3-hydroxylase (Risteli *et al.*, 1978) and prolyl 4-hydroxylase activities (see Tuderman *et al.*, 1975) were carried out with radioactively labelled procollagen as a substrate. In some experiments prolyl 4-hydroxylase activity was also measured by a procedure based on the assay of the $^{14}\text{CO}_2$ formed by the stoichiometric decarboxylation of 2-oxo[1- ^{14}C]glutarate with (Pro-Pro-Gly) $_{10}$, 9H $_2$ O as a substrate (see Tuderman *et al.*, 1975).

Other methods

Sodium dodecyl sulphate/polyacrylamide-disc-gel electrophoresis was performed after denaturation and reduction of the polypeptide chains (Weber &

Osborn, 1969). The gels were stained with Coomassie Brilliant Blue.

Double immunodiffusion was performed by standard procedures on microscope slides covered with 1% (w/v) agar in 0.02 M-barbital buffer, pH 8.6. The slides were stained with Coomassie Brilliant Blue and were examined both before and after staining.

The protein content of the enzyme samples was measured by peptide absorbance at 225 nm by using bovine serum albumin as a standard.

Fig. 1. Double immunodiffusion with antiserum prepared to pure chick-embryo lysyl hydroxylase (upper well, right) and with chick-embryo crude (lower well, right) or pure (left well) lysyl hydroxylase protein

In the standard assay 1:1000 of the amount of crude enzyme used gave 208 d.p.m. and 1:10 000 of the amount of pure enzyme used gave 253 d.p.m. For further details see the Experimental section.

Fig. 2. Inhibition of lysyl hydroxylase activity by various amounts of non-immune serum (○) or antiserum (●), with as substrate radioactively labelled procollagen (a) or a synthetic peptide substrate, L-I (b)

Assays were carried out as described in the Experimental section.

Results

Preparation and characterization of the antiserum

The lysyl hydroxylase preparations used to immunize the rabbits were entirely pure when studied by sodium dodecyl sulphate/polyacrylamide-disc-gel electrophoresis (results not shown). The sera of the rabbits gave no inhibition of lysyl hydroxylase activity for 2 weeks after the first injection, but a rapid increase in the inhibitory ability took place thereafter (results not shown). In double immunodiffusion the antiserum gave a single precipitation line of identity when tested with the pure or crude chick-embryo lysyl hydroxylase (Fig. 1).

The assays for inhibition of the enzyme activity were carried out with two different substrates: the radioactively labelled procollagen (non-hydroxylated procollagen) and the synthetic peptide L-I containing only 12 amino acids. Assays with the latter substrate are considerably less sensitive and hence require about 8 times more enzyme than do those with the natural procollagen substrate. The degrees of inhibition observed with these two substrates were compared by expressing the data as μ l of antiserum used per μ g of enzyme protein added (see legend to Fig. 2). No significant differences were found in the degree of inhibition of the enzyme activity with these two substrates (Figs. 2a and 2b).

Immunological cross-reactivity between lysyl hydroxylases from different chick-embryo tissues

The inhibition of lysyl hydroxylase from various chick-embryo tissues was tested with procollagen substrate at two antiserum concentrations (Table 1).

The enzyme preparations were extracts of crude tissue homogenates and the amounts used in the experiment corresponded to about the same amount of enzyme activity units in each case. No significant differences in the amounts of antiserum required for about a 40% inhibition of the same amount of lysyl hydroxylase activity were found between the different tissues, which included tendon, synthesizing exclusively type I collagen, sternum, synthesizing only type II, and several tissues synthesizing more than one collagen type, including types III and IV (Table 1). As reported previously (Ryhänen & Kivirikko, 1974), large differences are found between these tissues in their lysyl hydroxylase activity and in the ratio of lysyl hydroxylase to prolyl 4-hydroxylase activity (results not shown).

The immunological characteristics of the crude enzyme preparations from whole chick-embryo tendon, cartilage and kidney were also examined by double immunodiffusion. Precipitation lines of identity were seen between the antiserum and each of the enzyme preparations (Fig. 3).

