CHEMISTRY LETTERS, pp. 953-954, 1993.

Binding of Heme to Sperm Whale Myoglobin-(57-96)-Tetracontapeptide

Chikao HASHIMOTO* and Ichiro MURAMATSU†

Department of Chemistry, The Jikei University School of Medicine, 8-3-1, Kokuryo-cho, Chofu-shi, Tokyo 182

† Department of Chemistry, College of Science, Rikkyo University, 3-24-1, Nishi-ikebukuro, Toshima-ku, Tokyo 171

The binding of heme to sperm whale myoglobin-(57-96)-tetracontapeptide (**1**) was investigated. The appearance of a peak at 414 nm in the difference spectrum between a **1**-heme mixture and heme indicated the formation of a complex. The circular dichroism spectrum of the mixture showed that an α-helical structure is induced and stabilized by the formation of the complex.

The three dimensional structure of crystalline sperm whale myoglobin (Mb) is 77% in the α-helix form,[1] which is retained in solution.[2,3] Although removal of a heme from Mb brings on a decrease in helicity, the helical structure can be restored on addition of the heme to an apo-Mb.[4] The E and F helical regions of Mb include distal (E7: position 64) and proximal (F8: position 93) histidine residues which coordinate the heme iron atom and contribute to the physiological functions of Mb. In the previous papers,[5,6] we reported the synthesis of sperm whale Mb-(57-96)-tetracontapeptide (**1**) consisting mainly of E-EF-F region which contains both of the histidine residues as shown in Fig. 1. The results of circular dichroism (CD) spectra of **1** and the related

```
    57                                                  70
H-Ala-Ser-Glu-Asp-Leu-Lys-Lys-His-Gly-Val-Thr-Val-Leu-Thr-
    71                                                  84
  Ala-Leu-Gly-Ala-Ile-Leu-Lys-Lys-Lys-Gly-His-His-Glu-Ala-
    85                                          96
  Glu-Leu-Lys-Pro-Leu-Ala-Gln-Ser-His-Ala-Thr-Lys-OH
```

Fig. 1. Primary structure of sperm whale Mb-(57-96)-tetracontapeptide (**1**).

fragment peptides suggested that various interactions among amino acid residues located separately from each other in the primary structure contribute to stabilize the helical structure, and also that hydrophobic surroundings brought from regions other than the E-EF-F region play a very important role in helix formation of this region in the entire Mb molecule.[5-7]

In the present study, the interaction between **1** and a ferric heme was investigated by means of absorption spectra in the region of about 300-500 nm. The difference spectrum between a **1**-heme (1:12 molar ratio) mixture and the heme, and the absorption spectra of the heme and sperm whale Mb in 0.10 M (1 M=1 mol dm^{-3}) borate-HCl buffer (pH 9.20) are shown in Fig. 2. The spectrum of the **1**-heme mixture showed a strong peak at 414 nm (Soret band), which indicates that the heme binds to **1**.

Figure 3 shows the CD spectra of **1** in 0.10 M borate-HCl buffer (pH 9.20) with the heme and in 0.10 M phosphate buffer (pH 6.50) without heme. The spectrum of **1** without heme did not show unequivocal existence

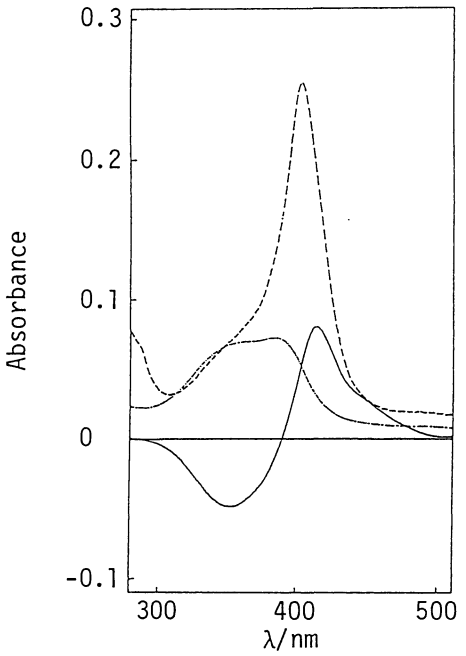

Fig. 2. Difference spectrum between peptide **1**–heme mixture (1:12) and heme (—— ; peptide **1**, 1.41 x 10^{-6} mol l^{-1}); absorption spectra of Mb (- - - - , 1.41 x 10^{-6} mol l^{-1}) and heme (—·—·— , 1.41 x 10^{-6} mol l^{-1}); in ferric state in 0.10 M borate–HCl buffer (pH 9.20).

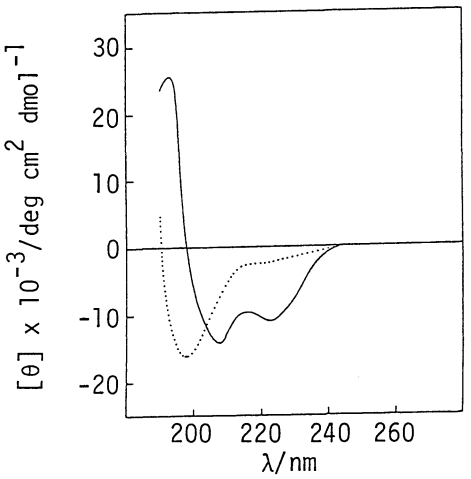

Fig. 3. CD spectra of peptide **1** in 0.10 M phosphate buffer (pH 6.50) (·····) and of peptide **1**–heme mixture in 0.10 M borate–HCl buffer (pH 9.20) (——).

of helical structure. The ferric complex provides a CD spectrum pattern typical for helical structure. The value of $[\theta]_{222}$ is estimated as −11000 and its helicity as 34%.[8] The theoretical helicity of **1** calculated from the result of an X-ray analysis[1] is 75%; the underlined amino acid residues in Fig. 1 take part in the helical structure in Mb. This result indicates that the binding of the heme to **1** induces α-helical structure, although the helicity of the **1**–heme complex is lower than the theoretical helicity. The stabilized helical structure of this ferric complex may be based on hydrophobic interactions between nonpolar side chains of **1** and the porphyrin ring of the heme, and/or on electrostatic interactions between basic groups of **1** and acid groups of the heme, as well known with regard to Mb and hemoglobin.

The authors thank Professor Machiko Tozawa, Associate Professor Tomoyoshi Takahashi, and emeritus Professor Tsunetaka Kushimoto of The Jikei University School of Medicine, and Professor Shosuke Sofuku of Rikkyo University for their continiuing interest and encouragement. We also thank the members of the Radiochemical Laboratory of Rikkyo University for CD measurements.

References

1) J. C. Kendrew, H. C. Watson, B. E. Strandberg, R. E. Dickerson, D. C. Phillips, and V. C. Shore, *Nature (London)*, **190**, 666 (1961).
2) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).
3) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).
4) E. Breslow, S. Beychok, K. D. Hardman, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 304 (1965).
5) C. Hashimoto and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, **62**, 1900 (1989).
6) C. Hashimoto and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, **66**, 181 (1993).
7) C. Hashimoto, *Bull. Chem. Soc. Jpn.*, **65**, 1268 (1992).
8) Minus 32000 was used as the value of $[\theta]_{222}$ for the completely helical structure in the calculation of the helical content: R. M. Epand and H. A. Scheraga, *Biochemistry*, **7**, 2864 (1968).

(Received March 9, 1993)