Inhibition of Ultraviolet-Induced Hydroxyproline Formation in Human Dermal Collagen

In previous studies[1,2] it has been demonstrated that UV-irradiation provokes an increase in hydroxyproline content of human dermal collagen. In addition to this UV-induced hydroxylation of proline, viscosity of collagen solution is reduced proportionally to the irradiation time. Although collagen research – so far – supplies no data which permit us to assume a direct connexion between these two effects described above, investigations seemed warranted to rule out such connexion. Therefore, irradiation experiments have been performed in vitro, under the influence of an inhibitor of hydroxyproline formation, i.e. dipyridyl[3,4].

Material and methods. Dermal tissue of human abdominal skin was separated from adherent subcutis and epidermis, minced, washed and extracted with 0.05 M phosphate buffer pH 7.2; thus, a solution of 'neutral salt soluble collagen' was obtained[5]. Into parts of this collagen solution ('control'), dipyridyl (2, 2'-dipyridyl = 2, 2'-bipyridin, $C_{10}H_8H_2$, $M = 156.19$) was introduced in concentrations of 10^{-3} and $10^{-5} M$. A $10^{-3} M$ solution of dipyridyl contains 156.19 µg/ml. All 3 solutions were irradiated with a Hanau S 200 from a distance of 20 cm under steady stirring and cooling. For 30 min, samples were withdrawn every 5 min. Changes in hydroxyproline content (method of STEGEMANN[6]) and changes in viscosity (Ostwald viscosimeter) were registered. All values collected are given as the mean of triple investigations, obtained in 5 irradiation experiments.

Results. 1. Hydroxyproline content: The initial value of hydroxyproline measured 36.5 µg/ml and was slightly higher than in the previous series[1]. UV-irradiation caused a statistically significant increase in hydroxyproline content to 47.4 µg/ml (+ 33% of control value, $P < 0.05$!). In the presence of $10^{-3} M$ dipyridyl, UV-irradiation provoked a weak augmentation in hydroxyproline content, only (39.7 µg/ml after 30 min irradiation). The values obtained in the experiments with $10^{-5} M$ dipyridyl did not differ significantly from the controls: after an irradiation of 30 min, hydroxyproline content measured 45.8 µg/ml. In Figure 1, the increase in hydroxyproline content is depicted over the 30 min of the experiments.

2. Viscosity: Viscosity of the neutral salt soluble collagen solution measured 3.3 sec; an irradiation for 30 min decreased this value to 0.6 sec. The presence of dipyridyl 10^{-3} or $10^{-5} M$ did not have any significant influence on this UV-induced decrease in viscosity. The diminuition of viscosity of dermal collagen solution under the influence of UV-light is depicted in Figure 2.

Discussion. The results demonstrate clearly that hydroxylation of proline (peptide-bound proline) under the influence of UV-light is inhibited by dipyridyl; a similar effect has already been encountered by other autors[3,4]. In vivo, hydroxyproline may be formed either directly via hydrogen peroxide or enzymatically[7,8]. In human dermal collagen both mechanisms may occur, since UV-irradiation is capable of provoking the formation of radical structures in the dermal layers[9], and it seems reasonable to assume that such energy may elicit the formation of peroxides, too. In the presence of strongly active chelating agents, UV-light provokes a weak increase in hydroxyproline content of neutral salt soluble collagen. It still remains to be elucidated over which mechanisms this 'residual' increase occurs.

UV-irradiation provokes a degradation of the primary collagen chains; thus, viscosity of collagen solutions is reduced[10]. In the presence of dipyridyl, viscosity is reduced as readily as in the experiments without chelating agent.

It may be concluded from the results collected in this study that oxydation of proline and degradation of the primary collagen chains – which both occur under the influence of UV-rays – are not directly connected.

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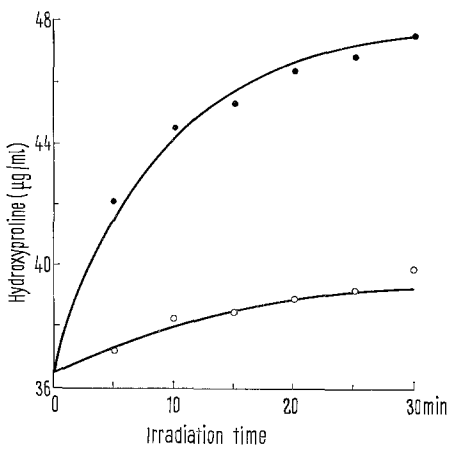

Fig. 1. The increase in hydroxyproline content of human dermal collagen following irradiation in vitro, as inhibited by dipyridyl. ●—●, untreated control; ○—○, with dipyridyl $10^{-3} M$.

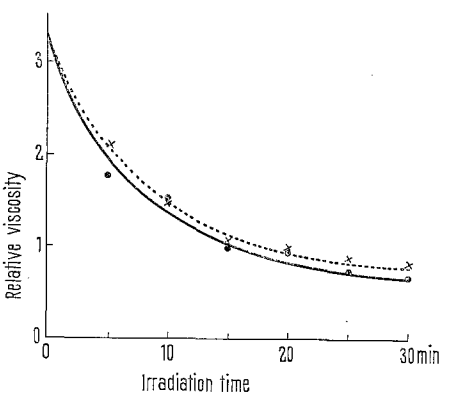

Fig. 2. The decrease in viscosity of neutral salt soluble collagen from human dermis following irradiation in vitro. ●—●, control; ×·····×, dipyridyl $10^{-3} M$.

