1. Introduction

To understand biological processes in detail, it is necessary to look at these processes in molecular detail. Biological macromolecules adopt specific three-dimensional structures, determined by their chemical composition and their particular environment in the living organism. The influence of the environment on the structure of a given macromolecule can have dramatic effects. For nucleic acids in particular, numerous examples of this phenomenon have been elucidated, commonly referred to as polymorphism (for an excellent review, see Rich, 1993).

Since structure determines function, there is a fundamental interest in understanding the rules governing the formation of structures in biological systems and the consequences thereof. X-ray crystallography, and in recent years nuclear magnetic resonance (NMR) spectroscopy as well, are the techniques that can provide structural data of biological macromolecules on the atomic level. This information allows for the understanding of precise structure-function relationships.

In 1979, with the publication of the first nucleic acid structure at atomic resolution (Wang *et al.*, 1979), one of the most prominent examples to date of polymorphism occurring in deoxyribonucleic acid (DNA) was presented. In the established DNA model at the time, developed by Watson & Crick and based on low resolution fiber diffraction data, DNA forms a right-handed double helix, with two DNA strands running in anti-parallel direction with adenine (A) : thymine (T) and guanine (G) : cytosine (C) base pairs forming specifically between the strands in the center of the helix (Watson & Crick, 1953). In contrast to this B-form of DNA, in the novel crystal structure of a 6-base pair duplex made of the palindromic sequence d(CGCGCG), a left-handed helix with perfect G:C base pairing was seen (Fig. 1.1). The sugar-phosphate backbone adopted a particular ‘zig-zag’ pattern, giving rise to the name Z-DNA.

