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The action of collagen on an inorganic pyrophosphatase in blood platelets

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SUMMARY

Intact human blood platelets suspended in an isoosmotic medium had no pyrophosphatase activity on inorganic pyrophosphate in the ambient fluid. In the presence of collagen sodium pyrophosphate was rapidly hydrolysed. This action was inhibited by NaF. Collagen may activate a latent inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1).

Collagen, guanine compounds and phosphoribosyl pyrophosphate (PRPP) were obtained from Sigma, [8–^{14}C] guanine from the Radiochemical Centre, Amersham; other reagents were B.D.H. Analar chemicals.

Collagen suspension was prepared by soaking 50 mg collagen in 10 ml of distilled water overnight at 4°. The ice-cooled suspension was homogenised for 1 min by an ultraterrex blender. Washed blood platelets were isolated as described by Ireland[1] and resuspended in isotonic saline (10^7/ml).

Inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) activity was assayed by adding 0.1 ml of platelet suspension to 1.0 ml of substrate solution containing 4 mM sodium pyrophosphate, 6 mM $MgCl_2$, 30 mM Tris–HCl, pH 7.3, and 130 mM NaCl. The mixture was incubated at 22° for 30 min with frequent shaking. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid containing 10 mM $CuSO_4$, and inorganic phosphate was determined in the deproteinised solution[2]. A similar assay included 0.05 ml collagen suspension in the substrate. The blank reaction for pyrophosphatase measurement contained no pyrophosphate. Contaminant orthophosphate in the collagen suspension was checked by an assay in which collagen was added after the trichloroacetic acid reagent. All the assays were done in duplicate. The experiment was performed on five different samples of platelets.

Collagen induced a pyrophosphatase activity of 0.112 ± 0.008 μmole phosphate liberated per mg platelet protein per min. No inorganic pyrophosphatase activity was detected without collagen or if collagen was added to a substrate containing 10^{-14} M NaF

The inorganic pyrophosphatase induced by collagen could be due to activation of a latent catalytic site or extrusion of an intracellular enzyme. The following experiment suggests the first explanation but does not exclude the second.

Platelets contain purine phosphoribosyltransferases (EC 2.4.2.8) which can catalyse: guanine + PRPP ⇌ GMP + PP_i. Hydrolysis of PP_i would increase the formation of GMP by removal of a potent inhibitor[3,4] and by mass action. Washed platelets were suspended (10^6/ml) in a medium containing 120 mM NaCl, 13.2 mM Tris–HCl, pH 7.4, 15.4 mM KCl, 2 mM $MgSO_4$, 6 mM glucose and carrier-free [^{14}C]guanine (55 mCi/μmole, 200 000 counts/min per ml). The mixture was incubated for 30 min at room temperature. 0.05 ml of collagen suspension was added to 1.0 ml of the mixture. The tubes were shaken for 3 min and further incubated at 37° for 15 min. Tubes without collagen were similarly treated. The tubes were then put in boiling water for 1 min. Proteins were separated by centrifugation. 0.1 ml of the supernatant was resolved on cellulose thin-layer chromatoplates developed in butanol–acetone–acetic acid–water–ammonia (35:25:15:25:2, by vol.). Areas corresponding to carriers (guanine, guanosine, GMP, GDP, GTP) were scraped for radioactivity measurement by liquid scintillation counting[5]. The distribution of radioactivity was as follows:

Control: guanine 90.18%, guanosine 0%, GMP 7.75%, GDP + GTP 2.07%.

Collagen: guanine 74.9%, guanosine 0%, GMP 23.1%, GDP + GTP 2.0%.

Collagen increased the incorporation of guanine in GMP by 3-fold. Assay of guanine phosphoribosyl transferase[6] excluded direct action of collagen on the enzyme.

The activation of inorganic pyrophosphatase by collagen and its inhibition by fluoride could be due to the water structure promoting effect of the former[7] and structure disruption by the latter[8]. Restriction of rotational and translational movements of water molecules by collagen may loosen a protein hydrophobic bonding concealing a pyrophosphatase site. A water structure breaker would increase the entropy change responsible for hydrophobic bonding.

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