Cross-reactivity of the antiserum with lysyl hydroxylase from different species

The antiserum to chick lysyl hydroxylase showed a considerable degree of species specificity in that it inhibited the activity of the pig and human enzymes only weakly and the rabbit, rat and mouse enzymes very weakly (Table 2). Similarly, only very weak precipitation lines were seen when the antiserum and crude extracts of pig skin, rat skin or kidney, rabbit tendon or kidney and mouse skin were examined by

Table 1. *Inhibition of lysyl hydroxylase activity from different chick-embryo tissues by antiserum prepared against the enzyme from whole chick embryos*

The enzyme activities were measured with radioactively labelled procollagen as a substrate. The 15000 g supernatants from the homogenates of the different tissues of 12-day-old chick embryos were all prepared at the same time, as also were the supernatants for the kidneys, where the embryos were 17 days old. The amount of enzyme activity tested was adjusted to give about 3000 d.p.m. in the absence of the antiserum in each case. Inhibition with 5 or 10 μ l of antiserum was calculated by comparison with a value found with the same amount of non-immune serum. The inhibition found with different amounts of antiserum was plotted against the amount of antiserum used, and the amount of antiserum required for 40% inhibition was obtained from this curve.

Tissue	Inhibition with 5 μ l of antiserum (%)	Inhibition with 10 μ l of antiserum (%)	Amount of antiserum required for 40% inhibition (μ l)
Whole chick embryo	27	60	6.3
Tendon	37	60	5.5
Sternum	30	60	6.7
Kidney	32	56	6.6
Skin	27	48	8.0
Lung	22	56	7.6
Liver	33	64	6.0
Tibia	32	44	8.2
Heart	37	53	6.0

Fig. 3. Double immunodiffusion of lysyl hydroxylase from various chick-embryo tissues

(a) The centre well contained the antiserum, the left well purified lysyl hydroxylase from whole chick embryos, the upper well crude enzyme from chick-embryo tendon, and the right well that from sternum. (b) The left well contained the purified whole chick-embryo enzyme, the right upper well the antiserum, and the lower well the crude enzyme from kidney. In the standard assay, 1:4000 of the amount of the purified enzyme used gave 250 d.p.m., and similar amounts of the tendon, sternum and kidney enzymes gave 680, 618 and 360 d.p.m. respectively.

Cross-reactivity between lysyl hydroxylase and other enzymes catalysing intracellular modifications of collagen biosynthesis

The antiserum to chick lysyl hydroxylase did not inhibit the activity of prolyl 3-hydroxylase, hydroxylysyl galactosyltransferase or galactosylhydroxylysyl glucosyltransferase from whole chick embryos (Table 3). It did partially inhibit pure prolyl 4-hydroxylase from chick embryos, but not human prolyl 4-hydroxylase when measured with either radioactively labelled protocollagen or the synthetic peptide substrate (Pro-Pro-Gly)₁₀ (Table 3). Correspondingly, the antiserum to pure chick embryo prolyl 4-hydroxylase significantly inhibited lysyl hydroxylase activity measured both with protocollagen (Table 3) and with the synthetic peptide substrate L-I (results not shown).

Since the antisera to lysyl hydroxylase and prolyl 4-hydroxylase were prepared by using homogeneous enzyme proteins, the data strongly suggest that there is a true cross-reactivity between these two enzymes from the same species. Additional experiments were carried out to elucidate this point further. Chick-embryo prolyl 4-hydroxylase was coupled to Sepharose 4B by the CNBr activation technique (Cuatrecasas & Anfinsen, 1971), the column was equilibrated with 0.1 M-potassium phosphate buffer, pH 7.5, and an immunoglobulin fraction purified by (NH₄)₂SO₄ precipitation and DEAE-cellulose chromatography from the antiserum to lysyl hydroxylase was passed through this column. The antibodies that did not become bound still inhibited prolyl 4-hydroxylase activity, the ratio of the degree of inhibition of this enzyme to the degree of inhibition of lysyl hydroxylase being the same as in the crude antiserum (results not shown). This suggests that the antiserum did not possess antibodies to prolyl 4-hydroxylase that might have been present in trace amounts as a non-detected contaminant in the lysyl hydroxylase preparations used to immunize the rabbits. Control experiments indicated that most of the antibodies to prolyl 4-hydroxylase present in the antiserum to this enzyme did become bound to the same immunoaffinity column.

