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DISSOCIATION AND REASSOCIATION OF PROLYL 4-HYDROXYLASE SUBUNITS AFTER CROSS-LINKING OF MONOMERS

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1. Incubation of prolyl 4-hydroxylase (prolyl-glycyl-peptide, 2-oxoglutarate : oxygen oxidoreductase (4-hydroxylating), EC 1.14.11.2) with H_2O_2 leads to a decrease of 50% in the specific activity of enzyme tetramers, followed by dissociation into inactive dimers in which the monomers are covalently cross-linked by S-S bridge formation. 2. Incubation of the enzyme with $K_3Fe(CN)_6$ leads to a comparable decrease in activity of enzyme tetramers. Addition of urea leads to dissociation into inactive dimers with similarly cross-linked monomers. 3. Removal of the dissociating agent leads to reassociation of cross-linked dimers to tetramers and to about 50% reactivation. The enzyme is further reactivated by preincubation with dithiothreitol. 4. Dissociation of the enzyme with dithiothreitol, urea or LiCl, or at low pH (4.15) produces inactive monomers, which could not be reassociated.

Introduction

In collagen synthesis prolyl 4-hydroxylase (prolyl-glycyl-peptide, 2-oxoglutarate : oxygen oxidoreductase (4-hydroxylating), EC 1.14.11.2) catalyses the *trans*-4-hydroxylation of proline in nascent or completed, unassembled pro-α-chains of pro-collagen (for reviews see Refs. 1–4). The enzyme requires iron and ascorbate for activity [5–8]. In a dioxygenase reaction it uses one atom of O_2 for the formation of the hydroxyl group of *trans*-4-hydroxyproline, whereas the other oxygen atom is used for stoichiometrically-coupled oxidative decarboxylation of 2-oxoglutarate and is incorporated into succinate [9–11]. In recent years the hypothesis has been developed that prolyl 4-hydroxylation is the rate-controlling step in the post-translational reactions involved in pro-collagen synthesis (see Ref. 2).

The enzyme is a tetramer of about 240 000 daltons [12–14] and consists of two different types of monomers of about 60 000 and 64 000 daltons, respectively [13–17]. The monomers differ in their isoelectric points (5.5 and 4.7), in their amino acid compositions (in particular with respect to methionine, tyrosine, phenylalanine and arginine) and in that most of the carbohydrate content of the enzyme is located on the larger subunit type [17]. It has been suggested on the basis of electron microscopy that rod-like monomers from V-shaped dimers which interlock to form a tetramer [18].

A large pool of monomer-size, enzymatically inactive, protein is present in cell cultures in addition to the tetramer. Under certain conditions this pool is involved in the formation of active tetramers [19–23]. Little is known, however, about the mechanism of the assembly of the enzyme, a process which may be of regulatory importance. Dissociation of the enzyme was achieved by incubation of the tetramers in 1 mM dithiothreitol [13,24], in 6 M urea/0.1 M 2-mercaptoethanol [13], by alkylation

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with 80 mM iodoacetic acid in 8 M urea with or without prior reduction by 20 mM dithiothreitol [17,25], by incubation with 5% sodium dodecyl sulphate (SDS) [13,14], and by dialysis against a buffer of low ionic strength [13]. Only the latter procedure can be expected to produce monomers in a native state, since the others would either disrupt intra-chain S-S bridges or lead to binding of SDS or acetic acid groups. We have dissociated the enzyme tetramer in several ways that do not change the primary structure, but we did not succeed in reassociating the monomers. We have, therefore, developed a method for cross-linking of monomers which enabled us to dissociate the enzyme into dimers and to study the reassociation at this level.

Materials and Methods

Materials

Prolyl 4-hydroxylase was isolated from chick embryos (age 13 days) by $(NH_4)_2SO_4$ fractionation (30–65%), affinity chromatography on poly L-proline Sepharose 4B and gel filtration as described previously [8]. The enzyme was pure according to polyacrylamide gel electrophoresis in the absence and the presence of SDS [8,26]. The synthetic polypeptide substrate (Pro-Pro-Gly)$_5 \cdot 4\,H_2O$ was from the Protein Research Foundation (Minoh-shi, Osaka, Japan); 2-oxo[1-^{14}C]glutarate was from New England Nuclear (Boston, MA).

Methods

Determination of enzyme activity.

