Effect of pH and Guanidine Hydrochloride on the Conformation of 57 kDa Rat Liver Nuclear Thyroid Hormone Binding Protein Measured by Fluorescence

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The denaturation of the 57 kilodalton (kDa) rat liver nuclear thyroid hormone binding protein (NTHB) by pH and guanidine hydrochloride (GdnHCl) has been investigated with the fluorescence method. The acid and alkaline fluorescence quenching suggests that the structure of NTHB is invariant in the relatively narrow pH region of approximately pH 7—9. A cooperative conformational transition occured in GdnHCl concentrations of 1.5—2.5 M. The apparent free energy of unfolding of NTHB, $\Delta G^{H_2O}_{app}$ was evaluated as 6.31 (±0.12) kcal·mol^{-1} at pH 7.7, 25 °C.

Keywords protein denaturation; nuclear thyroid hormone binding protein; guanidine hydrochloride; fluorescence

Introduction

The thyroid hormone is known to play a fundamental role in regulating mammalian development and metabolism in the nucleus through binding to the nuclear non-histone proteins.[1-3] Two molecular species with molecular weights of 57—47 kilodalton (kDa) have been reported as nuclear receptors.[4-6] Some physicochemical studies of these receptors have been reported,[4-19] but their structural features are not well known. In a preceding paper,[17] we reported the preparation and some physicochemical properties of the 57 kDa nuclear thyroid hormone binding protein (NTHB) from rat liver nuclei. In this study, the effect of pH and guanidine hydrochloride on the conformation of the 57 kDa NTHB was investigated using the fluorescence method to evaluate the stability of this protein.

Materials and Methods

The 57 kDa nuclear thyroid hormone binding protein (NTHB) was purified from livers of male Sprague–Dawley rats according to the method described in the preceding paper.[17] Guanidine hydrochloride (GdnHCl) and other reagents were of the highest quality and were obtained from Wako Pure Chemical Industry, Osaka.

Fluorescence measurements were performed with a Hitachi 850 spectrofluorometer. The excitation and emission wavelengths of the protein fluorescence were 280 and 330 nm, respectively. Assuming a two state mechanism, the equilibrium constant, K, at 25 °C was estimated from the change of the fluorescence at 330 nm according to Eq. 1,

$$K = e^{-\Delta G/RT} = (F_N - F)/(F - F_D) \quad (1)$$

where F is the observed fluorescence intensity at each GdnHCl concentration, F_N and F_D are the fluorescence intensities at the native and the unfolded state, respectively, and taken from the linear lines at low and high GdnHCl concentrations. According to Green and Pace,[20] the apparent free energy change in the absence of GdnHCl varies linearly as the equation

$$\Delta G = \Delta G^{H_2O}_{app} - m(\text{GdnHCl}) \quad (2)$$

where $\Delta G^{H_2O}_{app}$ is the apparent free energy in the absence of denaturant obtained from direct linear extrapolation of ΔG to (GdnHCl) = 0, and m is the gradient. The temperature of the cell holder was controlled by circulating water at a constant temperature regulated with a thermostat. The pH of the sample was adjusted using 0.1 M phosphate buffer containing 10% glycerol. The concentration of NTHR was determined by the Lowry method.[24]

Results and Discussion

Figure 1 shows fluorescence spectra of NTHB at various pH's. NTHB had an emission spectrum characteristic of tryptophan with a maximum near 330 nm. The fluorescence intensity varied at different pH's and quenched both in acid and alkaline pH's. The pH profile of the fluorescence maxima is shown in Fig. 2. Fluorescence quenching was observed in the pH regions below about 7 and above 9, and remained almost invariable between these pH's. The fluorescence quenching data indicate a

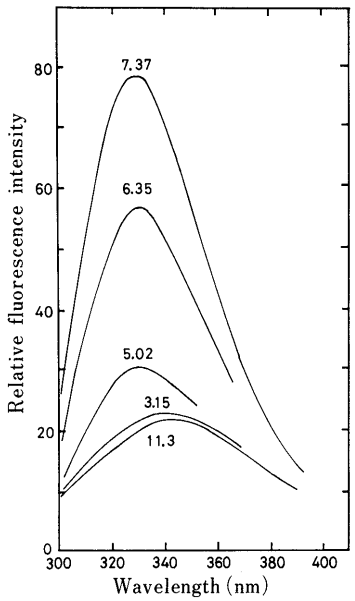

Fig. 1. Fluorescence Spectra of NTHB at the Various pH's Indicated

Solvents were 0.1 M sodium phosphate buffer between pH 5—9, 0.1 M sodium acetate buffer at pH 3.15 and 0.1 M sodium carbonate buffer at pH above 9, all of which contained 10% glycerol. The protein concentration was 2.14×10^{-6} M. The temperature was 25 °C. The excitation wavelength was 280 nm.

