CHAPTER 3

The Conformation of Glucagon

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A. Introduction

The biological activity of glucagon is mediated through binding, with high affinity and specificity, to a membrane receptor, implying extensive and well-defined intermolecular interactions (see Chap. 13). However, in dilute aqueous solutions glucagon has little defined secondary structure and almost certainly exists as a population of conformers in equilibrium. The formation of the receptor–hormone complex must involve either selection of one conformer from the population or induction of a conformer as the interaction takes place. Whatever the mechanism, the definition of the receptor-bound conformer, as well as the nature of the hormone–receptor interactions must be a primary objective in the understanding of the biology of glucagon and the design of glucagon agonists and inhibitors (BLUNDELL 1979; BLUNDELL and HUMBEL 1980). Unfortunately, the receptor has yet to be defined biochemically and so direct study of the receptor–hormone complex is not possible at present. Instead we must examine the conformation of glucagon in aqueous solution, in crystals, in lipid micelles and other environments in order to establish the nature of the conformational dependence on intermolecular interactions. Here, I first describe recent developments, especially in the use of X-ray diffraction and proton nuclear magnetic resonance (NMR) spectroscopy which have allowed description of the conformation in great detail under varied conditions. I then discuss the relevance of these conformations to the molecular biology of glucagon.

B. The Crystal Structure

I. Crystals

It is convenient to begin a description of the conformation of glucagon by reviewing the crystal structure analysis, for in the crystals the glucagon molecule has less flexibility, as it is stabilised by numerous intermolecular interactions, some of which may be maintained in other environments. The relatively ordered state allows a medium resolution analysis using the techniques of protein X-ray crystallography.

KING (1959) showed that rhombic dodecahedral crystals of glucagon contain 12 molecules packed with cubic symmetry (space group $P2_13$). Preliminary results (KING 1965; BLANCHARD and KING 1966) suggested a packing of α-helical rods and

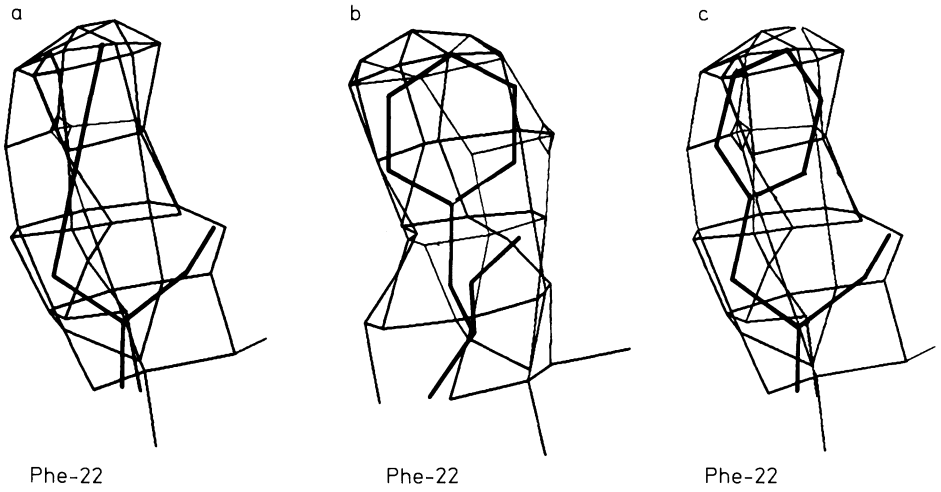

Fig. 1 a–c. The electron density at ~3 Å resolution for Phe-22. Orthogonal views (**a, b**) of the refined fit of the side chain to the density; rotation of 50° (**c**) around the β–γ bond (viewed in the same direction as **a**) leaves the side chain in the density, indicating rotational disorder in the crystals

this was detailed by SASAKI et al. (1975) who carried out a medium resolution X-ray analysis using the methods of isomorphous replacement and anomalous scattering.

