საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 16, №4, 2022
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 16, no. 4, 2022

*Biophysics*

# Influence of PH on Thermodynamic Stability of Rat Tail Type 1 Procollagen

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**We have carried out a DSC research of acid diluted procollagen of rat tail, which mainly contains type I collagen. The research was conducted within pH interval of 1.5 to 3.9 in presence of NaCl with physiological concentration. It is shown, that the melting process of procollagen in pH interval of 3.9-2.1 has two stages, and the melting process shifts to lower temperatures with decrease in pH, with minor changes in melting enthalpy (ΔHm). The melting parameters of procollagen at pH3.9 is $T_m^1$ = 32.6°C, $T_m^2$ = 36.7°C and ΔHm = 17.5±1 cal/g. Within pH interval of 1.7-1.5, the melting process has one stage, and at pH1.5 the melting parameters are: $T_m$ = 28.2°C, $\Delta T_m$ = 1.5°C, and $\Delta H_m$ = 15.2±0.9 cal/g. Decreasing in $\Delta H_m$ by 2 cal/g is in correlation with the dependence $\Delta H_m = f(t)$ that is commonly used for proteins. Thus, correspondence of the melting at pH1.5 with the melting enthalpy at pH3.9 and the high cooperativity of collagen melting process indicate that the individual triple helix chains of rat tail procollagen at pH1.5 preserves its native conformation. These results directly indicate that it is recommended to extract the procollagen at pH1.5 when collagen solubility is at least 10 times higher than at pH3.9. The abovementioned features of the rat tail procollagen, the physico-chemical parameters of which is close to all mammalian procollagens, and nativeness at pH1.5 are important as for pharmaceuticals so for food industry.** *© 2022 Bull. Georg. Natl. Acad. Sci.*

DSC, procollagen

Collagen is the major insoluble fibrous protein in the extracellular matrix and in the connective tissue. In fact, it is the single most abundant protein in the animal kingdom. There are, at least, genetically distinct 29 types of collagens. 80-90 percent of the collagen in the body consists of types I, II and III. These collagen molecules pack together to form long thin fibrils of similar structures. All collagens contain three polypeptide α-chains, and position of the polyproline II initiates right-handed supercoil conformation. Each polyproline chain has a repeating Gly-X-Y triplex which are held together by interchain hydrogen bonds [1]. A remarkable difference of collagen from globular proteins is the fact that, at increase in temperature above critical level, its regular structure breaks and the chains of

collagens separate and fold into random coils that do not have any residual structures [2]. The thermal stability of collagen expressed in its melting temperature (Tm) significantly depends on amino and amino acid composition, pH of solution, and presence of neutral salts in the solution [3-7]. This happens because collagen has both positive and negative charges. In a salt-free solution, procollagen melting proceeds in one stage with $T_m = 39 \pm 1°C$ [7]. However, in close to physiological conditions, in presence of 0.1M NaCl ion force of the solution, the melting process has 2 stages with $T_m = 33.0°C$ and $T_m = 37.8°C$. This process was studied at pH3.9 only [7,8]. It is worth mentioning that the thermodynamical stability of procollagen in presence and absence of NaCl 0.1M at pH3.9 is well studied, but the influence of various pH values on melting curve profiles and their thermodynamic parameters are not studied yet. In this work, we enlarged pH interval from 3.9 to 1.5 in order to better understand the procollagen unfolding process, which is important from both fundamental so practical viewpoints.

## Materials and Methods

In this work, we studied collagen extracted from the healthy rat tail tissue. Using the methods described in publication [7,8], the procollagen solutions were filtered through G-5 glass filter and centrifuged during 60 min at 20,000 x g. For clearing of collagen from polysaccharides, an additional treatment of the preparation was performed with chloroform. In our experiments, 0.1% solution of procollagen was centrifuged with 17,000 x g for 40 mins using centrifuge Prism R. In total, 96 DSC measurements were conducted on diluted procollagen solutions in 0.1 M NaCl with the pH range of 1.5 – 3.9. For evaluation of the isolated collagen, an absorption spectrum measurement in the wavelength range 200 to 300 nm was performed. The absence of absorption within the region 265 to 290 nm indicated absence of the globular proteins in our solution. The DSC investigation have been carried out with the DSC devise with sensitivity 0.5 μW [9]. The operative volume of measuring cells was 0.150 ml.

## Results and Discussion

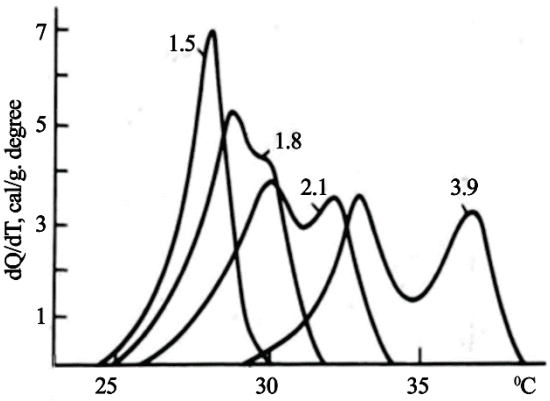


**Fig.** Dependence of heat absorption on temperature for type 1 procollagen solutions at various pH values (pH = 3.9, 2.1, 1.8, and 1.5), calculated for gram of collagen dray mass.

