Collagenase-like peptidase activity in serum from patients with rheumatoid arthritis

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Summary. The activities of collagenase-like peptidase, estimated by using (succinyl-Gly-Pro-Leu-Gly-Pro-Leu-Gly-Pro)-4-methylcoumaryl-7-amide as substrate, and of dipeptidyl-aminopeptidase IV were decreased in the sera from patients with rheumatoid arthritis. Both enzymes bring about the degradation of peptides derived from collagen. A significant positive correlation was observed between the activities of the two serum peptidases.
Key words. Rheumatoid arthritis; peptidase, collagen-like.

By using an artificial substrate for collagenase, 4-phenyl-azobenzyloxycarbonyl-L-Pro-L-Leu-Gly-L-Pro-D-Arg (PZ-peptide)[2], many tissues have been found to contain the collagenase-like (CL) peptidase (PZ-peptidase)[3] which is distinct from the animal collagenase that attacks native collagen[4], and there have been several reports suggesting a relationship between PZ-peptidase and the degradation of collagen in vivo[5–7]. However, since the method using PZ-peptide as substrate is not very sensitive, a highly sensitive fluorescence assay for CL-peptidase was developed using (succinyl-Gl-Pro-Leu-Gly-Pro)-4-methylcoumaryl-7-amide (Suc-GPLGP-MCA) as substrate[8], and we were able to measure the CL-peptidase activity in normal human sera by this method[8].

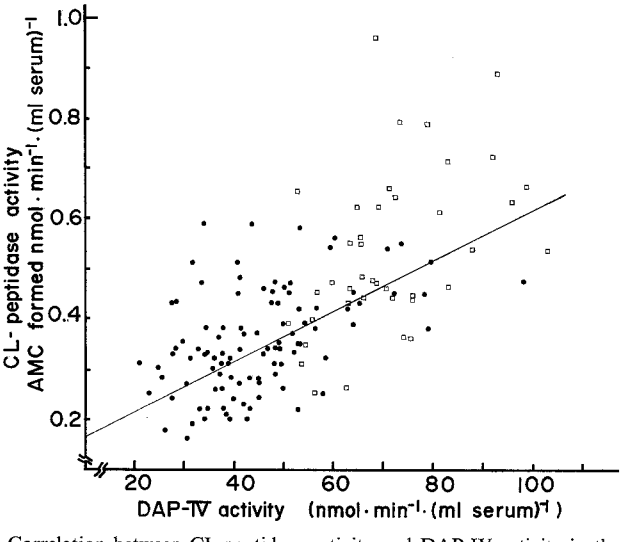
Fujita et al.[9] previously reported that serum dipeptidyl-aminopeptidase IV (DAP-IV) activity was decreased in patients with rheumatoid arthritis. DAP-IV is thought to be concerned in degradation of small peptides derived from collagen by successive degradation by animal collagenase and CL-peptidase.

We therefore examined serum CL-peptidase activity using Suc-GPLGP-MCA as substrate in patients with rheumatoid arthritis, and compared the CL-peptidase activity with that of DAP-IV. In order to investigate the effect of decreased activity of daily life (ADL) on the serum peptidases in the patients with rheumatoid arthritis, we also examined the enzyme activites in serum from patients with apoplexia.

Materials and methods. Suc-GPLGP-MCA was synthesized in our laboratory (Peptide Institute, Protein Research, Foundation)[8]. DAP-IV from the human submaxillary gland was purified to a homogeneous state according to the previously described method[10], dialyzed against 2 mM acetate buffer, pH 6.0, and stored at −80 °C. The specific activity of the pure DAP-IV toward Gly-Pro-p-nitroanilide[11] was 57.4 μmoles · min^{-1} · (mg $protein^{-1}$), and the concentration was 9.84 μg/ml. Human sera were kept at −80 °C until use. All other chemicals were of analytical grade.