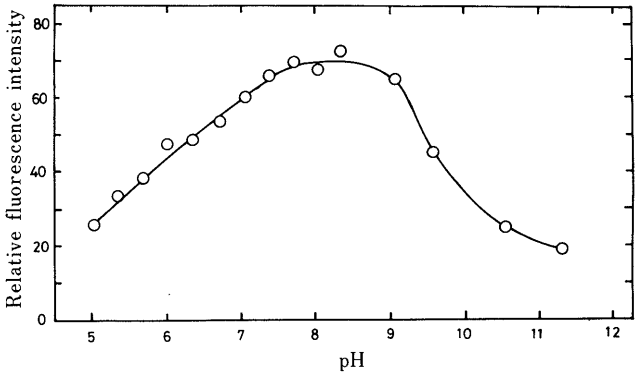

Fig. 2. pH Profile of the Fluorescence Maxima of NTHB

Conditions were the same as in Fig. 1

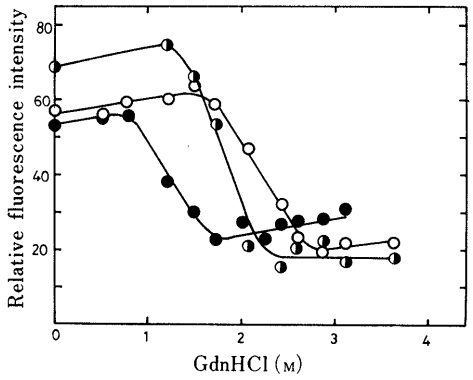

Fig. 3. Fluorescence Maxima of NTHB as a Function of GdnHCl at Various pH's

○, pH 7.7; ●, pH 6.0; ◑, pH 9.0. Solvent was 0.1 M sodium phosphate buffer containing 10% glycerol. Other conditions were the same as in Fig. 1.

change in the secondary and/or tertiary structure of NTHB under acid and alkaline pH conditions. The native conformation of NTHB is retained in the narrow pH region of approximately pH 7—9. It is interesting to note that the pH profile of the fluorescence intensity resembles the binding of 3,5,3′-triiodo-L-thyronine (T_3) to the solubilized nuclear receptor from rat liver,[18] in which binding was optimum in the narrow pH region between pH 7—8, and declined above pH 8.7 and below pH 7.[18] Although the declination of T_3 binding at alkaline pH values might be partly due to the ionization of the 4′-OH group,[23] the pH induced conformational change observed in this study might play an important role in the marked alteration of the T_3 binding ability. Figure 3 shows the denaturation curves for NTHB in the presence of GdnHCl at various pH's determined by measuring fluorescence intensity. At pH lower than 5 and higher than 11, no denaturation curves could be obtained by the fluorescence method because of the quenching in these regions (Fig. 2; data not shown here). At pH 6.0, 7.7 and 9.0, a large conformational transition of NTHB occurred with increasing in GdnHCl concentrations. The GdnHCl concentrations at half-maximum change of the transition, $(GdnHCl)_{1/2}$, which indicates the stability of the protein, were 1.2 M at pH 6.0, 2.2 M at pH 7.7 and 1.8 M at pH 9.0. These results indicate that the conformation of NTHB is stable at pH 7.7, but unstable at pH 6.0 and 9.0, especially under acidic conditions. The apparent free energy of denaturation in H_2O, $\Delta G_{app}^{H_2O}$ was calculated from the observed denaturation curve at pH 7.7, assuming a two-state mechanism.[20] The value of $\Delta G_{app}^{H_2O}$ and $(GdnHCl)_{1/2}$ of NTHB at pH 7.7 is listed in Table I together with the values reported for other globular proteins. $\Delta G_{app}^{H_2O}$ of NTHB resembles that of lysozyme, but $\Delta G_{app}^{H_2O}$ of lysozyme rose to 7.9 kcal·mol^{-1} at neutral pH.[21] $(GdnHCl)_{1/2}$ of NTHB is about 1 M lower than that of lysozyme. $(GdnHCl)_{1/2}$ of α-chymotrypsin and deoxyribonuclease I resemble that of NTHB, but their values of $\Delta G_{app}^{H_2O}$ are a few kcal·mol^{-1} higher. This suggests that the 57 kDa NTHB is not stable in its native conformation, since GdnHCl acted as a strong denaturant.