Method I. The activity of prolyl 4-hydroxylase was measured continuously at 37°C by polarographic determination of the oxygen consumption, essentially as described previously [8]. Unless otherwise stated a freshly prepared solution containing 3 mg bovine serum albumin/0.15 mg bovine liver catalase/75 μmol Tris-HCl buffer was added to the reaction vessel, followed by the addition of 1.5 μmol ascorbate, 7.5 nmol $FeSO_4$, 15 μg enzyme, 0.6 μmol 2-oxoglutarate and 0.93 μmol (Pro-Pro-Gly)$_5 \cdot 4\,H_2O$, in that order and with intervals of 30 s between the additions. The final volume was 1.5 ml. The final pH was 7.4 at 20°C. The solution of (Pro-Pro-Gly)$_5 \cdot 4\,H_2O$ was boiled for 5 min and cooled in ice before addition [14]. The $FeSO_4$ was dissolved just before addition to the reaction mixture to avoid precipitation of hydroxide.

Method II. The activity was determined as described previously [8] by measuring the $^{14}CO_2$ production caused by the oxidative decarboxylation of 2-oxo-[1-^{14}C]glutarate, which takes place stoichiometrically with the hydroxylation of peptidyl proline [9]. Unless otherwise stated, the reaction mixture contained (in a volume of 1 ml) 1 μg enzyme/2 mg serum albumin/0.1 mg catalase/0.1 mM dithiothreitol/1 mM ascorbic acid/5 μM $FeSO_4$/0.37 mM (Pro-Pro-Gly)$_5 \cdot 4\,H_2O$/0.1 mM 2-oxo[1-^{14}C]-glutarate (6.10^5 dpm/μmol)/50 mM Tris-HCl buffer. The final pH was 7.4 at 20°C. The order of additions was the same as described for Method I except that the reaction was started with 2-oxo[1-^{14}C]glutarate instead of (Pro-Pro-Gly)$_5 \cdot 4\,H_2O$ and dithiothreitol was added before ascorbate.

Protein determination. The concentration of enzyme protein was determined spectrophotometrically, making use of the relation $A_{230nm} = 7.73$ (mg/ml)$^{-1} \cdot$ cm^{-1} [27].

Gel electrophoresis. Gels were prepared with 7.5% polyacrylamide, 0.25% *N,N'*-methylene-bisacrylamide, 0.06% *N,N,N',N'*-tetramethyl-1,2,diaminoethane, 0.12% ammonium persulphate in 0.7 M Tris-HCl buffer, pH 8.3 (cf. Refs. 28, 29). The protein sample in the isolation buffer [8] was mixed with 10 μl 0.02% bromphenol blue and one drop of glycerol per 100 μl. The anode and cathode reservoirs contained 50 mM Tris-HCl/50 mM glycine, pH 8.5. 1.5 mA was applied to each gel for about 16 h at 4°C. The gels were stained in acetic acid/methanol/water (10 : 45 : 45, v/v), containing 0.25% Coomassie brillant blue R-250, de-stained in acetic acid/methanol/water (7.5 : 25 : 67.5, v/v) and stored in 5% acetic acid before photographs were taken.

Gel electrophoresis in the presence of SDS. Gels (cf. Ref. 30) were prepared with 13.5% polyacrylamide, 0.4% *N,N'*-methylene-bisacrylamide, 0.04% *N,N,N',N'*-tetramethyl-1,2-diaminoethane, 0.5% SDS, 0.1% ammonium persulphate in 0.3 M Tris-HCl buffer (pH 8.5).

Stacking gels (cf. Ref. 31) were prepared with 4% polyacrylamide, 0.1% *N,N'*-methylene-bisacrylamide, 0.1% SDS, 0.04% ammonium persulphate, 0.08% *N,N,N',N'*-tetramethyl-1,2-diaminoethane in 0.03 M Tris-HCl buffer (pH 6.8). To the protein sample in

the isolation buffer [8] were added: 5% glycerol, 0.2% SDS, 5% 2-mercaptoethanol, 0.02% bromphenol blue, 25 mM Na_2CO_3 and 0.5 mM iodoacetic acid (final concentrations).

The buffer used in the anode and cathode reservoirs was the same as described above for the 7.5% polyacrylamide gels except that 0.3 g SDS/l buffer was added to the latter. 1.5 mA was applied to each gel for 30 min at 17°C, followed by 3 mA for 4 h. Staining and de-staining were carried out as described above.

