NMR STUDIES OF THE CONFORMATION OF POLYPEPTIDE CHAINS BOUND TO LIPID MICELLES

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INTRODUCTION

The contents of the present paper relate to a particular aspect of the interactions between polypeptide chains and ordered lipids, i.e. investigation of the molecular conformations adopted by the lipid-bound polypeptide chains and comparison with the structures in different environments, such as in dilute aqueous solution or in single crystals. The main emphasis is on the description of the nuclear magnetic resonance (NMR) methodology used for structural studies of lipid-bound polypeptides. As an illustration, recent work with the polypeptide hormones glucagon and melittin is reviewed.

Glucagon is a hormone which consists of a linear polypeptide chain of 29 amino acid residues and has a molecular weight of 3500. The primary target organ for glucagon is the plasma membrane of liver and other cells. Specific binding to a plasma membrane receptor site mediates activation of glycogenolysis[1]. Evidence has been presented that recognition between glucagon and its receptor depends on the ordered lipid structures surrounding the receptor site in the membrane[2,3]. A possible avenue to further insights into the mode of action would be via knowledge of the conformations adopted by glucagon in the different environments encountered on the way from the sites of its synthesis in the islets of Langerhans to the complex formation with the receptor site. Previously, an α-helical conformation of glucagon was determined in single crystals[4], but ^{1}H NMR studies showed that the α-helical form was not preserved in aqueous solution of monomeric glucagon[5]. It appeared then of considerable interest to complement these structural data with a determination of the molecular conformation in a lipid-water interface, e.g. along the surface of lipid micelles.

Melittin is a polypeptide of 26 amino acid residues which constitutes about 50% of the dry weight of bee venom. Melittin has a variety of effects on natural membranes, including lysis[6], activation of exogenous or endogenous phospholipase A[7,8], alterations in mitochondrial respiration[9] and adenylate cyclase activity[10], and specific changes in coupling of photosynthetic systems[11]. These effects of melittin appear to result from the ability of the polypeptide to alter lipid organization[12,13], so that comparative studies of melittin in aqueous solution and in lipid-water interfaces are of particular interest with regard to the structural basis of its biological functions.