Additional experiments of another type were carried out by heat-inactivating pure chick-embryo prolyl 4-hydroxylase and adding 2 µg of this enzyme to a reaction mixture containing [¹⁴C]lysine-labelled protocollagen substrate, 0.1 µg of lysyl hydroxylase and 2 µl of antiserum to lysyl hydroxylase. The degree of inhibition of lysyl hydroxylase by the antiserum was decreased from 50% to 20% in the presence of prolyl 4-hydroxylase. Correspondingly, when heat-inactivated lysyl hydroxylase (0.3 µg) was added to a reaction mixture containing pure prolyl 4-hydroxylase (0.2 µg) and 10 µl of antiserum to lysyl hydroxylase, the inhibition of prolyl 4-hydroxylase decreased from 14% to about 0–3%.

double immunodiffusion (results not shown). When a relatively large amount of partially purified human placenta lysyl hydroxylase was tested with this technique, however, a distinct cross-reactivity was found (Fig. 4).

Table 2. *Inhibition of lysyl hydroxylase from different species by antiserum against the chick-embryo enzyme*

The enzyme activities were measured with radioactively labelled protocollagen as a substrate, as described in the Experimental section. The enzymes from the different sources tested were the $(\text{NH}_4)_2\text{SO}_4$ fractions (17–55% saturation) obtained from the 15000g supernatants of the tissue extracts. The enzyme activity found with the same amount of non-immune serum was considered as 100%.

Source of enzyme	Enzyme activity tested (d.p.m.)	Activity found with 8 μl of antiserum (%)	Activity found with 20 μl of antiserum (%)
Whole chick embryo	2580	60	25
Human placenta	2220	80	75
Rabbit cartilage	3120	100	90
Rat cartilage	1920	99	95
Mouse skin	2280	96	93
Pig skin	1560	92	70

Fig. 4. Double immunodiffusion with antiserum prepared to pure chick-embryo lysyl hydroxylase (centre well) and pure chick (left well) or partially purified human (lower well) lysyl hydroxylase

The human enzyme was purified 400-fold compared with the 15000g supernatant of the crude homogenate, and in both cases 1:1000 of the amount of enzymes used here gave about 120 d.p.m. in the standard assay. The upper well and the right well were empty.

These experiments again suggest that the two enzyme proteins react with a single species of the antibodies.

Discussion

Antibodies against chick-embryo lysyl hydroxylase were prepared here for the first time. The antiserum gave in double immunodiffusion a single precipitation line of identity when tested with the crude or purified chick-embryo lysyl hydroxylase. The antiserum also effectively inhibited lysyl hydroxylase activity when tested both with protocollagen and with the synthetic peptide substrate L-I. This suggests that the binding site of the antibody on the lysyl hydroxylase molecule is located in such a position that this binding prevents not only the reaction with the macromolecular substrate but also that with the synthetic peptide of only 12 amino acids. The antibodies prepared to prolyl 4-hydroxylase likewise inhibit effectively the reaction with both protocollagen and the relatively short synthetic peptide substrate (Pro-Pro-Gly), (Kuutti *et al.*, 1975), whereas antibodies prepared to galactosyl-hydroxylysyl glucosyltransferase (Myllylä, 1981) and collagenase (Hori & Nagai, 1979) inhibit the reaction much more readily with high-molecular-weight substrates than with ones of low molecular weight.

The antibodies to lysyl hydroxylase from chick embryos showed a considerable degree of species specificity when studied both by inhibition assays and by double immunodiffusion. These data are similar to those reported for chick-embryo prolyl 4-hydroxylase (Kuutti *et al.*, 1975) and galactosyl-hydroxylysyl glucosyltransferase (Myllylä, 1981) and indicate that the degree of species-specificity of the intracellular enzymes of collagen biosynthesis is considerably higher than that of the collagen itself (see Timpl *et al.*, 1977). It is noteworthy, however, that a distinct, although weak, cross-reactivity was found between the chick-embryo enzyme and those from all mammalian tissues tested. The cross-reactivity of the human placental enzyme is not very

Table 3. *Inhibition of other intracellular enzymes of collagen biosynthesis by antiserum to lysyl hydroxylase and inhibition of lysyl hydroxylase by antiserum to prolyl 4-hydroxylase*

The enzyme-activity assays were carried out as described in the Experimental section. Lysyl hydroxylase activity was measured with radioactively labelled protocollagen as a substrate. Chick prolyl 4-hydroxylase activity was measured with either protocollagen substrate or a synthetic substrate. The values shown here are from the experiments with the synthetic substrate, except for human prolyl 4-hydroxylase activity, which was measured with protocollagen substrate. The sera used were antisera to lysyl hydroxylase (anti-lh) or prolyl 4-hydroxylase (anti-ph), or non-immune serum (n.i.s.) (20 μ l in each case).