Protein bands in polyacrylamide gels were cut out and transferred to SDS-containing gels after one of the following treatments:

(a) the gel slice was extracted by homogenization with 0.4 ml of a solution (pH 7.8) containing 0.1 M NaCl/0.1 M glycine/10 μM dithiothreitol/5 mM Tris-HCl buffer/0.4% SDS/5% 2-mercaptoethanol/1% glycerol/0.002% bromphenol blue/6 mM Na_2CO_3/0.1 mM iodoacetic acid. Not more than 0.3 ml of the extract was applied to SDS-containing gel;

(b) in analogy with O'Farrell [32], the slice was incubated for 2 h at room temperature in a buffer containing 62.5 mM Tris-HCl/10% glycerol/5% 2-mercaptoethanol/2.3% SDS (pH 7.8). It was then attached to the top of an SDS-containing gel with the aid of the incubation buffer to which 1% agarose had been added.

After staining, gels were scanned at 540 nm with a Zeiss spectrophotometer equipped with an automatic gel-scanning attachment and a scale expander. Gel-scan areas were calculated by using a Tektronix 4954 graphics tablet coupled to a HP 2100 S mini computer equipped with a RTE-11 operating system.

Results

After incubation of prolyl 4-hydroxylase with 2 M H_2O_2 for 30 min at 0°C, the enzyme migrates more rapidly on a 7.5% polyacrylamide gel than the untreated tetrameric enzyme (cf. Fig. 1a and 1b), but less rapidly than enzyme fully dissociated with urea (see Fig. 3a). The single band shown in Fig. 1b was not resolved further on a gel of double the normal length. Prolonging the incubation with H_2O_2 (up to 20 h) did not lead to any further increase in mobility. Nor was this increased by dialysis of the H_2O_2-treated enzyme against 6 M urea. Reduction by dithiothreitol (1 mM), however, did lead to a substantial increase in mobility (Fig. 1c). Neither ascorbate (1 mM) nor NADH (1 mM) could replace dithiothreitol. Transfer of the protein in the gel band shown in Fig. 1b, after incubation with SDS and 2-mercaptoethanol (see

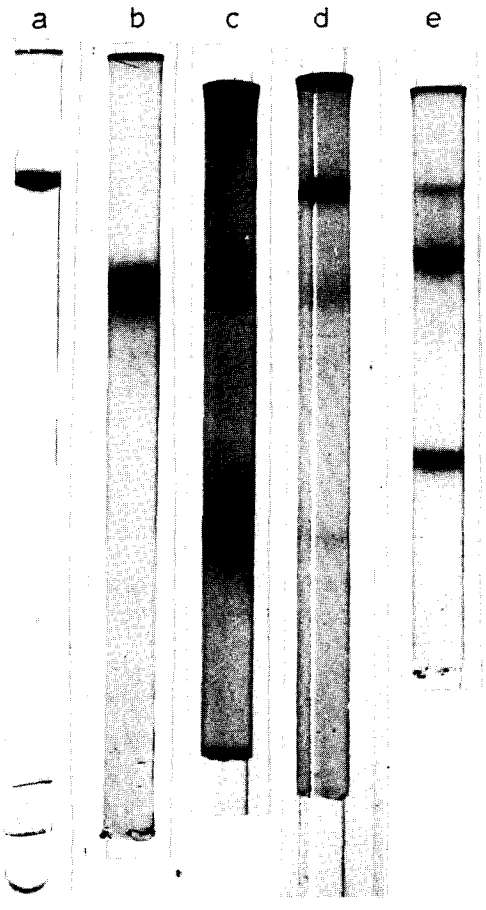

Fig. 1. 7.5% polyacrylamide gel electrophoresis of prolyl 4-hydroxylase; the influence of H_2O_2, urea and dithiothreitol. Gel a: the enzyme as isolated (11 μg). Gel b: the enzyme (23 μg) was incubated for 30 min at 0°C in the isolation buffer [8] plus 2 M H_2O_2. Gel c: the enzyme (34 μg) was treated as for gel b and then urea (3 M) was added and the sample dialysed three times for 1 h at 0°C against isolation buffer [8]/3 M urea, followed by dialysis for 1 h at 20°C against isolation buffer containing 1 mM dithiothreitol. Gel d: the enzyme (27 μg) was incubated for 3 h at 0°C in isolation buffer [8] plus 2 M H_2O_2, followed by dialysis against isolation buffer three times for 1 h. Gel e: the enzyme (34 μg) was treated as for gel d, but during the dialysis 1 mM dithiothreitol was present.

