

# Gold sodium thiomalate activates latent human leukocyte collagenase

Seppo Lindy, Timo Sorsa, Kimmo Suomalainen and Heikki Turto

*Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10, SF-00170 Helsinki, Finland*

Received 27 August

Gold sodium thiomalate, a drug used widely in the therapy of rheumatoid arthritis, was found to be an activator of latent human polymorphonuclear leukocyte collagenase. The activation was demonstrated by two distinct and independent collagenase assays: (i) by recording with a spectrophotometer at 227 nm the enzyme-induced increase in ultraviolet difference absorbance of native type I collagen connected to the cleavage of collagen at 37°C [(1986) *Eur. J. Biochem.* 156, 1–4] and (ii) by SDS-polyacrylamide gel electrophoresis analysis of formation of specific products of collagen resulting from collagenase cleavage at 25°C. Activation of latent collagenase by gold sodium thiomalate appeared to be of the same magnitude as by the known activator phenylmercuric chloride.

*Latent human leukocyte collagenase    Gold sodium thiomalate    Activation*  
*Ultraviolet difference absorbance technique*

## 1. INTRODUCTION

In rheumatoid arthritis the degradation of cartilage leading to erosion is believed to result from action of vertebrate collagenase(s) (EC 3.4.24.7). In clinical studies the antierosive effect of gold therapy has been established [1,2]. We report here that gold sodium thiomalate is surprisingly a potent activator of latent human leukocyte collagenase.

## 2. MATERIALS AND METHODS

Latent human leukocyte collagenase was purified as described [3–5]. A new spectrophotometric collagenase assay, recently described by us, based on an enzyme-induced increase in ultraviolet difference absorption at 227 nm of native type I collagen in solution was used [5]. The change in ultraviolet difference absorption was converted to mol of collagen degraded by the molar difference absorbance value ( $\Delta\epsilon_{227}$ ) of 465 000 l·mol<sup>-1</sup>·cm<sup>-1</sup> [5]. Collagenase activity was also measured by using SDS-polyacrylamide

gel electrophoresis (SDS-PAGE) for separation of the characteristic cleavage products from intact parent collagen [3]. The substrate solution consisted of 1.5  $\mu$ M rat type I collagen in 50 mM Tris-HCl, 0.2 M NaCl, 5 mM CaCl<sub>2</sub>, 1 M glucose, pH 7.5. Gold sodium thiomalate (GST) was purchased from Aldrich Chemie (Steinheim, FRG). Phenylmercuric chloride (PMC) was from EGA-Chemie (Steinheim/Albuch, FRG). Thiomalic acid was from Sigma (St. Louis, MO). All other chemicals were of reagent grade.

## 3. RESULTS

Latent human leukocyte collagenase was treated with 1.5 mM gold sodium thiomalate for 10 min at 37°C. After this samples were added into tandem cuvettes and the ultraviolet difference absorption at 227 nm was measured [5]. Tandem cuvettes were used as follows: the sample to be analyzed was added into (i) the substrate compartment of the sample cuvette and (ii) the buffer compartment of the reference cuvette. In this way concentrations were equal in both the sample and reference

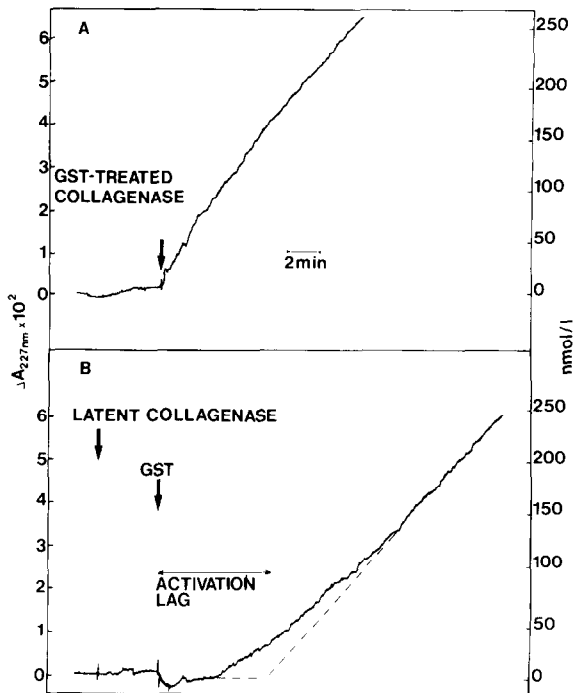


Fig.1. (A) Change in ultraviolet difference absorbance at 227 nm of collagen in solution at 37°C after addition of 1.5 mM GST-treated leukocyte collagenase (10  $\mu$ g enzyme protein). (B) Demonstration of the activation lag after addition of 1.5 mM GST to the mixture of latent leukocyte collagenase (10  $\mu$ g enzyme protein) and collagen. 1.5  $\mu$ M native type I collagen in buffer solution of 50 mM Tris-HCl, 0.2 M NaCl, 5 mM  $\text{CaCl}_2$ , 1 M glucose, pH 7.5, was used as a substrate.

