dans les groupes méthyle correspondent aux valeurs attendues.

La cohésion du cristal est assurée par des interactions de type van der Waals.

V. Conclusion

Le composé (8) étudié est le premier modèle, d'une structure phosphole pentacoordinée, analysé par diffraction de rayons X. Nous pensons que cette étude est justifiée par le fait que: plusieurs structures pentacoordinées contenant le cycle phosphole ont été décrites puis contestées; que, en ce qui concerne 'l'aromaticité' du cycle phosphole, il est intéressant de pouvoir comparer des modèles tricoordinés et tétracoordinés déjà décrits avec le modèle pentacoordiné objet de ce travail. Enfin, il est remarquable de constater que l'introduction d'un cycle insaturé à cinq atomes pratiquement plan, avec deux liaisons phosphore carbone dont une en position apicale, dans une structure pentacoordinée conduit à une géométrie très proche de la bipyramide trigonale idéale (8,3% de déformation) et non de la pyramide à base carrée ou du coin tors.

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The Structure of Des-Phe B1 Bovine Insulin

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Abstract

Des-Phe B1 bovine insulin crystallizes in space group $R3$, $a = 81{\cdot}6$ and $c = 34{\cdot}0$ Å, and is nearly isomorphous with the 2-zinc porcine insulin structure. The structure has been refined, using data to 2·5 Å resolution, through the use of fast Fourier refinement and the *MODELFIT* routine to a residual of 0·18 for 2128 data. The results of this refinement show that the removal of the B1 phenylalanine residue allows nearly free rotation about the C^{β}–C^{γ} bond of the A14 tyrosine side chain in one of the monomer units. This

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explains the discrepancies in the calculated and observed circular dichroism (CD) spectra since nearly half of the CD has been calculated to arise from B1 phenylalanine perturbing the A14 tyrosine.

Introduction

Various spectral studies of insulin have been carried out in order to determine the structure of the monomer in solution and also to describe better the aggregation properties. The aggregation properties are of some biological significance since the hormone is produced and stored as a hexamer but is active at the insulin receptor as the monomer. Circular dichroism (CD) is one of the techniques which has been employed. It has been particularly useful because the tyrosine's side chain makes a large contribution to the spectra and is buried in both dimer and hexamer formation. The A14 tyrosines, which are located on the surface of the monomer and dimer, participate in the formation of the hydrogen bonds and in that organization make van der Waals contacts to the B1 phenylalanine. Since in the near-UV (275 nm) as much as 44% of the total CD spectrum for the hexamer has been calculated to arise from B1 phenylalanine perturbing the A14 tyrosine (Wollmer *et al.*, 1980), it was expected that this residue might be a potential monitor of the aggregation properties of insulin. CD studies carried out on des-Phe B1 insulin, however, showed that the expected change in the spectra as a result of the loss of the A14–B1 interaction did not occur. This present study was undertaken in the hopes of providing a structural basis for this apparent anomaly.

Crystallization

Single crystals of des-Phe B1 bovine insulin were grown using the batch technique. A solution was made up from the following components: 5·5 mg des-Phe B1 insulin; 1·0 ml 0·02 *M* HCl; 0·1 ml 0·15 *M* zinc acetate; 0·5 ml 0·2 *M* sodium citrate; and 0·3 ml acetone. The largest and best crystals were grown from such a solution in which the pH was adjusted to 6·5, a pH slightly higher than that used for the crystallization of 2-zinc porcine insulin.

Data collection

Des-Phe B1 bovine insulin crystallizes in the rhombohedral space group $R3$, with cell dimensions $a = 81{\cdot}6$ and $c = 34{\cdot}0$ Å and is nearly isomorphous with that of the 2-zinc porcine insulin structure ($a = 82{\cdot}5$ and $c = 34{\cdot}0$ Å). A total of 2722 independent data, to a resolution of 2·5 Å resolution, were collected using Cu $K\alpha$ radiation from one crystal on a four-circle Hilger & Watts diffractometer controlled by a PDP-8 computer. Three standard reflections, measured after every 200 intensities were collected, showed a maximum variation of less than 20% over the course of the data collection. Data were collected using the ω-scan technique. Each peak was scanned using 25 to 40 steps of 0·02° in ω; the counting time for each step was 1·5 s. Backgrounds were measured at the beginning and end of the scan for each reflection for one-fifth of the peak counting time and were used to construct an average background over the various regions of reciprocal space. A total of 410 intensities were negative as a result of the application of the average background and were reset to zero. Intensities were corrected for absorption (North, Phillips & Mathews, 1968), Lorentz and polarization factors.