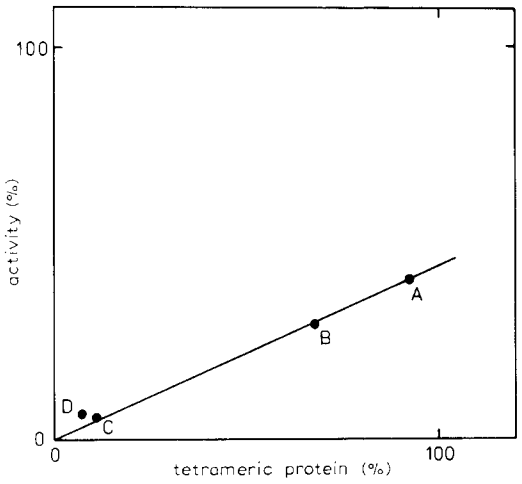

Fig. 2. The correlation between the percentage specific activity and the percentage of protein present as tetramers after treatment with H_2O_2. The enzyme (39 μg) was incubated for 30 min at 0°C with 0.1 M (A), 0.25 M (B), 1 M (C) and 2 M H_2O_2 (D) respectively. The specific activity was measured as μmol $^{14}CO_2 \cdot min^{-1} \cdot mg^{-1}$ (see Methods). The percentage tetramers was determined by 7.5% polyacrylamide gel electrophoresis, gel scanning and area calculation (see Methods).

Methods), revealed the bands of the two monomers (M_r 64 000 and 60 000) in about equal intensity (data not shown).

Treatment with H_2O_2 also leads to inactivation of the enzyme with a $t_{1/2}$ of 8 min when 0.5 M H_2O_2 is used at 0°C. Incubation with 0.1 M H_2O_2 for 30 min at 0°C inhibited the enzyme activity by about 50% with a minimal dissociation of the tetramer (see point A, Fig. 2). Treatment with different concentrations of H_2O_2 for 30 min at 0°C showed that the residual enzyme activity was proportional to the percentage of tetramer that remained (Fig. 2).

The dissociating effect of H_2O_2 on the enzyme was not found with other compounds of high dipole moment (0.5 M ethylene glycol (2.28 d), 0.5 M acetone (2.88 d) or 1 mM dimethyl sulphoxide (3.96 d) – cf. H_2O_2 (2.20 d)). Monomers were obtained by incubation with urea (Fig. 3a), with urea plus dithiothreitol and with urea plus 2-mercaptoethanol (data not shown). In all three cases one protein band was seen, which is in contrast to the results of Berg and Prockop [13] who found two monomer bands after treatment with urea plus 2-mercaptoethanol. In a control experiment the

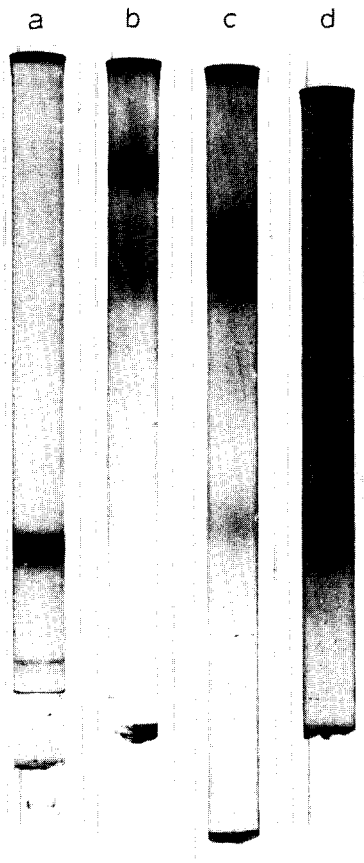

Fig. 3. 7.5% polyacrylamide gel electrophoresis of prolyl 4-hydroxylase; the influence of urea and $K_3Fe(CN)_6$. Gel a: the enzyme (25 μg) was incubated for 3 h at 0°C in the isolation buffer [8] plus 6 M urea. Gel b: the enzyme (23 μg) was incubated for 10 min at 37°C in isolation buffer [8] plus 50 mM $K_3Fe(CN)_6$. Gel c: the enzyme (23 μg) was treated as for gel b, followed by addition of urea (6 M) and incubation for 3 h at 0°C. Gel d: the enzyme (32 μg) was incubated for 10 min at 37°C in isolation buffer [8] plus 100 mM $K_3Fe(CN)_6$. The urea (3 M) was added and the sample was dialysed three times for 1 h against isolation buffer [8] plus urea (3 M) and dithiothreitol (2 mM).