Enzyme	Purity	Serum	Activity	
			(d.p.m.)	(%)
Lysyl hydroxylase	Pure	n.i.s.	10680	100
		anti-lh	2040	19
Lysyl hydroxylase	Pure	n.i.s.	960	100
		anti-ph	610	64
Prolyl 4-hydroxylase (chick)	Pure	n.i.s.	1640	100
		anti-lh	1190	73
Prolyl 4-hydroxylase (chick)	Pure	n.i.s.	1830	100
		anti-ph	120	7
Prolyl 4-hydroxylase (human)	Pure	n.i.s.	1700	100
		anti-lh	1620	95
Prolyl 3-hydroxylase	Highly purified	n.i.s.	1800	100
		anti-lh	1770	98
Galactosyltransferase	Crude	n.i.s.	1050	100
		anti-lh	1100	105
Glucosyltransferase	Crude	n.i.s.	2060	100
		anti-lh	2100	102

surprising, as lysyl hydroxylase recently purified to homogeneity from this tissue has been found to be very similar to the chick-embryo enzyme in its molecular characteristics (T. M. Turpeenniemi-Hujanen, U. Puistola & K. I. Kivirikko, unpublished work). Purification of lysyl hydroxylase to homogeneity has also been reported from pig skin, and it has been claimed that this enzyme differs distinctly from the chick-embryo enzyme in its structure (Miller & Varner, 1979). The specific activity of the crude pig skin extract is very similar to that of the chick-embryo extract (T. M. Turpeenniemi-Hujanen, U. Puistola & K. I. Kivirikko, unpublished work), and by comparing the degrees of purification, it can be calculated that the specific activity of the final pig skin enzyme preparation is about 100 times lower than that of the chick-embryo and human enzymes. The purity and properties of the pig skin enzyme may thus require additional studies, particularly as the present data suggest that this tissue contains an enzyme immunologically similar to chick-embryo lysyl hydroxylase.

Because of the large differences in the extent of lysyl hydroxylation between various collagen types, it was of special interest to compare the immunological characteristics of lysyl hydroxylases in tissues synthesizing different collagen types. All the chick-embryo tissues studied were found to contain an immunologically similar enzyme form. Essentially the same amount of the antiserum was required

to inhibit the same amount of lysyl hydroxylase activity units from various tissues by 40%, and in double immunodiffusion a precipitation line of complete identity was seen between the antiserum and the purified enzyme from whole chick embryos or the crude enzymes from chick-embryo tendon, cartilage and kidneys. The data thus do not support the hypothesis that tissues synthesizing genetically different collagen types have lysyl hydroxylase isoenzymes with markedly different specific activities or immunological properties. Nevertheless, additional studies with other techniques would be required to elucidate further the presence or absence of such isoenzymes.

Distinct differences are found between lysyl hydroxylase and prolyl 4-hydroxylase with respect to their molecular properties. The former enzyme appears to be a dimer consisting of only one type of subunit, with mol.wt. approx. 85 000 (Turpeenniemi-Hujanen *et al.*, 1980), whereas prolyl 4-hydroxylase is a tetramer consisting of two different types of subunit with mol.wts. of about 60 000 and 64 000 (Berg & Prockop, 1973; Kuutti *et al.*, 1975; Tuderman *et al.*, 1975). On the other hand, these two enzymes appear to have identical reaction mechanisms, and the kinetic constants for their co-substrates and many other catalytic properties are also very similar (Kivirikko & Myllylä, 1980; Puistola *et al.*, 1980a,b). It would thus seem possible, that there may be similarities between the two

hydroxylases in their catalytic sites. In the present study the antibodies to lysyl hydroxylase inhibited prolyl 4-hydroxylase activity, and the antibodies to prolyl 4-hydroxylase inhibited lysyl hydroxylase activity. In both cases the inhibition was seen when studied both with the macromolecular procollagen substrate and with the relatively low-molecular-weight synthetic peptide substrate. These findings suggest that there are distinct structural similarities between some parts of the two hydroxylase molecules, possibly close to or at their active sites.

