BIOCHEMICAL LETTERS

On the structure/function relationship of polymorphonuclear-leucocyte collagenase

In their recent paper, Knauper et al. [1] discuss the autoproteolytic degradation of polymorphonuclear-leucocyte (PMNL) collagenase. They were able to show that the enzyme is processed to two major fragments of 40 kDa and 27 kDa, corresponding to the enzymic core and to the hemopexin-like C-terminal domain respectively. By sequencing the N-terminus of each fragment, it was possible to deduce the autoproteolytic cleavage point, the peptide bond between either Gly²⁴²-Leu²⁴³ or Pro²⁴⁷-Ile²⁴⁸. As expected, both fragments lack the ability to cleave interstitial collagen. More interesting, however, was the fact that both fragments also lacked the ability of binding to collagen.

Our group has suggested that the sequence ²⁴⁴SSNPIQP²⁵⁰ in PMNL collagenase (or SQNPVQP in fibroblast collagenase) is important for its substrate-binding activity [2]. Using chimeric constructions, Hirose et al. [3] have confirmed that this region is important for the collagenolytic activity of the PMNL enzyme. In the Discussion of their paper, Knauper et al. [1] concluded that the sequence SSNPIQP would not be involved in collagen-binding activity, since after autoproteolytic cleavage the ensuing 40 kDa and 27 kDa fragments do not bind collagen. However, they did not notice that the cleavage point is exactly in the middle of the SSNPIQP sequence. In our opinion, the fact that the resulting fragments do not bind to collagen, whereas the intact molecule does, supports our suggestion that the sequence SSNPIQP is involved in collagen binding, rather than the opposite, as they contend. It seems clear, however, that the collagen-binding capacity of collagenases involves more than one peptide stretch. A great deal of evidence seems to indicate that additional sequences present in the hemopexin-like C-terminal domain are also involved in collagen-binding activity. For instance, mutants of neutrophil collagenase lacking a part of this domain show a very weak collagenolytic activity [3]. One interesting possibility is that these sequences are responsible for an 'unproductive binding' and that the SQNPVQP- or SSNPIQP-mediated binding could adjust the enzyme in the correct place. In agreement with this possibility is the fact that fibroblast collagenase chimaeras containing the C-terminal domain of stromelysin (and without the SQNPVQP sequence) also bind collagen and likewise lack the ability to cleave it [4]. The same is true for the native stromelysin.

Knauper et al. [1] remind us that autoproteolysis in collagenases takes place in a proline-rich region and that the cleavage point (Gly-Leu or Pro-Ile) resembles that of collagen. It is well known from Ramachandran's work that proline-rich regions can adopt a collagen-like conformation [5]. It is attractive to speculate that the proline-rich region of collagenases has a collagen-like conformation. Because the collagenase-sensitive site in collagen has a more relaxed triple helix, the collagen helix conformation of the proline repeat could enable the enzyme to bind to collagen. This would explain the fact that this region is always the target of autoproteolytic cleavage. If confirmed, this

would be one of the first cases where an enzyme mimics its substrate's conformation in order to cleave it.

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Protochlorophyllide reductase is homologous to human carbonyl reductase and pig 20 β -hydroxysteroid dehydrogenase

Protochlorophyllide reductase is an NADPH-dependent enzyme that catalyses the conversion of protochlorophyllide into chlorophyllide [1]. This is the first detectable light-dependent step towards the formation of the chloroplast. Synthesis and degradation of this enzyme are tightly regulated, due to its important role in the synthesis of chlorophyll in plants. The sequences of the enzymes from barley, oat, pea, pine and *Arabidopsis* have been determined without any reported homology to other enzymes [2–6]. My interest in the connection between animal and plant dehydrogenases [7,8] led me to compare the sequence of protochlorophyllide reductase with the protein database, which revealed that this enzyme is homologous to pig 20 β -hydroxysteroid dehydrogenase [9] and human carbonyl reductase [10], enzymes that belong to a protein superfamily that contains human 11 β -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase, 15-hydroxyprostaglandin dehydrogenase and *Drosophila melanogaster* alcohol dehydrogenase, as well as bacterial enzymes that are important in synthesis of antibiotics [11–15]. Much is known about these enzymes concerning residues that are important in binding of the nicotinamide cofactor [16], in preference for NADH and NADPH [17,18], and in catalysis [19–22], information that should be useful in understanding catalysis by protochlorophyllide reductase.

As noted by Spano et al. [6] the amino acid sequences of the different protochlorophyllide reductases are very similar to each other: the loblolly-pine enzyme is 76% identical with either barley or oat, 84% identical with pea, and 82% identical with *Arabidopsis* protochlorophyllide reductase. Thus, a comparison of one protochlorophyllide reductase sequence with that of other oxidoreductases is representative of that for other protochlorophyllide reductases. It is an ~140-residue segment in exon 3 of protochlorophyllide reductase that is most similar to human