protein of the single band produced by treatment with urea and dithiothreitol was found, by applying SDS-polyacrylamide gel electrophoresis, to contain both types of monomer. Monomers were also obtained by incubation with 2 M LiCl (cf. dissociation of F_1ATPase [33]) and by incubation at pH 4.15. We were not able to produce monomers by dialysis against a buffer with low ionic strength (cf. Ref. 13), as our preparations hardly dissociated

during this treatment, even when the temperature was raised from 4 [13] to 20°C.

Up to 0.2 M $K_3Fe(CN)_6$ was not able to mimic H_2O_2 (Fig. 3b). However, after treatment first with 0.05 M $K_3Fe(CN)_6$ and then with 6 M urea, a band was obtained similar to that found with 2 M H_2O_2 (Fig. 3c). Fig. 3d shows that dithiothreitol further increases the mobility of this preparation in the same way as shown above for enzyme treated with H_2O_2. Not shown is that a low concentration of H_2O_2 (0.1 M) followed by urea has the same effect as 2 M H_2O_2 or $K_3Fe(CN)_6$ plus urea.

By electrophoresis in the presence of SDS, it could be demonstrated that the molecular weight of the enzyme treated with 2 M H_2O_2 for 3 h at 0°C, followed by dialysis against urea is about 140 000, to be compared with about 70 000 (two species) found with untreated enzyme (data not shown). In the presence of 2-mercaptoethanol and iodoacetic acid, both treated and untreated preparations showed two bands at about 60 000 daltons.

Attempts, by dialysis against different (dithiothreitol-free) media, cf. Ref. 34), to reassociate the monomers produced by incubation with urea, dithiothreitol, LiCl, or by lowering the pH were not successful.

It is possible, however, to reassociate the dimers formed after H_2O_2 treatment by dialysis against a dithiothreitol-free medium (see Fig. 1d). When 1 mM dithiothreitol was present in the dialysis solution, both tetramers and monomers were formed (Fig. 1e). Within 5 min reassociation was obtained by removal of H_2O_2 by centrifugation on a bed of Sephadex G-25 [35]. Although ascorbate and 2-oxoglutarate influence the enzyme conformation [36], addition of ascorbate, Fe(II) or 2-oxoglutarate had no effect on the reassociation.

TABLE I

SPECIFIC ACTIVITY OF H_2O_2-TREATED AND REASSOCIATED PROLYL 4-HYDROXYLASE

Activity was measured as $^{14}CO_2$ production (see Method II). Reassociation was achieved by dialysis three times for 1 h against isolation buffer [8] without dithiothreitol, after previous dissociation by incubation for 30 min at 0°C in isolation buffer containing 2 M H_2O_2.

Experiment	Enzyme (μg)	Specific activity (μmol · min^{-1} · mg^{-1})
1	untreated (0.4)	0.98
	H_2O_2-treated (0.4)	0.06
	reassociated (0.5)	0.41
2	untreated (0.4)	0.76
	H_2O_2-treated (0.4)	0.09
	reassociated (0.5)	0.34

TABLE II

THE EFFECT OF PREINCUBATION WITH 1 mM DITHIOTHREITOL ON THE ACTIVITY OF OXIDIZED PROLYL 4-HYDROXYLASE

The activity was measured as μmol O_2 · min^{-1} · mg^{-1} (see Method I) and corrected for nonspecific oxidation. The order of additions was: ascorbate, $FeSO_4$, enzyme, (Pro-Pro-Gly)$_5$ · 4 H_2O and 2-oxoglutarate. When dithiothreitol was present, it was added before ascorbate. Because of the 30s intervals between the additions, the enzyme was preincubated for 1 min with 1 mM dithiothreitol. In Expts. 1 and 2 the enzyme was used as isolated, in Expts. 3 and 4 after oxidation and dissociation by H_2O_2 and reassociation by dialysis (see legend to Fig. 1). In Expts. 5 and 6 the enzyme was used after oxidation by $K_3Fe(CN)_6$ (see legend to Fig. 3) and dialysed as for reassociation.