As it is shown in Figure, decreasing in pH causes shifting of the heat absorption integral curve towards lower temperatures. In addition, we can observe redistribution of heat between the heat absorption peaks. In particular, the melting process at pH3.9 proceeds with two clear temperature intervals with $T_m^1 = 32.6°C$ and $T_m^2 = 36.7°C$ with transition enthalpies $\Delta H_m^1 = 9.0$ cal/g and $\Delta H_m^2 = 8.4$ cal/g, accordingly, while in case of pH1.8, there is only one peak at 29.1°C and a shoulder at 30.0°C. This happens so because the high temperature peak shifts toward lower temperatures more effectively than the lower one, and only deconvolution of the curve makes it possible to define $\Delta H_m$ of these transition stages. At pH1.5, the process has one stage in a narrow temperature interval with transition parameters $T_m = 28.2°C$, $\Delta T = 1.5°C$, and $\Delta H_m = 15.2$ cal/g. Taking into consideration the well-known dependence of protein melting enthalpy on temperature (including procollagen and collagen solutions in case of reasonable protein concentrations), the calculated $\Delta H_m = 15.2$cal/g for PH1.5 is in a good agreement with the dependence

$\Delta H_m$ f(t) [2]. So, correspondence of the melting at pH1.5 with melting enthalpy at pH3.9 and the high cooperativity of collagen melting process indicate that the triplex structure of procollagen type I of rat tail is native at pH1.5. According to the published data [4, 10], those results directly indicate that extraction of procollagen is advisable at pH1.5 where collagen solubility is at least 10 times more than at pH3.9. The determined fact about the rat tail procollagen nativeness on pH1.5 (parameters of which are close for all mammalian procollagens) is important for cosmetics and medical industry.

*ბიოფიზიკა*

# pH-ის გავლენა ვირთხის კუდიდან მიღებული I ტიპის კოლაგენის თერმოსტაბილურობაზე

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ჩვენ ჩავატარეთ მჟავაში განზავებული ვირთხის კუდის პროკოლაგენის დსკ კვლევა, რომელიც ძირითადად შედგება I ტიპის კოლაგენისგან. კვლევა ჩატარდა pH-ის ინტერვალში 1,5-3,9, NaCl-ის თანაობითა და ფიზიოლოგიური კონცენტრაციით. ნაჩვენებია, რომ პროკოლაგენის დნობის პროცესი pH-ის 3,9-2,1 ინტერვალში მიმდინარეობს ორ ეტაპად და pH-ის შემცირებასთან ერთად ინაცვლებს დაბალი ტემპერატურებისკენ, დნობის ენტალპიის ($\Delta H_დ$) მცირე ცვლილებით. პროკოლაგენის დნობის პარამეტრები pH3,9-ის შემთხვევაში არის $T^1_დ = 32,6°C$, $T^2_დ = 36,7°C$ და $\Delta H_m = 17,5±1$ კალ/გ. pH-ის 1,7-1,5 ინტერვალში დნობა მიმდინარეობს ერთ ეტაპად, ხოლო pH1,5-ზე დნობის პარამეტრებია: $T_დ = 28,2°C$, $\Delta T_დ = 1,5°C$ და $\Delta H_დ = 15,2±0,9$ კალ/გ. $\Delta H_დ$-ის 2 კალ/გ-ით შემცირება კორელაციაშია ფუნქციასთან $\Delta H_დ = f(t)$, რომელიც ჩვეულებრივ გამოიყენება ცილებისთვის. ამრიგად, pH1,5-ზე დნობის ენტალპიის შესაბამისობა pH3,9-ზე დნობის ენტალპიასთან და კოლაგენის დნობის პროცესის მაღალი კოოპერატიულობა მიუთითებს, რომ ვირთხის კუდის პროკოლაგენის სამმაგი სპირალის ცალკეული ჯაჭვი pH1,5-ზე ინარჩუნებს ნატიურ კონფორმაციას. ეს შედეგები პირდაპირ აჩვენებს, რომ პროკოლაგენის

ექსტრაქცია რეკომენდებულია pH1,5-ზე, როდესაც კოლაგენის ხსნადობა მინიმუმ 10-ჯერ მაღალია, ვიდრე pH3,9-ის შემთხვევაში. ვირთხის კუდის პროკოლაგენის ზემოთ მოცემული თვისებები, რომელთა ფიზიკურ-ქიმიური პარამეტრები ახლოსაა ყველა ძუძუმწოვრის პროკოლაგენთან და ნატიურობა pH1,5-ზე მნიშვნელოვანია როგორც კოსმეტიკის, ასევე სამედიცინო ინდუსტრიაში.

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*Received July, 2022*