CL-peptidase was assayed with Suc-GPLGP-MCA as substrate. The principle of the assay for CL-peptidase activity using Suc-GPLGP-MCA as substrate is based on the fluorometric measurement of 7-amino-4-methylcoumarine (AMC) liberated form the reaction product Gly-Pro-MCA by the 2nd enzyme reaction with DAP-IV: The standard incubation mixture (total volume 200 μl) contained 50 μl of 0.2 M Tris-maleate buffer (pH 8.0) with 2.4 mM Suc-GPLGP-MCA and 20 mM $CaCl_2$, 20 μl of DAP-IV (0.197 μg), and 50 μl of serum plus water. The blank and standard tubes contained water and 500 pmoles of AMC, instead of enzyme, respectively. A control tube without enzyme was run with each sample. Incubation was carried out at 37 °C for 1 h, and the reaction was stopped by adding 1.0 ml of 1 M sodium acetate buffer, pH 4.2. The same amount of enzyme was added to the control tubes after stopping the reaction. The fluorescence intensity was read at 460 nm with excitation at 380 nm, using a Shimadzu RF-500 spectrofluorophotometer. DAP-IV activity was assayed using Gly-Pro-p-nitroanilide (Gly-Pro-pNA)[11].

Results. The enzyme reaction of CL-peptidase was found to be linearly related to the incubation time at 37 °C for about 120 min, and to the amount of enzyme from 20 to 100 μl serum. Using Suc-GPLGP-MCA as substrate, CL-peptidase was optimally active at pH 6.7, and pH-activity curve had a small shoulder at pH 8.0 in 50 mM Tris-maleate buffer. There was a significant positive correlation ($r = 0.969$, $n = 17$) between the activity of the enzyme at pH 6.7 and 8.0. The K_m value of the enzyme for Suc-GPLGP-MCA was 2.8×10^{-4} M in Tris maleate buffer at pH 8.0.



Correlation between CL-peptidase activity and DAP-IV activity in the sera from 142 patients with rheumatoid arthritis (●) and with apoplexia (□). $r = 0.6552$, $p < 0.01$.

Activity of collagenase-like peptidase and dipeptidyl-aminopeptidase IV in sera from normal controls, from patients with rheumatoid arthritis and from patients with apoplexia

Enzyme activity[a]	Collagenase-like peptidase (nmoles · min^{-1} · ml^{-1})	Dipeptidyl aminopeptidase IV (nmoles · min^{-1} · ml^{-1})	Correlation coefficient between collagenase-like peptidase and dipeptidyl-aminopeptidase IV
Normal control	0.60 ± 0.03 (55)	70.1 ± 0.37 (117)	
Rheumatoid arthritis	0.36 ± 0.01[b] (107)	45.0 ± 1.37[b] (101)	0.4636[c]
Apoplexia	0.54 ± 0.03 (42)	75.9 ± 3.31 (45)	0.4634[c]

Numbers of patients in parentheses. [a] Values are mean ± SEM; [b] differs from control, $p < 0.01$; [c] statistically significant, $p < 0.01$.

The activities of CL-peptidase and DAP-IV in the sera from normal controls, from patients with rheumatoid arthritis and from patients with apoplexia are shown in the table. The activities of CL-peptidase and DAP-IV were significantly lower in patients with rheumatoid arthritis. The lowered activities are probably not due to decreased ADL, since patients with apoplexia, with a similar degree of decreased ADL, did not show any significant decrease in the two enzyme activities.

Significant positive correlation was found between the activity of CL-peptidase and that of DAP-IV in patients with rheumatoid arthritis or apoplexia (fig.) ($r = 0.655$, $p < 0.01$). There was no significant correlation between serum CL-peptidase activity and age (from 35 years to 80 years).

These results indicate that the activities of CL-peptidase and DAP-IV are decreased in the serum of patients with rheumatoid arthritis. This suggests that the degradation of collagen may be decreased in rheumatoid arthritis.