Expt.	Enzyme (μg)	Dithiothreitol	Reaction rate (%)
1	untreated (15)	–	100
2	untreated (15)	+	87
3	H_2O_2-oxidised, reassociated (9.6)	–	61
4	H_2O_2-oxidised, reassociated (9.6)	+	88
5	$K_3Fe(CN)_6$-oxidised (6.6)	–	66
6	$K_3Fe(CN)_6$-oxidised (6.6)	+	88

Table I shows that the specific activity for the reassociated tetramers is about 50% of the activity of untreated tetramers (ranging from 42–61% in five different experiments). This activity is comparable with that found in Table II for $K_3Fe(CN)_6$-oxidised tetramers.

Whereas comparison of lines 1 and 2 in Table II shows the partial inactivation of the enzyme by 1 min preincubation with 1 mM dithiothreitol, preincubation for 1 min with 1 mM dithiothreitol of enzyme reassociated after H_2O_2 treatment increased the

activity significantly (Table II, lines 3 and 4). $K_3Fe(CN)_6$-treated enzyme was also reactivated by 1 mM dithiothreitol (compare lines 5 and 6). Addition of 1 mM dithiothreitol under turnover conditions was not effective with either H_2O_2 or $K_3Fe(CN)_6$-treated enzyme (data not shown).

Discussion

Dissociation of prolyl 4-hydroxylase by H_2O_2 into subunits of about 140 000 daltons is a combination of two effects: oxidation and dissociation. For the dissociative effect of H_2O_2 a high concentration is required. This is not necessary for the oxidative part of the H_2O_2 effect, since treatment with 0.1 M H_2O_2 followed by 6 M urea has the same effect as high concentrations of H_2O_2. $K_3Fe(CN)_6$ mimics the oxidative part of the H_2O_2 effect (Fig. 3b and 3c). After treatment with high concentrations of H_2O_2, urea causes no further dissociation. From this it may be concluded that the oxidation by H_2O_2 produces a covalent bond between monomers. This covalent bond is most likely a S-S bridge since it can be broken by 1 mM dithiothreitol ($E_0' = -0.332$ V) (Fig. 1c) or 2-mercaptoethanol ($E_0' = -0.060$ V) (see Results), but not by 1 mM ascorbate ($E_0' = +0.060$ V) or 1 mM NADH ($E_0' = -0.320$ V). Electrophoresis in the presence of SDS shows that a subunit with a molecular weight of about 140 000 is formed by H_2O_2 treatment. This subunit is split into monomers by dithiothreitol (Fig. 1c). Since only one band was seen, both with and without SDS (Fig. 1b), even when long gels were used, probably only one type of subunit (α-β) is present after H_2O_2 treatment, suggesting $(\alpha \cdot \beta)_2$ as the tetramic structure in the native enzyme.

The inactivation of prolyl 4-hydroxylase by H_2O_2 is caused both by dissociaton and by oxidation. The results given in Fig. 2 show that the dimers are totally inactive, whereas the remaining tetramers with an $(\alpha$-$\beta)_2$ structure have 50% of the specific activity of untreated tetramers $(\alpha \cdot \beta)_2$. The fact that reassociation of H_2O_2-produced dimers can be achieved within 5 min by centrifugation on a bed of Sephadex [33] indicates that the cross-linked dimers are native.

After correction for the inactivation by dithiothreitol (see Table II) it can easily be seen that cross-linked, reassociated tetramers should regain full activity.

Although prolyl 4-hydroxylase can be completely dissociated into monomers by incubation with dithiothreitol, as has also been shown by others [22], the fact that the enzyme can also be dissociated by urea, LiCl and by lowering of the pH below the isoelectric point of the tetramers [12] and the monomers [17], shows that in the isolated enzyme no disulphide bonds exist between the monomers. This is in agreement with the conclusion of Berg and Prockop [25], based on dissociation by alkylation.

The failure to reassociate prolyl 4-hydroxylase monomers may indicate that the subunits can only associate in a precursor form. In fact it is reported that in vivo the β-subunit is present in a pre-β-form, in large excess awaiting the synthesis of the α-subunit [16,20,23]. Our results demonstrate that (cross-linked) dimers spontaneously reassociate to tetramers. Therefore, the possibility exists that regulation of the assembly of the enzyme from monomers is at the level of dimer formation.