lightpaths [5]. The change in ultraviolet difference absorption at 227 nm was followed with a Beckman recording spectrophotometer model DK at 37°C; 10% substrate was degraded in about 6 min (fig.1A).

When latent collagenase was added similarly into the tandem cuvettes, no change in ultraviolet difference absorption occurred (fig.1B). However, after addition of 1.5 mM GST, an increase in ultraviolet difference absorption of equal magnitude as in fig.1A was observed after a few minutes lag period (fig.1B).

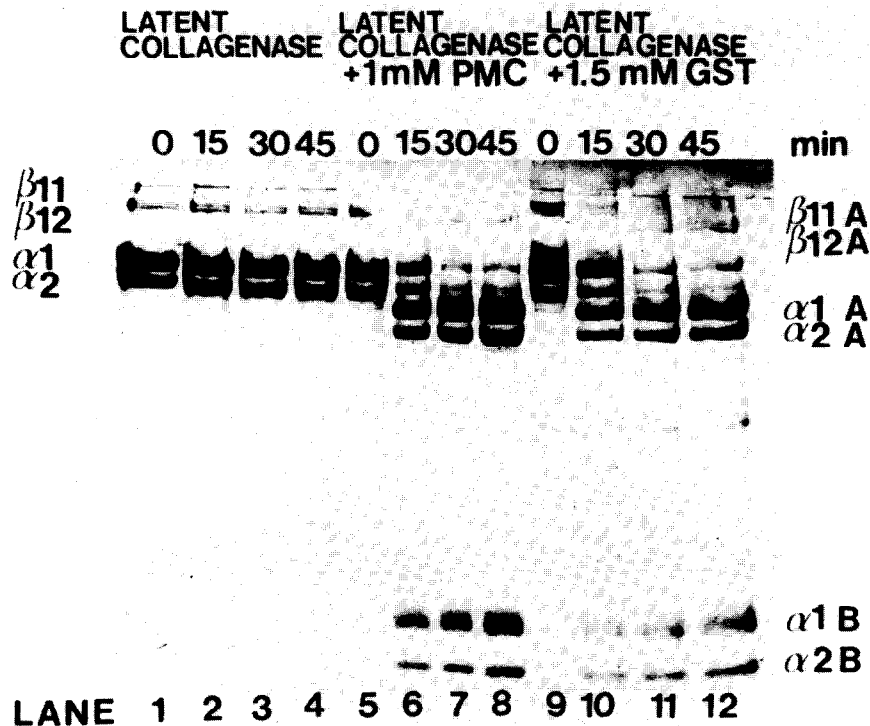


Fig.2. SDS-PAGE (10%) analysis of the effect of PMC and GST on latent human leukocyte collagenase. Latent enzyme (10  $\mu$ g enzyme protein) was treated with buffer, 1 mM PMC and 1.5 mM GST for 10 min at 37°C and then incubated with 1.5  $\mu$ M native type I collagen in 50 mM Tris-HCl, 0.2 M NaCl, 5 mM  $\text{CaCl}_2$ , 1 M glucose, pH 7.5, at 25°C for indicated time periods.  $\beta_{11}$  and  $\beta_{12}$  indicate undegraded dimers and  $\alpha_1$  and  $\alpha_2$  indicate undegraded monomers of collagen.  $\beta_{11A}$  and  $\beta_{12A}$  indicate 3/4-degradation products of dimers.  $\alpha_1A$  and  $\alpha_2A$  indicate 3/4-degradation products of monomers;  $\alpha_1B$  and  $\alpha_2B$  indicate 1/4-degradation products of monomers.

