# ENTHALPY OF INTERPEPTIDE HYDROGEN BONDS IN COLLAGEN

$\Delta H_{CO..NH}$ = ?

**Background:** Collagen is the most abundant protein in mammals and a main component of the connective tissue matrix. Triple helical tropocollagen molecules are the main units of collagen. Tropocollagen is composed of repeating tripeptides -(Gly-X-Y)-, where X=Pro in 37,8% and Y=4-Hyp in 28,8% of cases. The factors of the collagen triple helix stability include interpeptide $N_1H_1\cdots C_2O_2$ hydrogen bonds, hydrogen bonds of $C_1O_1$ and $C_3O_3$ groups with water, hydrophobic and Van der Waals interactions.

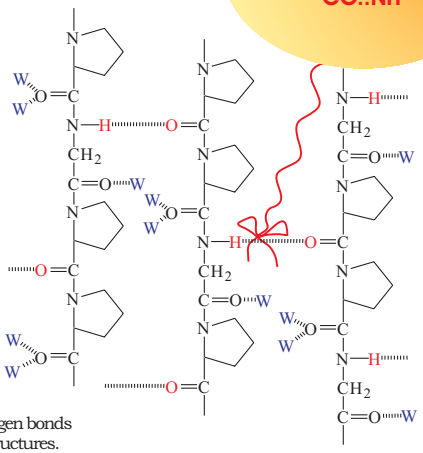

**Fig. 1.** Hydrogen bonds in collagen structures.

**Objective:** to determine the enthalpy of interpeptide $N_1H_1\cdots C_2O_2$ hydrogen bonds in collagen and poly(Pro-Gly-Pro).

## Theory

Enthalpy $\Delta H_{H\text{-}bond}$ of H-bonds, formed by C=O groups, is linearly dependent upon frequency shift $\Delta\nu_{H\text{-}bond}$ of correspondent stretching vibrations, caused by formation of these bonds [1],

$$\Delta H_{H\text{-}bond}=\Delta\nu_{H\text{-}bond}\cdot 0.205\ \text{kJ·cm/mol} \quad (1)$$

In our previous work [2] it was demonstrated that for $C_2O_2$ groups $\Delta\nu_{H\text{-}bond}=\nu^{hydr}-\nu_0-\Delta\nu_{res}=-37\pm5\ cm^{-1}$

Where

$\nu_0$ is frequency of unperturbed carbonyl vibration ($\nu_0$=1693 $cm^{-1}$ for $C_2O_2$ in poly(Pro-Gly-Pro) ) [3]

$\nu^{hydr}$ is the observed frequency of $C_2O_2$ streching vibrations for poly(Pro-Gly-Pro) in hydrated state $\nu^{hydr}$=1643±2 $cm^{-1}$ for $C_2O_2$ in poly(Pro-Gly-Pro) [3]

$\Delta\nu_{res}$=13±0.5 $cm^{-1}$ is the frequency shift of the carbonyl vibration caused by dynamic resonance interactions of carbonyl transition dipoles [2]

$$\Delta H_{CO...NH}=7.6\pm1.0\ \text{kJ/mol}$$

## Experiment (IR spectroscopy)

Dehydrated collagen structures retain the interpeptide $N_1\text{-}H_1\ldots O_2\text{=}C_2$ bond while the helical parameters of dehydrated triple helix differ from the ones observed in a well hydrated state [4].

The enthalpy of $N_1\text{-}H_1\ldots O_2\text{=}C_2$ bond in dehydrated state may be calculated by formula (2), where $\Delta\nu_{H\text{-}bond}=\Delta\nu^{dry}=\nu_0^{\nu}-\nu^{dry}$

$\nu_0$=1693 $cm^{-1}$ is the unperturbed frequency of $C_2O_2$ -group stretching vibrations (random coil in non-polar solvents) [3]

$\nu^{dry}$= 1664±2 $cm^{-1}$, it is the observed frequency of $C_2O_2$ -group stretching vibrations in dehydrated triple helix [3]

Thus $\Delta\nu^{dry}$= -28±2 $cm^{-1}$ and $\Delta H^{dry}$=5.74±0.4 kJ/mol

Hydration of the sample leads to down-shift of Amide I band frequency and up-shift of Amide II frequency, caused by conformational reorganization of the triples helix and strengthening the of interpeptide H-bond (Fig.2,3).