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References

1 Bornstein, P. (1974) Annu. Rev. Biochem. 43, 567–603
2 Prockop, D.J., Berg, R.A., Kivirikko, K.I. and Uitto, J. (1976) in Biochemistry of Collagen (Ramachandran, G.N. and Reddi, A.H., eds.), pp. 163–273, Plenum Press, New York
3 Fessler, J.H. and Fessler, L.I. (1978) Annu. Rev. Biochem. 47, 129–162
4 Prockop, D.J., Kivirikko, K.I, Tuderman, L. and Guzman, N.A. (1979) New Engl. J. Med. 301, 13–23 and 77–85
5 Hutton, J.J., Tappel, A.L. and Udenfriend, S. (1967) Arch. Biochem. Biophys. 118, 231–240
6 Kivirikko, K.I. and Prockop, D.J. (1967) J. Biol. Chem. 242, 4007–4012
7 Myllylä, R., Kuutti-Savolainen, E.-R. and Kivirikko, K.I. (1978) Biochem. Biophys. Res. Commun. 83, 441–448
8 Nietfeld, J.J. and Kemp, A. (1980) Biochim. Biophys. Acta 613, 349–358
9 Rhoads, R.E. and Udenfriend, S. (1968) Proc. Natl. Acad. Sci. U.S.A. 60, 1473–1478
10 Lindblad, B., Lindstedt, G., Tofft, M. and Lindstedt, S. (1969) J. Am. Chem. Soc. 91, 4604–4606

11 Cardinale, G.J., Rhoads, R.E. and Udenfriend, S. (1971) Biochem. Biophys. Res. Commun. 43, 537–543
12 Pänkäläinen, M., Aro, H., Simons, K. and Kivirikko, K.I. (1970) Biochim. Biophys. Acta 221, 559–565
13 Berg, R.A. and Prockop, D.J. (1973) J. Biol. Chem. 248, 1175–1182
14 Tuderman, L., Kuutti, E.-R. and Kivirikko, K.I. (1975) Eur. J. Biochem. 52, 9–16
15 Kuutti, E.-R., Tuderman, L. and Kivirikko, K.I. (1975) Eur. J. Biochem. 57, 181–188
16 Cheng-Kiang, S., Cardinale, G.J. and Udenfriend, S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4420–4424
17 Berg, R.A., Kedersha, N.L. and Guzman, N.A. (1979) J. Biol. Chem. 254, 3111–3118
18 Olsen, B.R., Berg, R.A., Kivirikko, K.I. and Prockop, D.J. (1973) Eur. J. Biochem. 35, 135–147
19 Chichester, C.O., Fuller, G.C. and Cardinale, G.J. (1976) Biochem. Biophys. Res. Commun. 73, 1056–1062
20 Tuderman, L., Oikarinen, A. and Kivirikko, K.I. (1977) Eur. J. Biochem. 78, 547–556
21 Majamaa, K., Kuutti-Savolainen, E.-R., Tuderman, L. and Kivirikko, K.I. (1979) Biochem. J. 178, 313–322
22 Kao, W.W.-Y. and Chou, K.-L.L. (1980) Arch. Biochem. Biophys. 199, 147–157
23 Berg, R.A., Kao, W.W.-Y. and Kedersha, N.L. (1980) Biochem. J. 189, 491–499
24 Tuderman, L., Kuutti, E.-R. and Kivirikko, K.I. (1975) Eur. J. Biochem. 60, 399–405
25 Berg, R.A. and Prockop, D.J. (1974) Abstr. 22nd Colloquium on Protides of the Biological Fluids, Brugge, Belgium
26 Nietfeld, J.J. and Kemp, A. (1979) Abstr. 11th Intern. Congr. Biochem. Toronto, p. 459
27 Berg, R.A. and Prockop, D.J. (1976) in The Methodology of Connective Tissue Research (Hall, D.A., ed.), pp. 187–196, Joynson-Bruvvers Ltd., Oxford
28 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. U.S.A. 121, 404–427
29 Knowles, A.F. and Penefsky, H.S. (1972) J. Biol. Chem. 247, 6617–6623
30 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
31 Maurer, H.R. (1971) Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis, 2nd Edn., W. de Gruyter, Berlin
32 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021
33 Verschoor, G.J., Van der Sluis, P.R. and Slater, E.C. (1977) Biochim. Biophys. Acta 462, 438–449
34 Hatefi, Y. and Hanstein, W.G. (1974) Methods Enzymol. 31, 770–790
35 Penefsky, H.S. (1977) J. Biol. Chem. 252, 2891–2899
36 Nietfeld, J.J. and Kemp, A. (1981) Biochim. Biophys. Acta 657, 159–167