In fig.2 the activation of latent leukocyte collagenase is followed by SDS-PAGE [3]. Without any activator no formation of cleavage products of collagen could be seen (fig.2, lanes 1–4). However, when latent enzyme was treated with 1 mM PMC, known to activate latent human leukocyte collagenase [4–7], or with 1.5 mM GST, the SDS-PAGE analysis showed that the intact  $\beta$ - and  $\alpha$ -chains were converted to characteristic cleavage products  $\beta$ A-,  $\alpha$ A- and  $\alpha$ B-chains (fig.2, lanes 5–8 for PMC; lanes 9–12 for GST), indicating that both compounds activated the latent enzyme. 1.5 mM thiomalate did not activate or inhibit human leukocyte collagenase.

#### 4. DISCUSSION

Present data indicate that GST is a potent activator of latent human leukocyte collagenase. The results also show, that the ultraviolet difference absorption technique [5] is a rapid and convenient way to study the effects of activators and/or inhibitors on collagenases.

The mechanism of activation of latent human leukocyte collagenase by GST is unclear, as is the general activation mechanism(s) of leukocyte collagenase [4,6–10]. Latent human leukocyte collagenase can be activated by mercurial compounds [4,6,7], probably reacting with sulfhydryl groups of the latent enzyme. Organic gold (I) compounds have affinity towards thiol groups [11,12]; attack on the free sulfhydryl groups of the enzyme by the heavy metal may lead to activation of the enzyme by a conformational change [4,6], which might be followed by a cleavage of the polypeptide chain [13].

Although variable effects of gold salts on the function of collagenases have been reported [14–16], in the conditions used by us GST-treated enzyme remained active for several hours. The significance of our finding in vivo, when the latent and active collagenases are exposed to a variety of proteases, remains to be seen.

#### ACKNOWLEDGEMENTS

This work was supported by grants from Emil Aaltonen Foundation, Finnish Dental Society and Medical Scientific Foundation. The authors wish to thank Ms Kaija Tiilikka and Ms Annikki Saulamo for excellent secretarial work.

#### REFERENCES

- [1] Research Subcommittee of the Empire Rheumatism Council (1961) *Ann. Rheum. Dis.* 20, 315–334.
- [2] Sigler, J.W., Bluhm, G.B., Duncan, H., Sharp, J.T., Ensign, D.C. and McCrum, W.R. (1974) *Ann. Intern. Med.* 80, 21–26.
- [3] Turto, H., Lindy, S., Uitto, V.-J., Wegelius, O. and Uitto, J. (1977) *Anal. Biochem.* 83, 557–569.
- [4] Sorsa, T., Suomalainen, K., Turto, H. and Lindy, S. (1985) *Med. Biol.* 63, 66–72.
- [5] Lindy, S., Sorsa, T., Suomalainen, K., Lauhio, A. and Turto, H. (1986) *Eur. J. Biochem.* 156, 1–4.
- [6] Uitto, V.-J., Turto, H., Huttunen, A., Lindy, S. and Uitto, J. (1980) *Biochim. Biophys. Acta* 613, 168–177.
- [7] Murphy, G., Bretz, U., Baggiolini, M. and Reynolds, J.J. (1980) *Biochem. J.* 192, 517–525.
- [8] Tschesche, H. and Macartney, H.W. (1981) *Eur. J. Biochem.* 120, 183–190.
- [9] Weiss, S.J., Peppin, G., Ortiz, X., Ragsdale, C. and Test, S.T. (1985) *Science* 227, 747–749.
- [10] Hasty, K.A., Hibbs, M.S., Kang, A.H. and Mainardi, C.L. (1986) *J. Biol. Chem.* 261, 5645–5650.
- [11] Sadler, P.J. (1976) in: *Structure and Bonding* vol.29 (Dunitz, J.D., Hemmerich, P., Ibers, J.A., Jørgensen, C.K., Neilands, J.B., Reinen, D. and Williams, R.J.P. eds) pp.171–219, Springer, Berlin.
- [12] Brown, K.R., Parish, R.V. and McAuliffe, C.A. (1981) *J. Am. Chem. Soc.* 103, 4943–4945.
- [13] Stricklin, G.P., Jeffrey, J.J., Roswit, W.T. and Eisen, A.Z. (1983) *Biochemistry* 22, 61–68.
- [14] Wojtecka-Lukasik, E. and Danciewicz, A.M. (1974) *Biochem. Pharmacol.* 23, 2077–2081.
- [15] Woolley, D.E., Glanville, R.W., Roberts, D.R. and Evanson, J. (1978) *Biochem. J.* 169, 265–276.
- [16] Spalding, D.M., Darby, W.L., iii and Heck, L. (1986) *Arth. Rheum.* 29, 75–81.