PLASMA HYDROXYPROLINE-CONTAINING PROTEIN (HYPRO-PROTEIN)

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1. Introduction

Hydroxyproline is present in only some proteins especially collagen and elastin [1]. LeRoy et al. [2] found hypro-proteins in plasma of some mammalian species which they suggested were derivatives of tissue collagens. Preliminary experiments [2, 3] showed plasma hypro-proteins to be heterogenous, they occurred in both the albumin and globulin fractions and migrated with various electrophoretic velocities.

In this work we have fractionated hypro-proteins from bovine plasma.

2. Materials and methods

Citrated bovine plasma was clotted with thrombin and the fibrin removed by centrifugation. The globulin fraction was then precipitated by 40% saturated $(NH_4)_2SO_4$. This 40% $(NH_4)_2SO_4$ fraction was subjected to several purification procedures.

2.1. *Gel-filtration*

The 40% $(NH_4)_2SO_4$ precipitate was dissolved in 0.05 M citrate buffer, pH 3.6, and dialysed overnight against the same buffer. The protein solution was applied to a Sephadex G-200 column (75 X 2.5 cm) and the proteins eluted with the same buffer. The flow rate was 20 ml/hr and 5 ml fractions were collected.

2.2. *Ion-exchange chromatography*

The 40% $(NH_4)_2SO_4$ precipitate was dissolved in 0.02 M citrate buffer, pH 4.4, and dialysed against the same buffer. The protein solution was applied to a CM-cellulose column (75 X 1 cm) and the proteins fractionated by stepwise elution with 0.0–0.5 M NaCl in the same buffer. Proteins remaining on the column were eluted with 0.5 M NaOH.

The pooled 0.2 M NaCl fractions from three CM-cellulose column runs were concentrated by vacuum dialysis against 0.05 M sodium bicarbonate buffer, pH 8.0, and the resulting protein solution applied to a DEAE-cellulose column (75 X 1 cm). The proteins were fractionated by stepwise elution with 0.0–0.5 M NaCl in the same buffer. Proteins remaining on the column were eluted with 0.5 M NaOH.

Protein concentrations were determined by the method of Lowry and others [4]. Hydroxyproline contents were assayed by the method of Prockop and Udenfriend [5], contaminating interfering material being separated from the protein hydrolysates by the method of LeRoy et al. [2]. Plasma protein digestions by pronase (Calbiochem, B-grade) were by the method of LeRoy et al. [2].

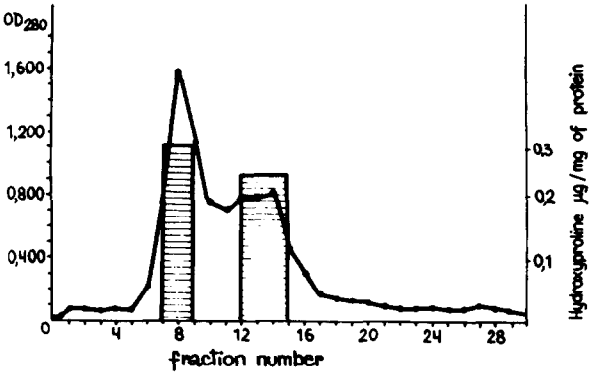

Fig. 1. Gel-filtration on Sephadex G-200 of plasma proteins insoluble in 40% saturated $(NH_4)_2SO_4$. Continuous line, optical density at 280 nm; hatched bars, hydroxyproline content. ▬ Eluant, 0.05 M citrate buffer, pH 3.6

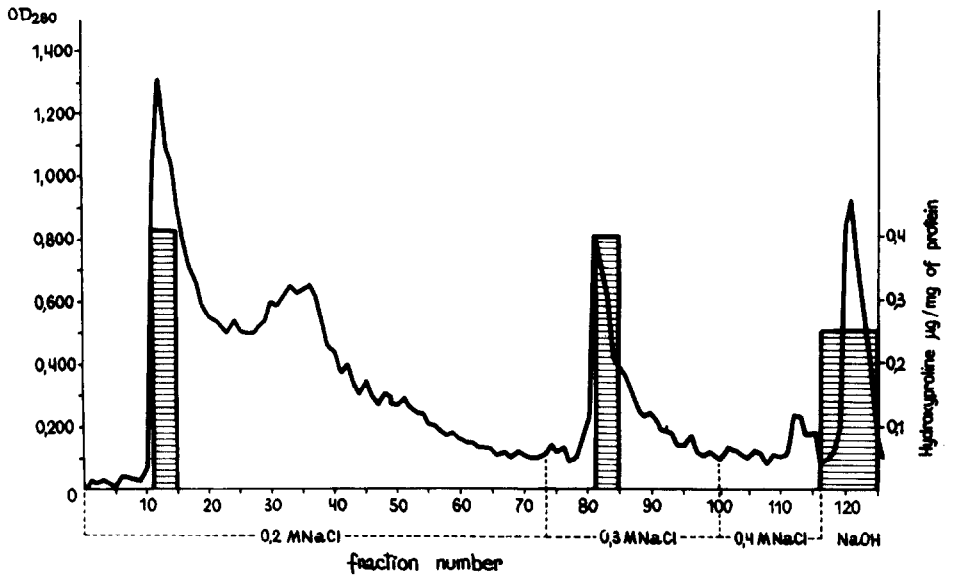

Fig. 2. Column chromatography on CM-cellulose of plasma proteins insoluble in 40% saturated $(NH_4)_2SO_4$. Continuous line, optical density at 280 nm; hatched bars, hydroxyproline content. Eluant, 0.02 M citrate buffer, pH 4.4, with added NaCl as shown. Last fractions eluted with 0.5 M NaOH.