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Sympathectomy enhances the substance P-mediated breakdown of the blood-aqueous barrier in response to infrared irradiation of the rabbit iris[1]

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Summary. Rabbits were subjected to infrared irradiation of the iris 1 month after unilateral cervical sympathectomy. The resulting breakdown of the blood-aqueous barrier was greatly enhanced on the sympathectomized side. In contrast, the response to intravitreally injected substance P (SP) was the same in both eyes. The enhancement of the response to IR irradiation could be abolished by pretreatment with an SP antagonist, (D-Pro^2, D-Trp^{7,9})-SP.

Key words. Rabbit iris; cervical sympathectomy, blood-aqueous barrier; infrared irradiation; substance P antagonist; miosis.

The response of the eye to local injury includes miosis (constriction of the pupil) and breakdown of the blood-aqueous barrier, with consequent leakage of protein into the aqueous humor.

The response of the rabbit eye to laser irradiation of the pigmented iris appears to be mediated partly by E-type prostaglandins (PGs) and partly by a noncholinergic neurogenic factor[3,4]. Similar mechanisms seem to be involved in the breakdown of the blood-aqueous barrier evoked by infrared irradiation (IR) of the rabbit iris, since this breakdown is reduced after topical or retrobulbar anesthesia[5] and after indomethacin[6]. However, Butler and Hammond[7] emphasized the key role of the neurogenic pathway in the response to all types of noxious stimuli, since trigeminal denervation was found to abolish the response not only to laser irradiation of the iris[8] but also to PGE_1. The response to substance P (SP) was not abolished. SP has been put forward as a candidate mediator of the ocular response to injury, since it evokes symptoms of ocular injury[9–11], and since it exists in the trigeminal nerve from which it is released into the aqueous humor upon stimulation[9]. Moreover, destruction of the trigeminal ganglion is followed by reduced SP levels in the uvea[12]. Also, a substance P antagonist, (D-Pro^2, D-Trp^{7,9})-SP, has been shown to reduce the breakdown of the blood-aqueous barrier to IR of the iris[11] and to PGE_2[13] and PGE_1[14].

Following sympathectomy, the ocular response to laser irradiation of the iris is increased[15,16]. Concomitantly, the SP levels in the uvea of the rabbit are raised significantly[17]. This gives indirect support to the theory that SP plays a key role in the ocular response to injury.

In the present study, the miosis and breakdown of the blood-aqueous barrier caused by IR of the iris was studied after sympathectomy and the involvement of SP was tested by the use of an SP antagonist.

Methods. Five adult pigmented rabbits (3–5 kg) underwent extirpation of the right cervical ganglion during pentobarbital (Mebumal®) anesthesia. Successful sympathectomy was indicated 3 weeks later by the supersensitivity of the mydriatic response to topically applied phenylephrine (Neosynephrine®). 1 month after sympathectomy the rabbits were subjected to IR of the iris of both eyes for 2 min. For details see Dyster-Aas and Krakau[18]. The pupil diameter was measured with a transparent plastic ruler under standardized light conditions immediately after IR and then at 30-min intervals. The time course of the barrier damage was followed by photoelectric measurement[6] of the aqueous flare response (AFR) every 30 min. This response is a Tyndall phenomenon, reflecting protein leakage into the anterior chamber. A correlation between the AFR and the protein concentration has been established[19]. The results are expressed in arbitrary units with reference to a standard. A week later the right eye was pretreated with 300 nmoles (D-Pro^2, D-Trp^{7,9})-SP, topically every 15 min for 1.5 h before IR of the iris of both eyes, and the AFR was measured. 1 month later the same animals were given 30 pmoles (9 µl) SP intravitreally to both eyes.

Results. The pupil of the sympathectomized eye was significantly smaller than that in the control eye before IR of the iris, 4.9 mm ± 0.33 versus 6.5 ± 0.22, $p < 0.0025$ (Student's t-test). There was no significant difference in the IR-induced pupillary