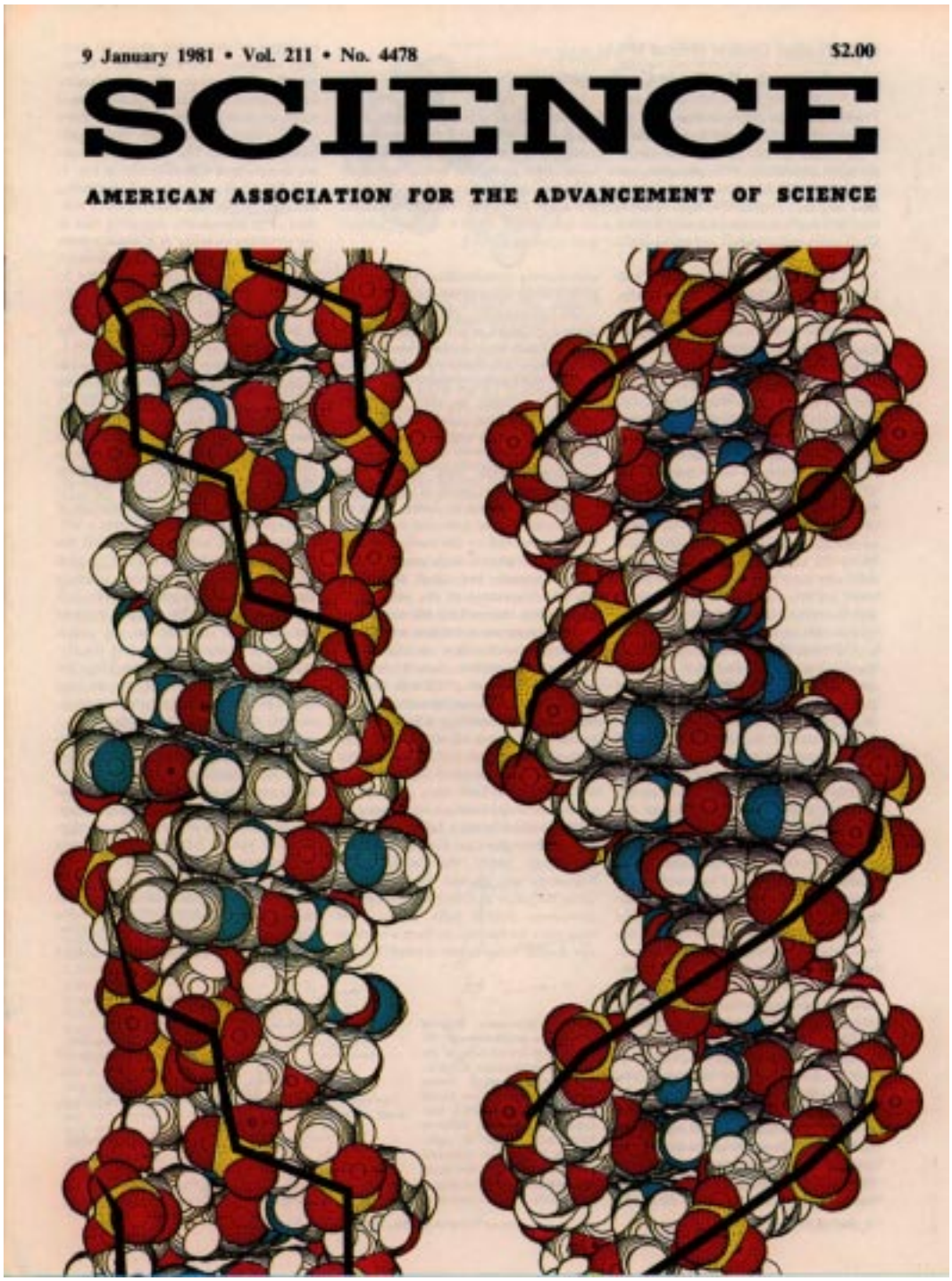

Figure 1.1 Structures of B- and Z-DNA.
This cover photo from *Science* illustrates both Z-DNA (left) and B-DNA (right). Characteristic of Z-DNA are the left-handed 'zig-zag' pattern of the sugar-phosphate backbone, the absence of a major groove and the deep and shallow minor groove. Typical for B-DNA are the smooth right-handed backbone trace and the exposure of base edges in the major and the minor groove.

The discovery of this form of DNA initiated a new field of research, which aimed for a thorough understanding of alternate DNA conformations in

general, and Z-DNA in particular. Among many questions, a major focus was to search for proteins binding specifically to Z-DNA, in an attempt to determine a possible biological function.

After a long and tedious effort, an activity called Zα was identified, which binds Z-DNA with high affinity and specificity (Herbert *et al.*, 1993). It was shown that the activity is located in a protein ADAR1, an RNA editing enzyme. This discovery opened two crucial questions. First, the functional relationship between Z-DNA binding and RNA editing had to be revealed; second, the structural basis for Z-DNA binding needed to be elucidated.

The focus of this thesis work was to obtain an understanding of the structural basis for this interaction. Biochemical and molecular biological methods were employed to understand the structural organization and the binding behavior of the Z-DNA binding domain *in vitro*. These studies were the basis for the main goal of this work — namely, to obtain a high-resolution structure of the complex between the Z-DNA binding domain and its substrate. This three-dimensional structure should reveal the fold of the protein and which parts of it are involved in DNA interaction. The molecular basis for specificity and high-affinity binding of Z-DNA was of central interest. The relationship to other known protein structures was another important aspect to focus on.

The determination of the desired protein-DNA complex was undertaken by x-ray crystallography. As a first step it was necessary to obtain the components pure and in high amounts. Crystals suitable for x-ray diffraction experiments had to be obtained. Next, x-ray diffraction data were collected. Finally, the structure was solved and analyzed.

The resulting information provides a basis for Z-DNA recognition by the protein domain Zα. It largely explains the characteristics of this interaction seen in biochemical studies. The Zα-Z-DNA complex serves as a prototype for Z-DNA recognition, providing rules as to how such a specific recognition is possible. These findings might help to find other proteins that also bind Z-DNA rather than other nucleic acid conformations.

This dissertation has been organized in the following manner. Chapter 2 comprises two parts. First, the components of the examined protein-DNA complex are presented; then the theoretical background for the employed experimental techniques is introduced. The detailed documentation of materials and methods employed in this investigation follow in chapters 3, 4

and 5. In chapters 6 and 7, the results of this thesis work are presented. These are the basic results from the biochemical studies in chapter 6 and the detailed results from the x-ray crystal structure analysis in chapter 7. In chapter 8, the results are discussed; the comparison with related proteins that bind B-DNA is a major focus here. A summary of the work undertaken in this thesis is presented in English in chapter 9 and in German in chapter 10.