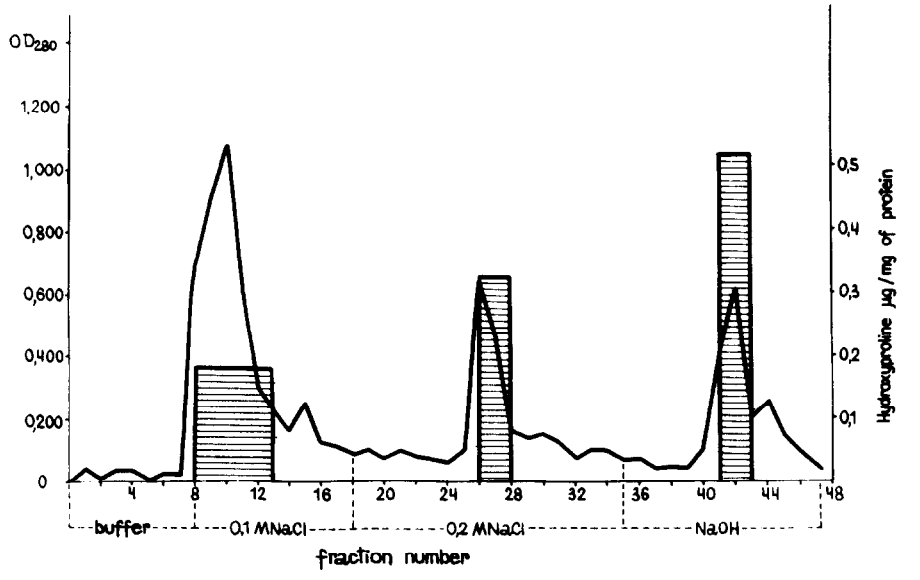

Fig. 3. Column chromatography on DEAE-cellulose of the fraction eluted by 0.2 M NaCl from the CM-cellulose column (fig. 2). Continuous line, optical density at 280 nm; hatched bars, hydroxyproline content. Eluant, 0.05 M sodium bicarbonate buffer, pH 8.0, with added NaCl as shown. Last fractions eluted with 0.5 M NaOH.

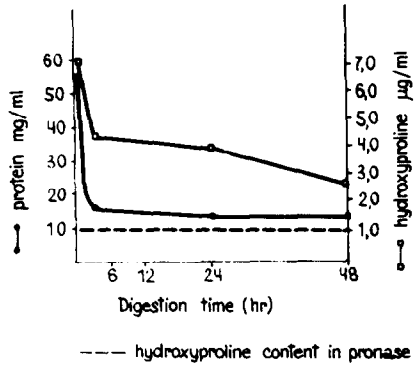

Fig. 4. Non-dialysable protein and hydroxyproline during the digestion of whole plasma proteins by pronase. Conditions as in LeRoy et al. [2].

3. Results and discussion

The plasma proteins precipitated by 40% $(NH_4)_2SO_4$ saturation can be fractionated into two hypro-protein components by gel-filtration (fig. 1) and into three components by CM-cellulose chromatography (fig. 2). The first fraction from CM-cellulose chromatography can be further resolved into three hypro-protein fractions by chromatography on DEAE-vellulose (fig. 3).

In fig. 4 are shown our results of the digestion of whole plasma proteins by pronase. In the first 6 hr the non-dialysable protein and hydroxyproline decreased by 75% and 36% respectively, results roughly in agreement with those of LeRoy et al. [2]. These authors found that after 6 hr digestion the non-dialysable hydroxyproline concentration remained constant and they argued that this was evidence for the existence in plasma of a specific hypro-protein, resistant to pronase. As can be seen from fig. 4, in our experiment the non-dialysable hydroxyproline concentration continued to decrease up to 48 hr; it had fallen by 72% in that time.

From the results presented here we conclude that various plasma proteins, especially globulins, contain small amounts of hydroxyproline. While we cannot exclude the possibility that collagen degradation products may contaminate all plasma protein fractions, we believe our results do not support the existence of a specific hypro-protein in plasma.

References

[1] I.Banga, Structure and function of elastin and collagen (Akademiai Kiado, Budapest, 1966).
[2] E.C.LeRoy, A.Kaplan, S.Udenfriend and A.Sjoerdsma, J. Biol. Chem. 239 (1964) 3350.
[3] A.Sjoerdsma, S.Udenfriend, H.Keiser and E.C.LeRoy, Ann. Internal Med. 63 (1965) 672.
[4] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[5] D.J.Prockop and S.Udenfriend, Anal. Biochem. 1 (1960) 228.