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References

- Berg, R. A. & Prockop, D. J. (1973) *J. Biol. Chem.* **248**, 1175–1182
- Bornstein, P. & Traub, W. (1979) in *The Proteins* (Neurath, H. & Hill, R. L., eds.), vol. 4, 3rd edn., pp. 411–632, Academic Press, London
- Cuatrecasas, P. & Anfinsen, C. B. (1971) *Methods Enzymol.* **22**, 345–358
- Eyre, D. R. (1980) *Science* **207**, 1315–1322
- Hori, H. & Nagai, Y. (1979) *Biochim. Biophys. Acta* **566**, 211–221
- Kivirikko, K. I. & Myllylä, R. (1979) *Int. Rev. Connect. Tissue Res.* **8**, 23–72
- Kivirikko, K. I. & Myllylä, R. (1980) in *The Enzymology of Post-translational Modifications of Proteins* (Freedman, R. B. & Hawkins, H. C., eds.), pp. 53–104, Academic Press, London
- Kivirikko, K. I. & Prockop, D. J. (1972) *Biochim. Biophys. Acta* **258**, 366–379
- Kivirikko, K. I., Shudo, K., Sakakibara, S. & Prockop, D. J. (1972) *Biochemistry* **11**, 122–129
- Krane, S. (1980) in *Gene Families of Collagen and Other Proteins* (Prockop, D. J. & Champe, P., eds.), pp. 57–69, Elsevier/North-Holland, New York
- Kuutti, E.-R., Tuderman, L. & Kivirikko, K. I. (1975) *Eur. J. Biochem.* **57**, 181–188
- Livingston, D. M. (1974) *Methods Enzymol.* **34**, 723–731
- Miller, R. L. & Varner, H. H. (1979) *Biochemistry* **18**, 5928–5932
- Myllylä, R. (1981) *Biochim. Biophys. Acta* in the press
- Myllylä, R., Risteli, L. & Kivirikko, K. I. (1975) *Eur. J. Biochem.* **52**, 401–410
- Myllylä, R., Risteli, L. & Kivirikko, K. I. (1976) *Eur. J. Biochem.* **61**, 59–67
- Pinnell, S. R., Krane, S. M., Kenzora, J. E. & Glimcher, M. J. (1972) *N. Engl. J. Med.* **286**, 1013–1020
- Prockop, D. J., Kivirikko, K. I., Tuderman, L. & Guzman, N. A. (1979a) *N. Engl. J. Med.* **301**, 13–23
- Prockop, D. J., Kivirikko, K. I., Tuderman, L. & Guzman, N. A. (1979b) *N. Engl. J. Med.* **301**, 77–85
- 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
- Risteli, J. & Kivirikko, K. I. (1976) *Biochem. J.* **158**, 361–367
- Risteli, J., Tuderman, L. & Kivirikko, K. I. (1976) *Biochem. J.* **158**, 369–376
- Risteli, J., Tryggvason, K. & Kivirikko, K. I. (1978) *Anal. Biochem.* **84**, 423–431
- Risteli, L., Risteli, J., Ihme, A., Krieg, T. & Müller, P. (1980) *Biochem. Biophys. Res. Commun.* **96**, 1778–1784
- Ryhänen, L. (1976) *Biochim. Biophys. Acta* **438**, 71–89
- Ryhänen, L. & Kivirikko, K. I. (1974) *Biochim. Biophys. Acta* **343**, 121–128
- Timpl, R., Wick, G. & Gay, S. (1977) *J. Immunol. Methods* **18**, 165–182
- Tryggvason, K., Majamaa, K., Risteli, J. & Kivirikko, K. I. (1979) *Biochem. J.* **183**, 303–307
- Tuderman, L., Kuutti, E.-R. & Kivirikko, K. I. (1975) *Eur. J. Biochem.* **52**, 9–16
- Turpeenniemi, T. M., Puistola, U., Anttinen, H. & Kivirikko, K. I. (1977) *Biochim. Biophys. Acta* **483**, 215–219
- Turpeenniemi-Hujanen, T. M., Puistola, U. & Kivirikko, K. I. (1980) *Biochem. J.* **189**, 247–253
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412