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Role of His16 in the structural flexibility of the C-terminal region of human endothelin-1

Hirotsugu Hiramatsu, Hiroki Aduma, Yuriko Tanaka, Takashi Miura, Hideo Takeuchi *

Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Sendai 980-8578, Japan

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ABSTRACT

The biological activity of endothelin-1 (ET-1), a 21-residue vasoconstrictive peptide hormone, has been reported to largely increase upon substitution of Ala for His16. We have investigated possible differences in structural properties between wild type ET-1 and its H16A mutant by using circular dichroism, ultraviolet resonance Raman, visible Raman, and infrared absorption spectroscopy. The C-terminal region of ET-1 spanning from His16 to Trp21 is found to be sensitive to the environment and folds into an α-helical structure under hydrophobic conditions. The environmental sensitivity is elevated in the H16A mutant. A pH decrease from 7.0 to 5.5 does not affect the secondary structure of WT ET-1 but induces an α-helical structure in the H16A mutant. These observations indicate that the mutation of His16 to Ala significantly increases the flexibility of the C-terminal region. The increased flexibility of the H16A mutant may be advantageous for efficient but not for specific binding to receptors. His16 may play an important role in maintaining the structural flexibility of the C-terminal region at an appropriate level and keeping a high specificity to the endothelin receptors.

1. Introduction

Endothelin-1 (ET-1) is a 21-amino acid peptide isolated from the culture medium of endothelial cells [1]. The peptide chain of ET-1 contains four Cys residues at positions 1, 3, 11, and 15, which form outer (Cys1–Cys15) and inner (Cys3–Cys11) disulfide bridges to make a loop structure as shown in Fig. 1. ET-1 is characterized by a potent vasoconstrictive activity and exerts a number of physiological functions including smooth muscle contraction and neural crest cell development [2,3]. The peptide is also implicated in various pathological conditions such as hypertension, cardiac failure, and cancer [2–5]. The diverse activity of ET-1 is exerted by binding to the cell surface receptors, ET_A and ET_B [6,7], though the structures of the membrane-bound receptors and their interactions with ET-1 are little understood.

To elucidate the structure–activity relationships of ET-1, fragments and mutants of ET-1 have been assayed for the activity as a vasoconstrictor [8–10]. Removal of residues from the C-terminus one by one reduced the activity in a stepwise manner, and the peptide fragment lacking the C-terminal five residues completely lost the activity [8]. Destruction of the loop structure by reduction, in particular at the Cys1–Cys15 disulfide bridge, also caused activity loss [8,9]. Mutation of negatively charged Asp8 and Glu10, or bulky Phe14 to uncharged or non-bulky residues resulted in complete loss of the activity [9]. Furthermore, an alanine scan study showed that the mutations of Asp8 and Leu17 to Ala caused a substantial loss of the agonist contracting activity, while those of Tyr13, Phe14, and Trp21 largely reduced the receptor binding affinity as well as the agonist contracting activity [10]. Generally, mutations reduced either or both of the binding and contracting activities. Interestingly, however, the His16 → Ala (H16A) mutant showed more than threefold enhancement of both binding and contracting activities [10], implying a unique suppressive role of His16 in the activity of ET-1.

The molecular structure of ET-1 has been solved in the crystalline state by X-ray diffraction (Protein Data Bank, code 1EDN) [11] and in acidic solution by nuclear magnetic resonance (NMR) spectroscopy (1V6R) [12]. (ET-1 is amenable to NMR analysis only at acidic pH because of solubility problem.) The structural models obtained in solid and solution are in substantial agreement with each other in the N-terminal loop region, where a strong constraint is imposed by the Cys1–Cys15 and Cys3–Cys11 disulfide bridges. On the other hand, the structure of the C-terminal region, His16–Trp21, significantly differs between the two models. The solid-phase model contained a long α-helix extending from Lys9 to Ile20 and covering almost the entire C-terminal region. In contrast, the solution-phase model contains a short α-helix involving only Glu10–Phe14, and the C-terminal His16–Trp21 region is disordered as shown in Fig. 2. The structural difference between the solid and solution phases suggests that the C-terminal region has a large structural flexibility, which might play an important role in

* Corresponding author. Tel./fax: +81 22 795 6855.
E-mail address: takeuchi@mail.tains.tohoku.ac.jp (H. Takeuchi).

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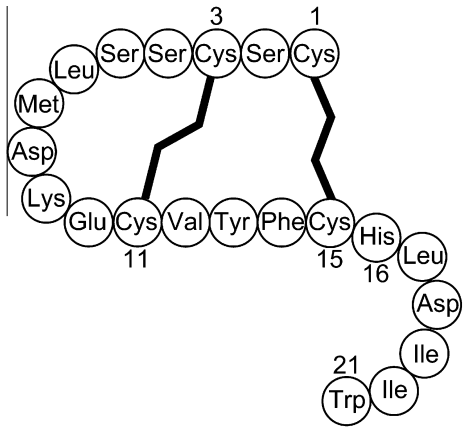

Fig. 1. Amino acid sequence of ET-1. Disulfide bridges are indicated with zigzag lines.

the binding to receptors. A ligand–receptor docking model study suggested that the C-terminal region could adopt varying conformations to fit in the cleft of the receptor protein, ET_A or ET_B [13]. His16 at the interface between the N-terminal loop and the C-terminal flexible region may play an important role in controlling the structure and activity of ET-1.