Crystals of glucagon are most easily obtained at high pH (~9.2) where the glucagon is more soluble than at physiological pH. However, SASAKI et al. (1975) showed that these crystals undergo a phase change on lowering the pH to around 6.5, involving a retention of the cubic symmetry, but a shortening of the cubic cell parameter from 47.9 to 47.1 Å. Similar crystals are obtained by careful crystallisation at pH 6.5. A further crystal form of the same symmetry, but of a different cell dimension (48.7 Å) is found at lower pH (~3) (DOCKERILL 1978). These observations are a reflection of the flexibility of the glucagon conformation and especially its dependence on the pH which is also observed in solution. Various metal ion complexes cause similar changes in the crystals, giving rise to complications in the use of the method of isomorphous replacement.

Even the best crystals are disordered relative to those of most globular proteins. The nominal resolution is ~3 Å, which implies that the general conformation can be defined although the details are often unclear. For instance, the precise orientation of the carbonyls of the peptide groups and the side chains are not defined. Figures 1 and 2 show some typical side chain electron densities. Figure 1 shows that the phenyl ring of Phe-22 may be rotated by $\pm 50°$ around the β–γ bond from the optimal position and still remain in the electron density. As the resolution of the crystals is only ~3 Å, this implies that even in the better ordered parts of the crystal structure there is considerable disorder. Other groups such as Leu-26 have a poorly defined conformation and the crystal probably contains a population of each of the three conformers with a staggered arrangement around the β–γ bond. Other parts of the molecule are even more disordered and these include residues 1–4 at the NH_2-terminus and 28 and 29 at the COOH-terminus as well as the side chains of Asp-9, Lys-12, and Arg-18.