$\Delta\nu^{wet\text{-}\to dry}$=9±1 $cm^{-1}$ is the frequency shift of Amide I band caused by hydration of the sample ,

$\Delta H^{wet\text{-}\to dry}$=1.84±0.2 kJ/mol is growth of enthalpy of interpeptide H-bond upon hydration

$$I\Delta H_{CO...NH}=\Delta H^{dry}+\Delta H^{wet\text{-}\to dry}=7.58\pm1.2\ \text{kJ/mol}$$

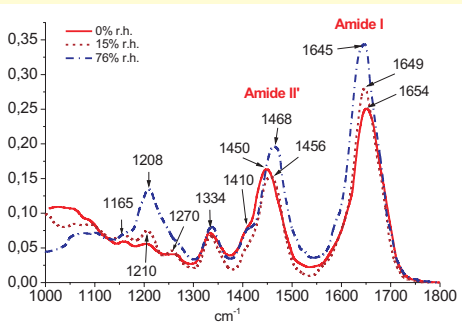


**Fig. 2.** IR spectrum of deuterated poly(Pro-Gly-Pro) films in dehydrated (0% r.h.) and hydrated (15% r.h., 76% r.h. ) states.

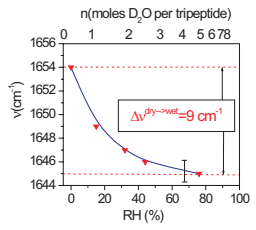


**Fig.3.** Frequency of Amide I band at different hydration levels in deuterated poly(Pro-Gly-Pro) film.

## Calculation of enthalpy of interpeptide hydrogen bond $\Delta H_{CO...NH}$

| Poly(Pro-Gly-Pro) | Collagen |
|---|---|
| Theoretical calculations | Calorimetry+ IR spectroscopy |
| 7.6±1.0 kJ/mol | 5.6±1.2 kJ/mol |
| Experiment (IR spectroscopy) | Calorimetry |
| 7.58±1.2 kJ/mol | 6.5±0.4 kJ/mol |
| Average values | |
| 7.6 ±1.2 kJ/mol | 6.0 ±1.2 kJ/mol |

## Calorimetry+IR spectroscopy

Dehydrated collagen retain the interpeptide $N_1\text{-}H_1\ldots O_2\text{=}C_2$ bond [4], enthalpy of $N_1\text{-}H_1\ldots O_2\text{=}C_2$ bond in dehydrated collagen $\Delta H^{dry}$ may be found from the dependence ΔH(n) of enthalpy of helix-coil transition from the rate of the sample hydration (Fig. .6)obtained in [5]

$$\Delta H^{dry}=2.93\pm0.4\ \text{kJ/mol}$$

As well as in poy(pro-Gly-Pro), hydration of collagen leads to down-shift of Amide I band frequency and up-shift of Amide II frequency, caused by conformational reorganization of the triples helix and strengthening of the interpeptide H-bond.

$\Delta\nu^{wet\text{-}\to dry}$=13±1 $cm^{-1}$ is the frequency shift of Amide I band caused by hydration of the sample

$\Delta H^{wet\text{-}\to dry}$=2.67±0.2 kJ/mol is the correspondent growth of enthalpy of interpeptide H-bond

$$\Delta H_{CO...NH}=5.6\pm1.2\ \text{kJ/mol}$$

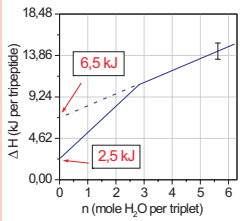


**Fig. 4.** Dependence of enthalpy of collagen helix-coil transition upon hydration level [5].

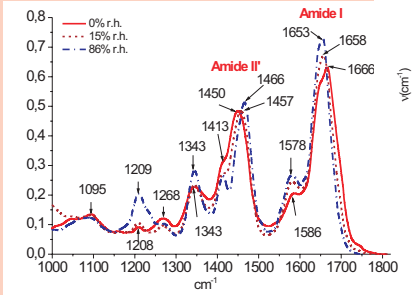


**Fig. 5.** IR spectrum of deuterated collagen films in dehydrated (0% r.h.) and hydrated (15% r.h., 86% r.h. )

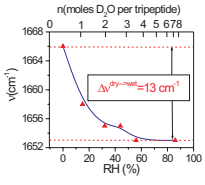


**Fig.6.** Frequency of Amide I band at different hydration levels in deuterated collagen film.

## Calorimetry

To exclude the impact of bound water into the total enthalpy of collagen helix-coil transition, we have performed extrapolation of the dependence ΔH(n) to n=0 (Fig. 6) and thus obtained the enthalpy of interpeptide H-bond $\Delta H_{CO...NH}$=6.5±0.4 kJ/mol

**Conclusion:** Enthalpy of interpeptide $N_1H_1\cdots C_2O_2$ hydrogen bond were calculated for poly(Pro-Gly-Pro) and collagen. The results obtained can be used for evaluation of the impacts of energies of interpeptide hydrogen bonds into the total energy of stabilization of native triple helical collagen and poly(Pro-Gly-Pro).


**References:**

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5) Gasan, A. I. *et al.* (1990). **Studia Biophysica, 136 (2-3)**, 171-178.



**Ye. P. Boryskina,** Institute of Radiophysics and Electronics NAS of Ukraine, Department of Biophysics
61085, Kharkov, Ac. Proskura str, 12. E-mail: selena@ire.kharkov.ua, WWW: www.ire.kharkov.ua