TABLE I. Parameters Characterizing GdnHCl Denaturation at 25 °C

	pH	$\Delta G_{app}^{H_2O}$	$\lvert GdnHCl\rvert_{1/2}$
NTHB	7.7	6.3±0.1	2.17
Ribonuclease[a]	6.6	9.7±1.7	3.01
Lysozyme[a]	2.9	6.1±0.4	3.07
α-Chymotrypsin[a]	4.3	8.3±0.4	1.90
β-Lactoglobulin[a]	3.2	11.7±0.8	3.23
Deoxyribonuclease I[b]	7.0	9.3±0.3	2.10

a) From Ref. 20. *b*) From Ref. 22.

References

1) J. Robbins, S. Y. Cheng, M. C. Gershengorn, D. Glinoer, H. J. Cahnmann, and H. Edelhoch, *Recent Prog. Horm. Res.*, **34**, 477 (1978).
2) L. J. DeGroot, A. Nakai, A. Sakurai, and E. Macchia, *J. Endocrinol. Invest.*, **12**, 843 (1989).
3) V. Cody, *Endocrinol. Rev.*, **1**, 140 (1980).
4) A. Pascual, J. Casanova, and H. H. Samuels, *J. Biol. Chem.*, **257**, 9640 (1982).
5) D. Dozin, H. J. Cahnmann, and V. M. Nikodem, *Biochemistry*, **24**, 5197 (1985).
6) J. Casanova, Z. D. Horowitz, R. P. Copp, W. R. Intyre, A. Pascual, and H. H. Samuels, *J. Biol. Chem.*, **259**, 12084 (1984).
7) M. B. Bolger and E. C. Jorgensen, *J. Biol. Chem..*, **255**, 10271 (1984).
8) V. M. Nikodem, S. Y. Cheng, and J. E. Rall, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 7064 (1980).
9) A. J. Perlman, F. Stanley, and H. H. Samuels, *J. Biol. Chem.*, **257**, 9640 (1982).
10) B. van der Walt, V. M. Nikodem, and H. J. Cahnmann, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 3508 (1982).
11) R. Somack, T. A. Andrea, and E. C. Jorgensen, *Biochemistry*, **21**, 163 (1982).
12) A. Inoue, K. Nakagawa, and S. Morisawa, *Eur. J. Biochem.*, **114**, 509 (1981).
13) H. C. Towle, C. N. Mariash, H. L. Schwartz, and J. J. H. Oppenheimer, *Biochemistry*, **20**, 3486 (1981).
14) D. B. Jump, P. Narayan, H. C. Towle, and J. H. Oppenheimer, *J. Biol. Chem.*, **259**, 2789 (1984).
15) J. Brtko, J. Knopp, and L. J. DeGroot, *Endocrinol. Exp.*, **21**, 251 (1987).
16) R. C. Jaffe, *Mol. Cell. Endocrinol.*, **52**, 137 (1987).
17) N. Okabe and K. Goto, *J. Biochem.* (Tokyo), **106**, 1064 (1989).
18) B. D. Wilson and W. L. Gent, *Biochem. J.*, **232**, 663 (1985).
19) A. Anselmet, J. Bismuth, and J. Torresani, *Biochim. Biophys. Acta*, **739**, 291 (1983).
20) R. F. Green and C. N. Pace, *J. Biol. Chem.*, **249**, 5388 (1974).
21) K. C. Aune and C. Tanford, *Biochemistry*, **8**, 4579 (1969).
23) N. Okabe, E. Fujita, and K. Tomitra, *Biochim. Biophys. Acta*, **700**, 165 (1982).
23) K. R. Latham, J. C. Ring, and J. D. Baxter, *J. Biol. Chem.*, **251**, 7388 (1976).
24) O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).