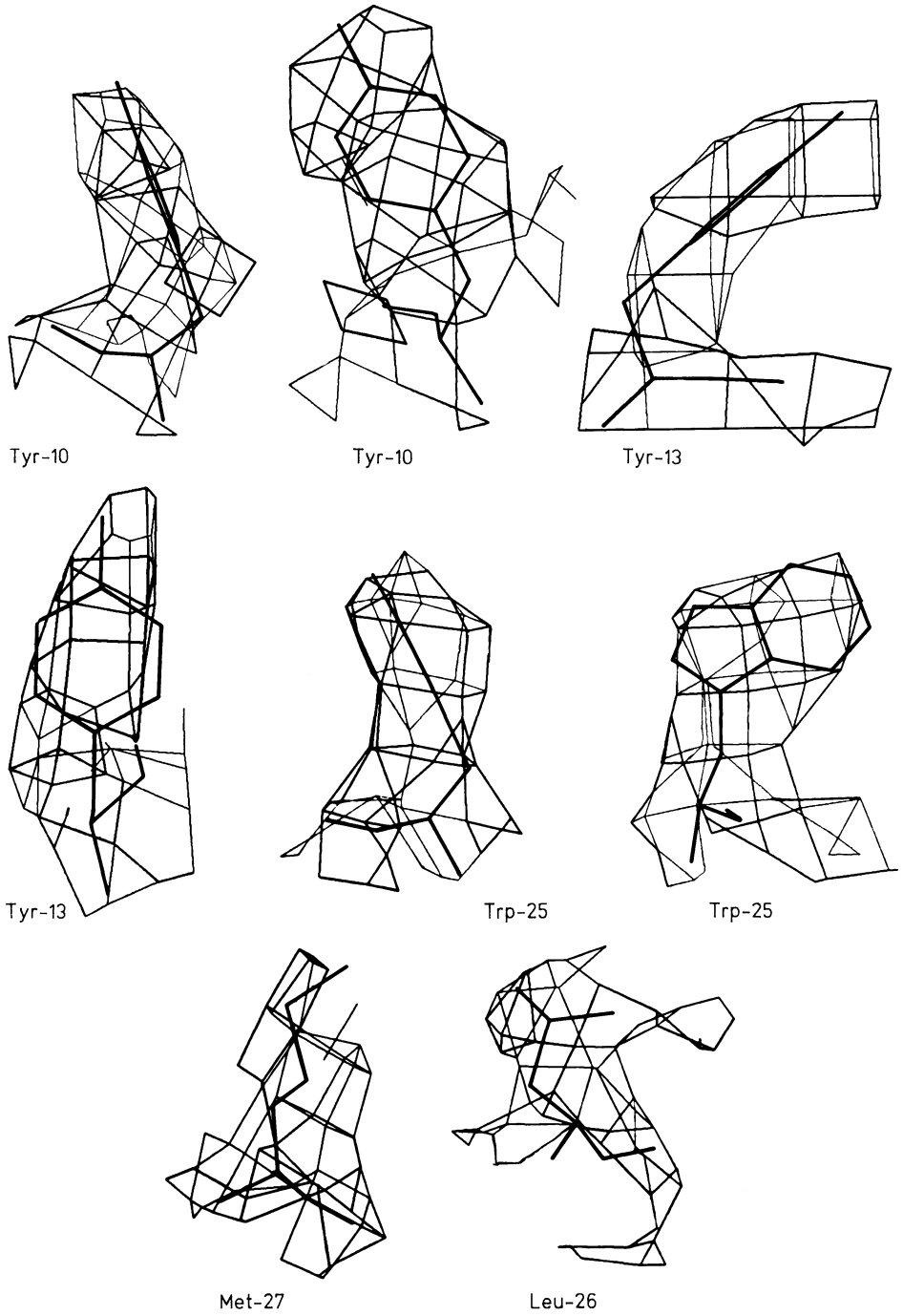

Fig. 2. The electron densities for a selection of side chains at ~3 Å resolution with the side chain positions from the least-squares refinement. Note that the ends of some side chains, e.g. Met-27 C^{ε} have no density, indicating complete disorder, while others such as Leu-26 are not unambiguously defined, indicating several possible conformations