In this study, we have investigated the difference in structural properties between wild type (WT) ET-1 and its H16A mutant by examining the effect of solvent and pH on the secondary structure by circular dichroism (CD) spectroscopy. Ultraviolet resonance Raman (UVRR), visible Raman, and infrared (IR) absorption spectra were also utilized to study the protonation states, environments, and conformations of some amino acid side chains. These spectroscopic methods have an advantage over NMR in the ability to resolve fast-exchanging conformers at low concentrations. The results clearly show that His16 plays a role in maintaining the structural flexibility of the C-terminal region, which is otherwise folded into an α-helical structure by an increase in environmental hydrophobicity or by protonation of certain other residues.

2. Experimental

2.1. Materials

HPLC-purified WT ET-1 and its H16A mutant (acetic acid salt, >95% pure) were purchased from Peptide Institute Inc. (Osaka, Japan) and used without further purification. The concentration of ET-1 was determined from the UV absorption intensity at 279 nm by using the molar extinction coefficient, $\varepsilon_{279} = 7250\ M^{-1}\ cm^{-1}$ [14]. Since the UV absorption at this wavelength arises from Trp, Tyr, and the disulfide bridge [15], the substitution of Ala for His16 would not significantly affect the extinction coefficient. Thus, the same molar extinction coefficient was used for the H16A mutant.

Chemicals for preparing buffers including tris(hydroxymethyl)aminomethane (Tris), 2-(*N*-morpholino)ethanesulfonic acid (MES) and 1,4-piperazine-*N*,*N*′-bis(3-propanesulfonic acid) (PIPPS) were purchased from commercial sources.

Samples for CD measurements were prepared by dissolving the peptide (30 μM) in 20 mM phosphate (pH 5.5 and 7.5) or 5 mM Tris–HCl (pH 8.5) buffer. For IR and UVRR measurements, the peptide was dissolved at a concentration of 130 μM in 5 mM MES/PIPPS D_2O buffer (pD 3.3, 5.5, 7.0, and 8.5). The peptide concentration was raised to 1.0 mM for visible Raman measurements at pH 7.0 (20 mM phosphate buffer).

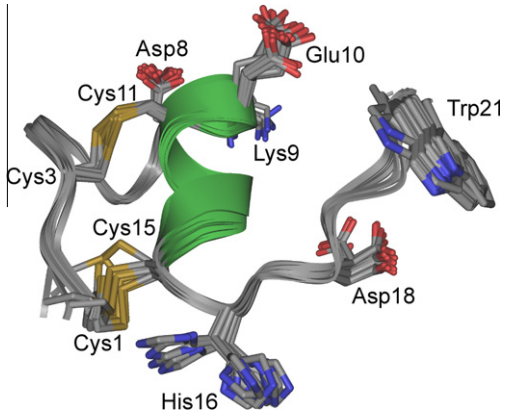

Fig. 2. Molecular model of ET-1 obtained by NMR spectroscopy in acidic solution [12]. The atomic coordinates are taken from the Protein Data Bank (code, 1V6R) and 20 possible structures are overlaid. Amino acid side chains are shown only for Asp, Cys, Glu, His, Lys, and Trp.

2.2. Acquisition of spectral data

CD spectra were recorded on a JASCO J-820 spectropolarimeter using a 1-mm cell. IR spectra were recorded at a resolution of $4\ cm^{-1}$ on an Avatar 360 (Thermo Scientific) equipped with an MCT detector. For UVRR excitation, the 229-nm laser line from a frequency-doubled Ar^+ laser (Innova 300 FReD, Coherent) was used and the scattered Raman light was dispersed and detected on a UV Raman spectrometer (TR-600UV, JASCO). Visible Raman spectra were excited at 488 nm and recorded on an NR-1800 spectrometer (JASCO). Wavenumber calibration for Raman spectra was effected by using indene in visible excitation and a 1:1 mixture of cyclohexanone and acetonitrile in UV excitation. The peak positions were reproducible to within $\pm 1\ cm^{-1}$.

3. Results and discussion

3.1. Solvent effect on the CD spectrum

To investigate the structural sensitivity of ET-1 to environments, we have recorded CD spectra of WT ET-1 and the H16A mutant dissolved in H_2O (phosphate buffer, pH 7.0), 50% 2,2,2-trifluoroethanol (TFE), 50% ethanol (EtOH), and 50% isopropyl alcohol (iPrOH). As Fig. 3a shows, the spectrum of WT ET-1 in H_2O solution exhibits a single broad negative band peaking at 206 nm, which is known to shift to 198 nm upon disruption of the Cys1–Cys15 and Cys3–Cys11 disulfide bridges by reduction [16] and is regarded as a marker of the N-terminal loop structure. In 50% TFE solution, the negative peak undergoes a small red-shift of about 1 nm, a positive peak appears around 195 nm, and the negative intensity in the 220–230 nm region is enhanced, as seen more clearly in the difference spectra (Fig. 3b). These spectral changes correspond to those expected for a partial structural transition from irregular to α-helical [17] and are consistent with the properties of TFE that induces and stabilizes α-helices in peptide segments having intrinsic α-helical propensity [18,19]. Possibly, the C-terminal flexible region takes, at least partly, α-helical structure in the presence of TFE. The spectral change from H_2O solution is also evident in 50% EtOH and 50% iPrOH, though the magnitude of the change is a little smaller than in 50% TFE. The environmental hydrophobicity, which increases in organic solvents, may be a factor that induce α-helical structure in the C-terminal region of WT ET-1.

Comparison of the CD spectra of the H16A mutant in pure H_2O and 50% organic solvents is shown in Fig. 3c and d. The spectrum of the H16A mutant in H_2O (Fig. 3c) is very close to that of the WT