Solution and refinement

Because of the near isomorphism with the 2-zinc porcine structure, the 2-zinc insulin phases calculated from the 1·5 Å spacing refinement were employed for the calculation of the initial Fourier maps. As an initial check on the similarities of the two structures, the first map to be calculated was a difference map, using as amplitudes $||F_{\text{des-Phe}}| - |F_{2\text{Zn}}||$ and, as phases, those of the 2-zinc insulin structure. The dominant feature of this map was a very large negative region in both monomer units where the B1 Phe residues would have been located. Closer examination of the map also revealed negative regions in the vicinity of A8 and A10 of both monomer units. In porcine insulin, A8 is a threonine while alanine is present at this position in bovine insulin. The loss of the γ hydroxy and methyl groups is thus responsible for the observed negative density. A sequence difference also exists at A10 where isoleucine is replaced by valine and the loss of the δ methyl group again explains the observed negative density.

A difference map calculated at this stage with residues A13–A17, B2–B4 and B20–B23 (both molecules) removed from the structure factor calculation showed low or negative density for: A17 glutamate of molecule I; A13 leucine and A14 tyrosine of molecule II; and B22 arginine of molecule II. In addition, no density was observed for the entire B2 valine residue of both molecules. The coordinates of these atoms, along with 12 water molecules which were located in negative regions of the difference map, were therefore excluded from the starting positional and thermal parameters obtained from the 2-zinc porcine insulin structure. Positional parameters were refined by the fast Fourier block-diagonal least-squares method (Agarwal, 1978) using data for which $d < 6$ Å. The residual at the beginning of the refinement was 0·28. Following each cycle of fast Fourier refinement, the structure was made to conform to idealized peptide geometry by the

application of the *MODELFIT* routine (Dodson, Isaacs & Rollett, 1976) and the positional and thermal parameters of the water molecules were reset to the values obtained from the 2-zinc porcine insulin refinement. Because at low resolution the water molecules have unreasonable shifts, the model of the water structure determined from the 1·5 Å resolution 2-zinc porcine refinement was used. After four cycles of least-squares refinement, the residual was reduced to 0·20 for the regularized structure. A difference map calculated at this point provided tentative positions for both B2 valine residues as well as the side chain for A17 molecule I. The structure was then subjected to two additional cycles of least-squares refinement of the positional parameters.

Difference maps were then calculated and examined with the aid of the MMS-X graphics system. In this technique, a portion of the protein molecule (eight to ten residues) is excluded from the structure factor calculations; a difference map is then calculated and compared to the portion of the molecule which was excluded. The results of such a comparison indicate errors in side-chain orientations and misplaced atoms. Although these maps provided positional parameters for the side chains of the A13 leucine and the B22 arginine residues of molecule II, no density was observed for the side chain of the A14 tyrosine of molecule II. Furthermore, these difference maps showed that only B22 arginine of molecule I and B29 lysine of molecule II had two distinct side-chain conformations. These maps also suggested that B4 glutamine of molecule I may be disordered to the extent of 20 to 30%. There was no evidence for alternative side-chain orientations for the remainder of the residues which were found to be disordered in the 2-zinc porcine insulin structure (B12 valine and B21 glutamate of molecule I and B22 arginine of molecule II).

In native 2-zinc bovine insulin and des-Phe B1 bovine insulin different residues appear to be disordered. A comparison of the 2-zinc porcine insulin at 2·5 and at 1·5 Å resolution showed that the disorder can only be adequately described at high resolution. Refinement of the positional parameters and the thermal parameters was continued and converged at a residual of 0·18 for 2128 data.* The r.m.s. shift following regularization was 0·16 Å [parameters used in *MODELFIT* were σ(bond) = 0·02 Å and σ(angle) = 3·0°] and the r.m.s. deviation of all bonds from their ideal value was 0·20 Å.

Discussion

A comparison of the structures of 2-zinc porcine insulin (Dodson, Dodson, Hodgkin & Reynolds, 1979) and des-Phe B1 bovine insulin shows that the two structures are quite similar. The main-chain atoms of A11 to A19 and B9 to B18 of the des-Phe structure were fitted by a least-squares procedure to the corresponding atoms of the 2-zinc porcine insulin structure. The r.m.s. displacements of the atoms used in the least-squares fit were 0·16 Å (0·42 Å for all main-chain atoms) for molecule I and 0·23 Å (0·45 Å for all main-chain atoms) for molecule II and the largest displacements are found to occur at the N and carboxy terminus of both B chains, at the A4 and B21 glutamates of molecule I and A21 asparagine, B4 glutamine and B29 lysine of molecule II.