Zusammenfassung. In Gegenwart des Chelatbildners Dipyridyl ist die unter der Einwirkung von UV-Bestrahlung eintretende Oxydation von Prolin zu Hydroxyprolin gehemmt, wie hier am Beispiel von neutralsalzlöslichem dermalem Kollagen des Menschen gezeigt werden konnte. Die unter den gleichen Bedingungen einsetzende Degradation der primären Kollagenketten wird jedoch durch die Gegenwart von Dipyridyl nicht gestört, so dass ein direkter Zusammenhang zwischen den beiden Effekten ausgeschlossen werden kann.

W. P. Raab

Department of Medical Chemistry, Vienna University Medical School, Waehringerstrasse 10, A-1090 Vienna (Austria), 20 April 1970.

The Effect of Anaerobic Incubation Upon 2,3-Diphosphoglycerate Synthesis in vitro

It has been observed that 2,3-diphosphoglycerate (2,3-DPG) binds with greater affinity to reduced than to oxygenated adult human hemoglobin[1–4]. The binding of 2,3-DPG by deoxyhemoglobin within the adult human erythrocyte relieves 2,3-diphosphoglycerate mutase (D-1,3-diphosphoglyceric acid: D-3-phosphoglycerate phosphotransferase, EC 2.7.5.4) from inhibition by its product and facilitates further synthesis of 2,3-DPG[5]. While studying the effects of added deoxy- and carboxyhemoglobin upon 2,3-DPG synthesis, it was noted that the generation of 2,3-DPG from fructose-1,6-diphosphate was accelerated under nitrogen even in the absence of added reduced hemoglobin[6]. It is the purpose of this communication to describe the mechanism of this effect.

Hemoglobin-free enzyme mixture which contained red cell 2,3-DPG mutase and was free of 2,3-DPG phosphatase (D-2,3-diphosphoglycerate 2-phosphohydrolase, EC 3.1.3.13), was prepared according to the method described elsewhere[5]. Synthesis of 2,3-DPG was studied in an incubation medium containing red cell 2,3-DPG mutase; fructose-1,6-diphosphate, 100 μmoles; NAD, 20 μmoles; 3-phosphoglycerate, 10 μmoles; EDTA, 35 μmoles; triethanolamine buffer, pH 7.6, 360 μmoles; potassium disodium phosphate, 70 μmoles; aldolase (ketose-1-phosphate aldehyde-lyase, EC 4.1.2.7), 9 units; and glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate: NAD oxidoreductase, EC 1.2.1.12, G-3-PD), 36 units; in a total volume of 10.5 ml. The generation of 2,3-DPG was studied in air, 100% nitrogen and 100% carbon monoxide. Incubation mixtures were allowed to equilibrate with the appropriate atmosphere for 30 min prior to the initiation of the reaction by the addition of fructose-1,6-diphosphate. Compressed air, nitrogen and carbon monoxide were washed via a fritted glass dispersion tube immersed in distilled water prior to delivery into the reaction mixture. One ml samples were removed periodically and deproteinized by immersion in boiling water. The supernatants were assayed for 2,3-DPG using the Schroter and Heyden[7] modification of the method devised by Krimsky[8].

The rates of synthesis of 2,3-DPG in incubation mixtures supplemented with enzyme protein containing adult red cell 2,3-DPG mutase are shown in Figure 1. The total synthesis of 2,3-DPG was approximately 18% greater in anaerobic incubation mixtures, maintained under either nitrogen or carbon monoxide, than in mixtures incubated in air. The increase in 2,3-DPG synthesis induced by anaerobic incubation was best observed at phosphate concentrations of 7 m*M* or greater. When phosphate was omitted from the reaction mixture only approximately 15% as much 2,3-DPG was synthesized and the differential effect of anaerobic incubation was not observed.

While 2,3-DPG synthesis appears to be a function of phosphate concentration, 2,3-DPG mutase is not known to be phosphate dependent[9]. However, G-3-PD is sensitive to phosphate concentrations[9,10]. G-3-PD activity

Effect of phosphate concentration upon glyceraldehyde-3-phosphate dehydrogenase activity

Phosphate concentration (m*M*)	Glyceraldehyde-3-phosphate reduced after 1 h (nmoles/ml)
0	32
0.7	39
3.5	58
7.0	72

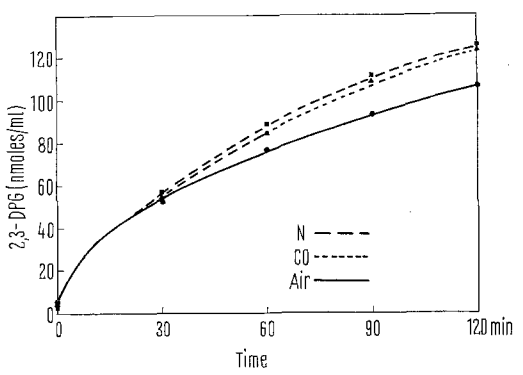

Fig. 1. The rate of 2,3-DPG synthesis in incubation mixtures maintained under air, carbon monoxide and nitrogen. Each point represents the average value of 5 experiments. See text for experimental details. In these experiments phosphate final concentration was 7 m*M*.

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