rophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP) (*8, 9*). This result is consistent with the observation that defensins form voltage-dependent channels in lipid bilayers, a process that apparently involves aggregation of two to four molecules (*10*). These data strongly suggest that defensins kill by permeabilizing the membrane; so does their broad specificity, which includes enveloped but not naked viruses (7).

To understand further the mechanism of defensin action we have determined the crystal structure of the defensin HNP-3 by the method of isomorphous replacement (Table 1) and refined the atomic model to an *R* factor of 0.19 against 1.9 Å data (Table 2). The structure we see is remarkable in being quite unlike that of other membrane-permeabilizing proteins. Other lytic or membrane-permeabilizing peptides have been characterized, including melittin (*11*), cecropin (*12*), magainin (*13*), alamethicin (*14*), and δ-hemolysin (*15*). All of these are amphiphilic α helices that contain no β sheet and have no disulfide bonds. In sharp contrast, defensin is an all–β-sheet protein with no α helix, and it is stabilized by three disulfide bonds. The few membrane-permeabilizing proteins that do contain significant amounts of β sheet are either circular peptides, such as gramicidin S (*16*), or contain D-amino acids, such as gramicidin A (*17, 18*), or, like porin (*19*), are much larger than defensin.

Defensin is an elongated, ellipsoidal molecule with overall dimensions of 26 Å by 15 Å by 15 Å (Fig. 1A). The structure is

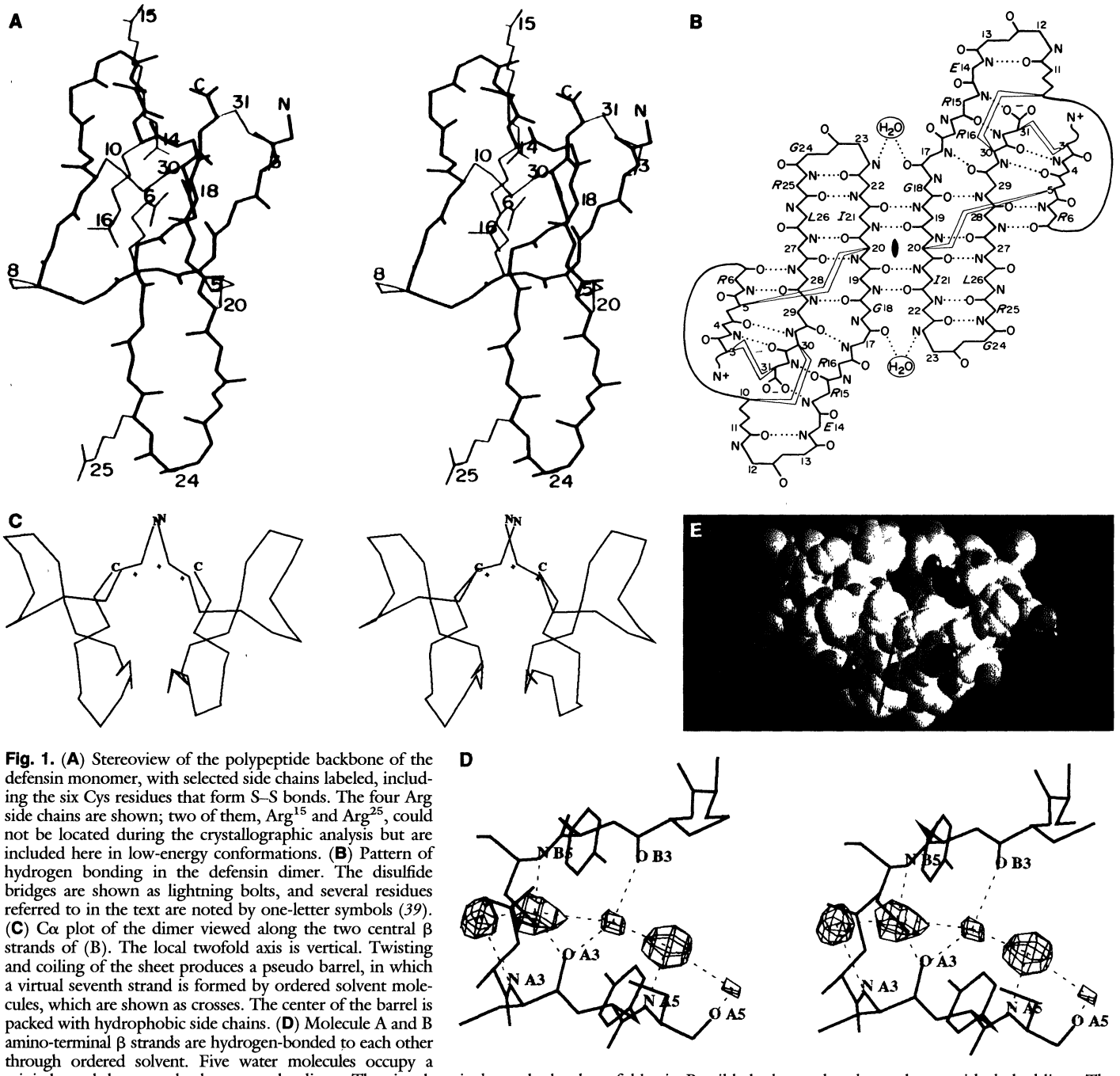

Fig. 1. (**A**) Stereoview of the polypeptide backbone of the defensin monomer, with selected side chains labeled, including the six Cys residues that form S–S bonds. The four Arg side chains are shown; two of them, Arg^{15} and Arg^{25}, could not be located during the crystallographic analysis but are included here in low-energy conformations. (**B**) Pattern of hydrogen bonding in the defensin dimer. The disulfide bridges are shown as lightning bolts, and several residues referred to in the text are noted by one-letter symbols (*39*). (**C**) Cα plot of the dimer viewed along the two central β strands of (B). The local twofold axis is vertical. Twisting and coiling of the sheet produces a pseudo barrel, in which a virtual seventh strand is formed by ordered solvent molecules, which are shown as crosses. The center of the barrel is packed with hydrophobic side chains. (**D**) Molecule A and B amino-terminal β strands are hydrogen-bonded to each other through ordered solvent. Five water molecules occupy a mini-channel that completely crosses the dimer. The view here is down the local twofold axis. Possible hydrogen bonds are shown with dashed lines. The $F_{obs} - F_{calc}$ map was computed with all water molecules deleted from the model; it was contoured at 3.5 times the root-mean-square deviation. (**E**) Space-filling representation of the dimer with the local twofold axis vertical and viewed along the solvent mini-channel, which can be seen at the top center. Carbon atoms, yellow; sulfur, brown; uncharged nitrogen, light blue; charged nitrogen, blue; uncharged oxygen, pink; and charged oxygen, red. Notice that the base of the basket is apolar, as shown by the hydrophobic moment (black arrow), which points toward the base.