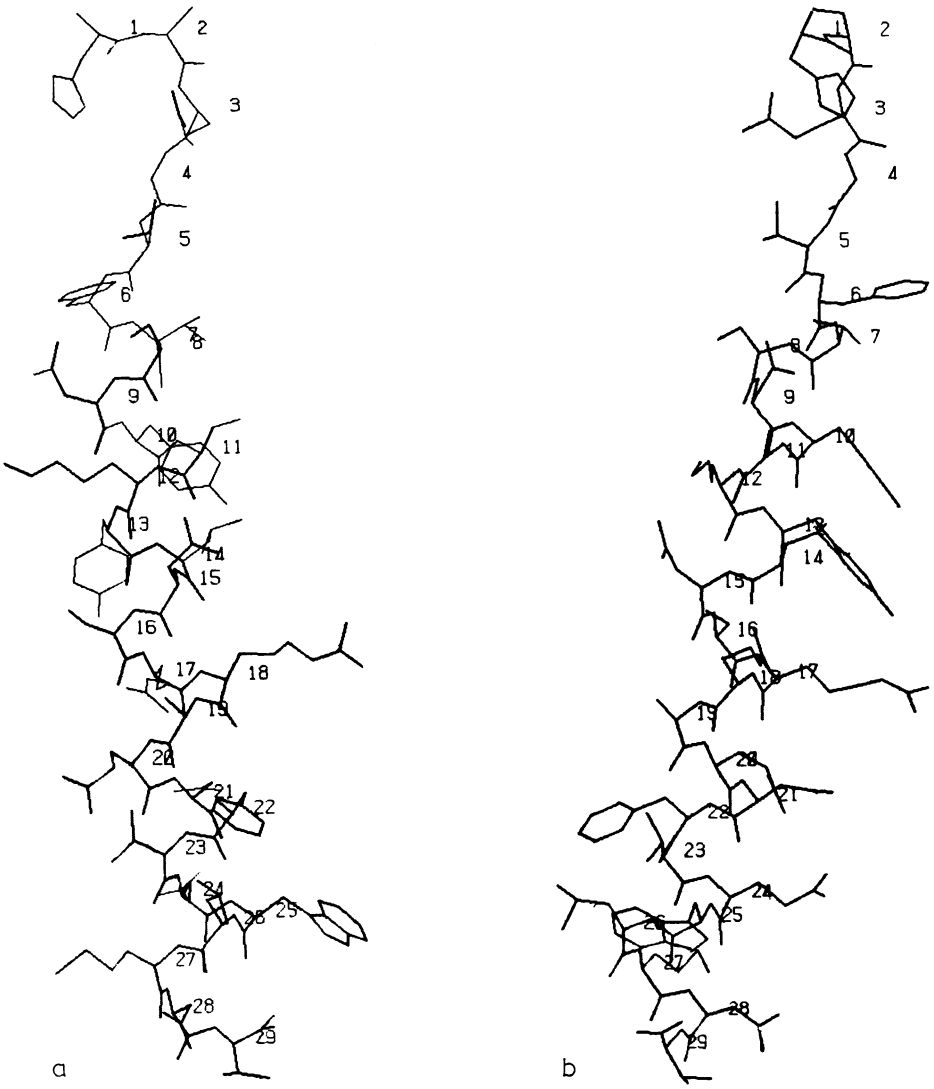

Fig. 3 a, b. Two orthogonal views (**a, b**) of the conformation of the glucagon molecule (protomer) in the crystals. Note the amphipathic nature of the helical conformer with two hydrophobic regions involving Phe-6, Tyr-10, Tyr-13, and Leu-14 towards the NH_2 terminus and Ala-19, Phe-22, Val-23, Trp-25, Leu-26, and Met-27 at the COOH terminus

The model defined by X-ray analysis and described in this chapter is an average of the conformers present. In fact, the poor resolution of the crystals poses problems in the refinement of the molecular structure using least-squares techniques. The model presented by SASAKI et al. (1975) was a best fit to the electron density at pH 6.5, obtained by the method of isomorphous replacement with anomalous scattering using an optical comparator (see BLUNDELL and JOHNSON 1976 for a review). The first set of coordinates deposited with the Brookhaven Protein Structure

Data Bank were refined using the methods considered optimal in 1975: cycles of real space refinement and calculation of electron density. More recently, J. MOREIRA, I.J. TICKLE, and T.L. BLUNDELL (1981) unpublished work) have shown that restrained least-squares refinement gives improved coordinates and these are used in this discussion.

II. Protomer Conformation

Figure 3 illustrates the conformation of the glucagon molecule (a protomer) in the cubic crystals. Table 1 gives the main chain torsion angles (ϕ, ψ, and ω) which show that residues 6–28 are in an approximately α-helical conformation. Table 2 gives the lengths and angles subtended at the peptide oxygen and hydrogen atoms of possible hydrogen bonds. The variations in lengths and angles are partly a reflection of the conformational disorder although the existence of a 3_{10} helix in residues 5–11 is probably real. At Gly-4 the chain becomes poorly defined, indicating

Table 1. The torsion angles defining the main chain conformation of the glucagon molecule from the medium resolution X-ray analysis of crystals at pH ~ 6.5

Residue	Φ	Ψ	ω
His-1	–	117	−173
Ser-2	− 90	6	−177
Gln-3	−109	− 69	−177
Gly-4	114	137	−177
Thr-5	−162	8	178
Phe-6	− 53	− 29	178
Thr-7	− 31	− 49	−179
Ser-8	− 39	− 61	−179
Asp-9	− 63	− 14	174
Tyr-10	− 71	− 56	177
Ser-11	− 52	− 28	178
Lys-12	− 73	− 53	178
Tyr-13	− 46	− 39	−179
Leu-14	− 68	− 22	180
Asp-15	− 76	− 42	−179
Ser-16	− 62	− 30	180
Arg-17	− 85	− 46	179
Arg-18	− 49	− 42	−176
Ala-19	− 79	− 44	−180
Gln-20	− 66	− 31	179
Asp-21	− 80	− 26	179
Phe-22	− 77	− 22	177
Val-23	− 72	− 16	178
Gln-24	− 76	− 48	178
Trp-25	− 67	− 55	−178
Leu-26	− 51	− 54	−176
Met-27	− 76	− 62	−179
Asn-28	− 35	− 66	−180
Thr-29	174	−169	

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