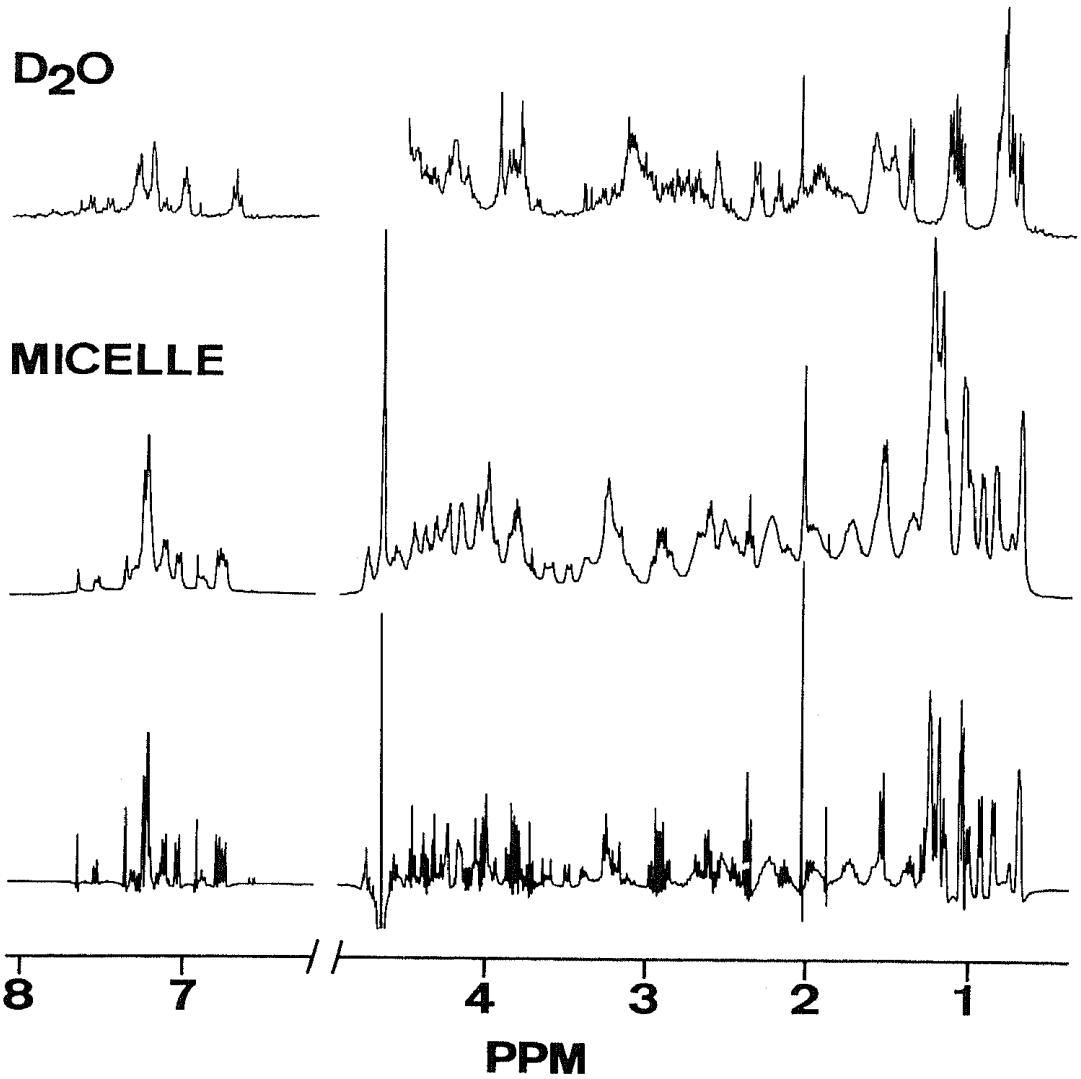

FIGURE 1. 360 MHz ^1H NMR spectra at p^2H 10.8 and 37°C of monomeric glucagon in ^2H_2O solution and glucagon bound to deuterated dodecylphosphocholine micelles. (A) $1 \cdot 10^{-4}$ M glucagon (monomeric). (B) $1.2 \cdot 10^{-3}$ M glucagon plus $6 \cdot 10^{-2}$ M [$^2H_{38}$]dodecylphosphocholine. (C) Same as B except that the spectral resolution was improved by applying a phase-shifted sine bell to the free induction decay. (Reproduced with permission from ref.[14]).