However, the most striking difference between the two structures is that of the A14 tyrosine residue. The immediate environment of A14 in the 2-zinc hexamer is illustrated in Fig. 1 and shows that B1 phenylalanine lies adjacent to both the A13 and A14 residues. Furthermore, contacts between A13, A14 and B1 are important for holding dimer units together to form the hexamer. In fact, pairs of A14 hydroxyl functions are evidently involved in hydrogen bonds across the dimer–dimer interface to each other. These interactions between dimer units are illustrated in Fig. 2.

However, when the B1 phenylalanine residue is removed, as in des-Phe B1 bovine insulin, no side-chain density for the A14 tyrosine of molecule II can be located indicating that this side chain is moving freely. That considerable mobility is allowed in this region is reflected in the difficulty which was encountered in obtaining positional parameters for the side chain of A13 leucine, which was located only after the refinement had progressed. Furthermore, the density in the region of the A13 side chain was considerably weaker than that of the side chain preceding it, A12. The

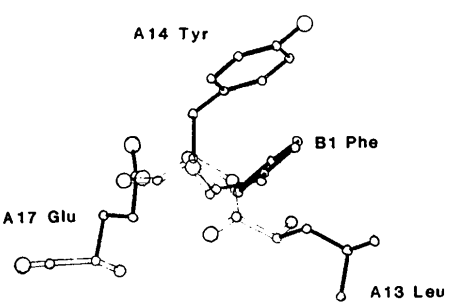

Fig. 1. The immediate environment of A14 tyrosine in 2-zinc insulin.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 2INS DES-PHE B1 and R2INSSF DES-PHE B1), and are available in machine-readable form from the Protein Data Bank at Brookhaven or one of the affiliated centers at Cambridge, Melbourne or Osaka. The data have also been deposited with the British Library Lending Division as Supplementary Publication No. SUP 37006 (2 microfiche). Free copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

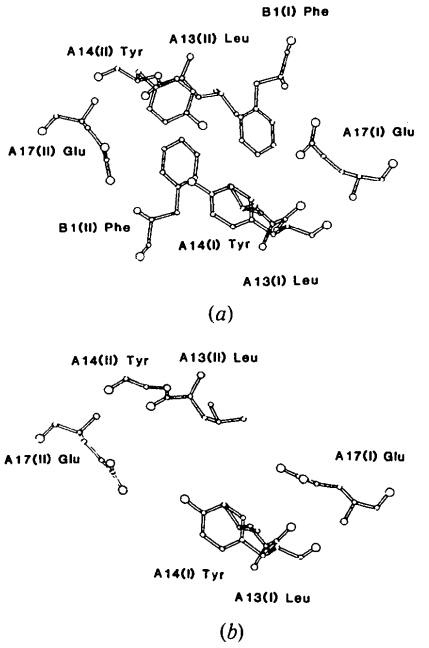

Fig. 2. Interactions between dimer units in (*a*) 2-zinc porcine insulin and (*b*) des-Phe B1 bovine insulin hexamers.

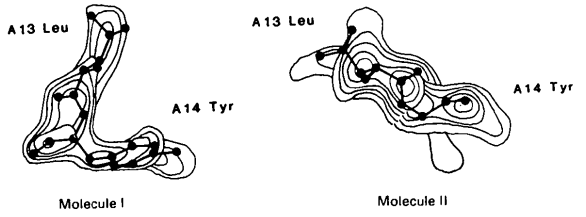

Fig. 3. Residues A13 and A14 of molecules I and II of des-Phe B1 insulin and their respective electron densities.

glutamine residue, A15, was also observed to have low side-chain density. These regions, along with representations of the electron density, are illustrated in Fig. 3. Interestingly enough, these regions are well defined in molecule I.

These results are of particular interest in trying to explain the CD results obtained for 2-zinc porcine insulin and des-Phe B1 bovine insulin in solution. Based upon theoretical calculations, it has been estimated that 44% of the total CD for the insulin hexamer arises from the B1 phenylalanine residue perturbing the A14 tyrosine residue (Strickland & Mercola, 1976; Wollmer *et al.*, 1977). It was therefore expected that the result of removing the N-terminal phenylalanine residue of the B chain would have significant spectral consequences. However, this was not the case and the spectra were not significantly altered. Early attempts to explain this discrepancy invoked both rotational motion and thermal vibration of the phenylalanine group.

