Impact of Protein–Protein Contacts on the Conformation of Thrombin-bound Hirudin Studied by Comparison with the Nuclear Magnetic Resonance Solution Structure of Hirudin(1-51)

T. Szyperski¹, P. Güntert¹, S. R. Stone¹, A. Tulinsky³, W. Bode³, R. Huber⁴ and K. Wüthrich¹

¹Institut für Molekularbiologie und Biophysik
 Eidgenössische Technische Hochschule Hönggerberg
 CH-8093 Zürich, Switzerland

²University of Cambridge, Department of Haematology
 MRC Centre, Hills Road, Cambridge CB2 2QH, U.K.

³Department of Chemistry, Michigan State University
 East Lansing, MI 48824, U.S.A.

⁴Max-Planck-Institut für Biochemie
 D-8033 Martinsried, Germany

(Received 26 May 1992; accepted 19 August 1992)

The impact of protein–protein interactions on the conformation of the N-terminal hirudin domain consisting of residues 1 to 51 in the X-ray crystal structure of a hirudin–thrombin complex was investigated through comparisons with the nuclear magnetic resonance solution structure of hirudin(1-51). The close overall similarity observed between these two structures contrasts with the behavior of the C-terminal 17 residue polypeptide segment of hirudin, which is flexibly disordered in solution but exhibits a defined conformation in the complex with thrombin. Localized structural differences in the N-terminal domain include that residues 1 to 3 of hirudin in the crystalline complex form a hydrogen-bonding network with thrombin that is reminiscent of a parallel β-sheet. Moreover, the backbone conformation of residues 17 to 20 in the complex does not contain the characteristic hydrogen bond observed for the type II reverse turn in the solution structure, and the side-chains of Ser19 and Val21 have significantly different orientations in the two structures. Most of these structural changes can be related directly to thrombin–hirudin contacts, which may also be an important factor in the mechanism of hirudin action. In this context, it is of special interest that other residues that also make numerous contacts with thrombin, e.g. Thr4, Asp9 and Asn20, have identical conformations in free hirudin and in the complex.

Keywords: hirudin; thrombin; NMR; X-ray crystallography; protein–protein interactions

1. Introduction
Motivated by the biomedical interest in the regulation of thrombin activity by hirudin (Lenti, 1986; Märki & Wallis, 1990), considerable effort has been focused on structure determinations of the individual components as well as hirudin–thrombin complexes. As a result, X-ray crystal structures are available for thrombin (Bode et al., 1989) and two 1:1 hirudin–thrombin complexes (Grüter et al., 1990; Rydel et al., 1990, 1991). Nuclear magnetic resonance (NMR) solution structures have been published for natural hirudin (Chere et al., 1987), which contains a sulfated tyrosine residue in position 63, recombinant desulfatohirudin (Folker et al., 1989; Hanyama & Wüthrich, 1989), the mutant Lyv47 → Glu of recombinant desulfatohirudin (Folker et al., 1989) and the N-terminal 51-residue domain, hirudin(1-51) (Szyperski et al., 1992, accompanying paper). Comparative studies of these

† Abbreviations used: NMR, nuclear magnetic resonance; HV1, hirudin variant 1; HV2-K47, hirudin variant 2 containing Lys in position 47; hirudin(1-51), N-terminal 51-residue domain of hirudin variant HV1; r.m.s.d., root-mean-square deviation.

1200

© 1992 Academic Press Limited