However, the results of this crystallographic study nicely account for the discrepancies in the calculated and observed CD spectra. Energy calculations for rotations about χ^2 of A14, molecule II, show that this residue in des-Phe B1 bovine insulin has more freedom of rotation in the hexamer than does the same residue in the monomer or dimer of normal 2-zinc porcine insulin (Wollmer *et al.*, 1980). Thus, the removal of the B1 phenylalanine residue allows free rotation of the A14 tyrosine side chain about the C^{β}–C^{γ} bond. However, in 2-zinc bovine insulin the A14 tyrosine side chains are well localized at 2·3 Å resolution (Reynolds, Smith, Wood, Dodson & Duax, 1982). If this observed free rotation of A14 of molecule II is taken into account, the calculated CD for des-Phe B1 insulin ($-2{\cdot}81 \times 10^{-3}$ mol^{-1} cm^2) is nearly that calculated for native insulin ($-3{\cdot}01 \times 10^{-3}$ mol^{-1} cm^2) for identical concentrations in the hexameric state at 275 nm (Wollmer *et al.*, 1980). If the free rotation of the A14 is imposed upon both molecules, then the calculated CD is in excellent agreement ($-2{\cdot}99 \times 10^{-3}$ mol^{-1} cm^2), explaining why the presence or absence of B1 phenylalanine does not affect the spectra (Wollmer *et al.*, 1980).

The results of this study have also shown that a very limited amount of data, 2·5 Å resolution for example, will suffice to define the structure of a complicated molecule. This was illustrated clearly at the beginning of the structural analysis by the fact that sequence differences at A8 and at A10 could be observed on the electron density difference maps. These results suggest that if a good model such as 2-zinc porcine insulin is used as the starting point, the structure can be successfully refined through the use of the fast Fourier least-squares methods (Agarwal, 1978) and *MODELFIT* (Dodson, Isaacs & Rollett, 1976).

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The Structure and Absolute Configuration of Viridicatumtoxin: 2′ *S*-(2′α,7′a*β*,11′a*β*,12′*β*)-7′,7′a,8′,11′,11′a,12′-Hexahydro-5′,6′,7′a,10′,11′a,12′-hexahydroxy-3′-methoxy-2,6,6-trimethyl-7′,8′-dioxospiro[2-cyclohexene-1,2′(1′*H*)-cyclopenta[*de*]naphthacene]-9′-carboxamide Methanolate

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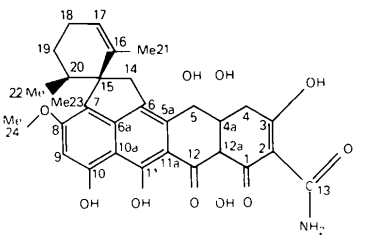
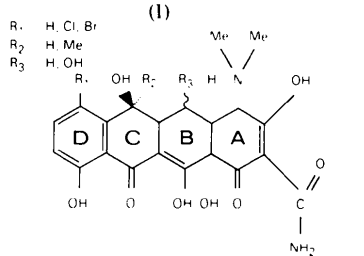
Abstract

$C_{30}H_{31}NO_{10}.2(?)CH_3OH$, M_r = 565·58 + 2(?) × 32·04, habit: prismatic, *I*2 (No. 5), Cu $K\alpha$ (graphite monochromator), λ = 1·5418 Å, a = 12·9784 (12), b = 7·8029 (8), c = 29·3152 (25) Å, β = 99·309 (9)°, V = 2929·63 Å^3, Z = 4, D_x = 1·428 g cm^{-3}, crystal size: 0·25 × 0·15 × 0·15 mm, 3202 ($482 < 1\sigma$) reflections, maximum $\sin\theta/\lambda$: 0·6233 Å^{-1}. The structure was determined by direct methods and has been refined to R = 0·031. The absolute configuration, determined by anomalous scattering, is the same as the tetracyclines at comparable atoms. The bond lengths associated with the carboxamide moiety indicate π-conjugation.



(I)



(II)

Introduction

Details of the isolation and characterization of the title compound (I), which will hereafter be referred to as viridicatumtoxin, are given by Hutchison, Steyn & van Rensburg (1973). The atomic numbering has been chosen to conform to that used in the tetracyclines and does not correspond to the *Chemical Abstracts* name given in the title.

A description of the chemical structure and relative configuration, determined by crystal-structure analysis with very little chemical information, has been given by Kabuto, Silverton, Akiyama, Sankawa, Hutchison, Steyn & Vleggaar (1976). The original X-ray data refined to an R factor of 0·059 which was considered too high for a reliable determination of absolute