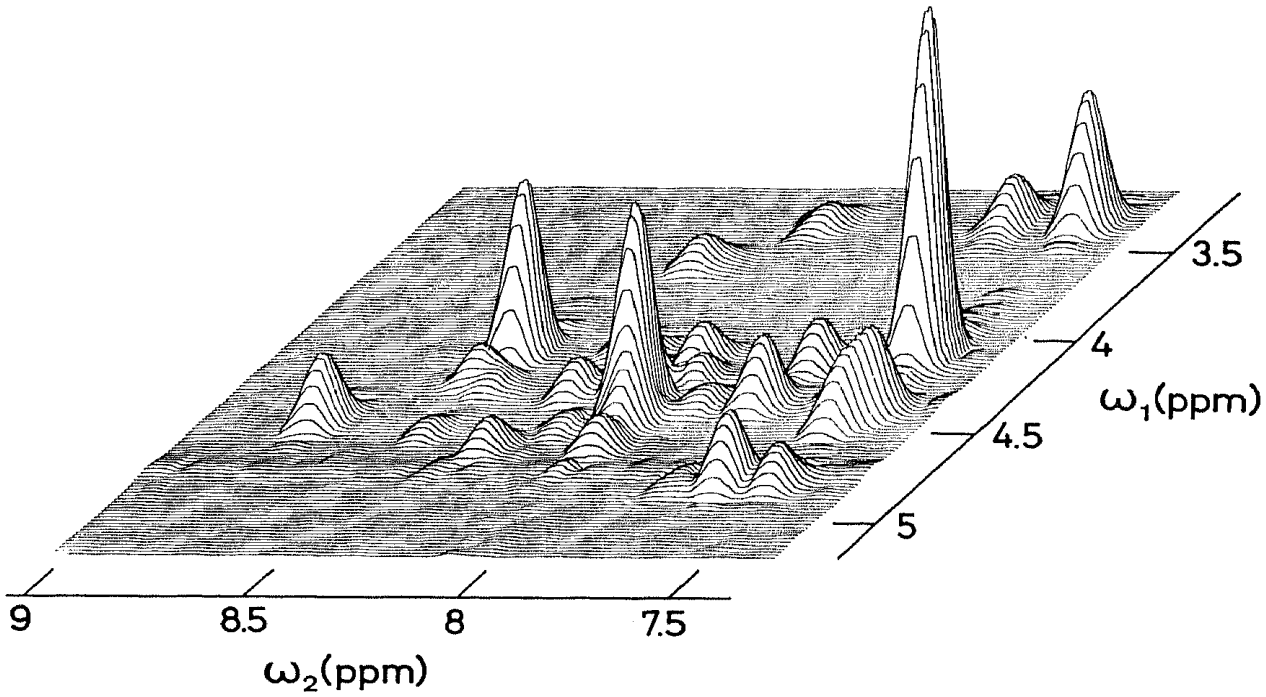

FIGURE 2. Contour plot of the spectral region (3.1 to 5.2ppm) x (7.4 to 9.1ppm) of a 360 MHz ^{1}H COSY (two-dimensional correlated spectroscopy) spectrum of glucagon bound to perdeuterated dodecylphosphocholine micelles. The sample contained 0.015 M glucagon, 0.7 M [$^2H_{38}$]dodecylphosphocholine and 0.05 M phosphate buffer in a mixed solvent of 90% H_2O and 10% ^2H_2O, pH = 6.0, T = 37°C. COSY spectra manifest J-coupling connectivities. The peaks in this particular spectral region correspond to the spin-spin couplings between the amide protons and the C^α-protons of residues 3-29 in micelle-bound glucagon[17].

^{1}H NMR SPECTROSCOPY OF POLYPEPTIDES BOUND TO PERDEUTERATED LIPID MICELLES.

By means of ultracentrifugation and quasi-elastic light-scattering, it was found that stoichiometrically well defined complexes were formed between glucagon and perdeuterated dodecylphosphocholine micelles, consisting of one glucagon molecule and approx. 40 detergent molecules and with a molecular weight of ca. 17'000[14]. Corresponding mixed micelles are formed of one molecule of melittin and ca. 40 molecules of dodecylphosphocholine[15]. Well resolved ^{1}H NMR spectra can be obtained for the polypeptide chains in the mixed micelles (Figs. 1 and 2)[14-17], which are thus amenable for all the NMR techniques which are generally used for conformational studies of proteins in solution[18].

While the NMR spectral features in Figs. 1 and 2 correspond largely to those for a globular protein of comparable size to the mixed micelles, it was found that spin diffusion by proton-proton cross relaxation[19,20] is much less pronounced for the lipid-bound polypep-

tides. As an illustration, Fig. 3 shows a series of TOE (truncated driven nuclear Overhauser enhancement) difference spectra obtained by selective preirradiation of the α-proton resonance of Val-23 in micelle-bound glucagon for different times. In this experiment negative NOE's are seen to build up[20] in the aliphatic and aromatic regions of the spectrum. Yet, even after preirradiation for 1 sec, cross relaxation effects were limited to a small number of amino acid side chains

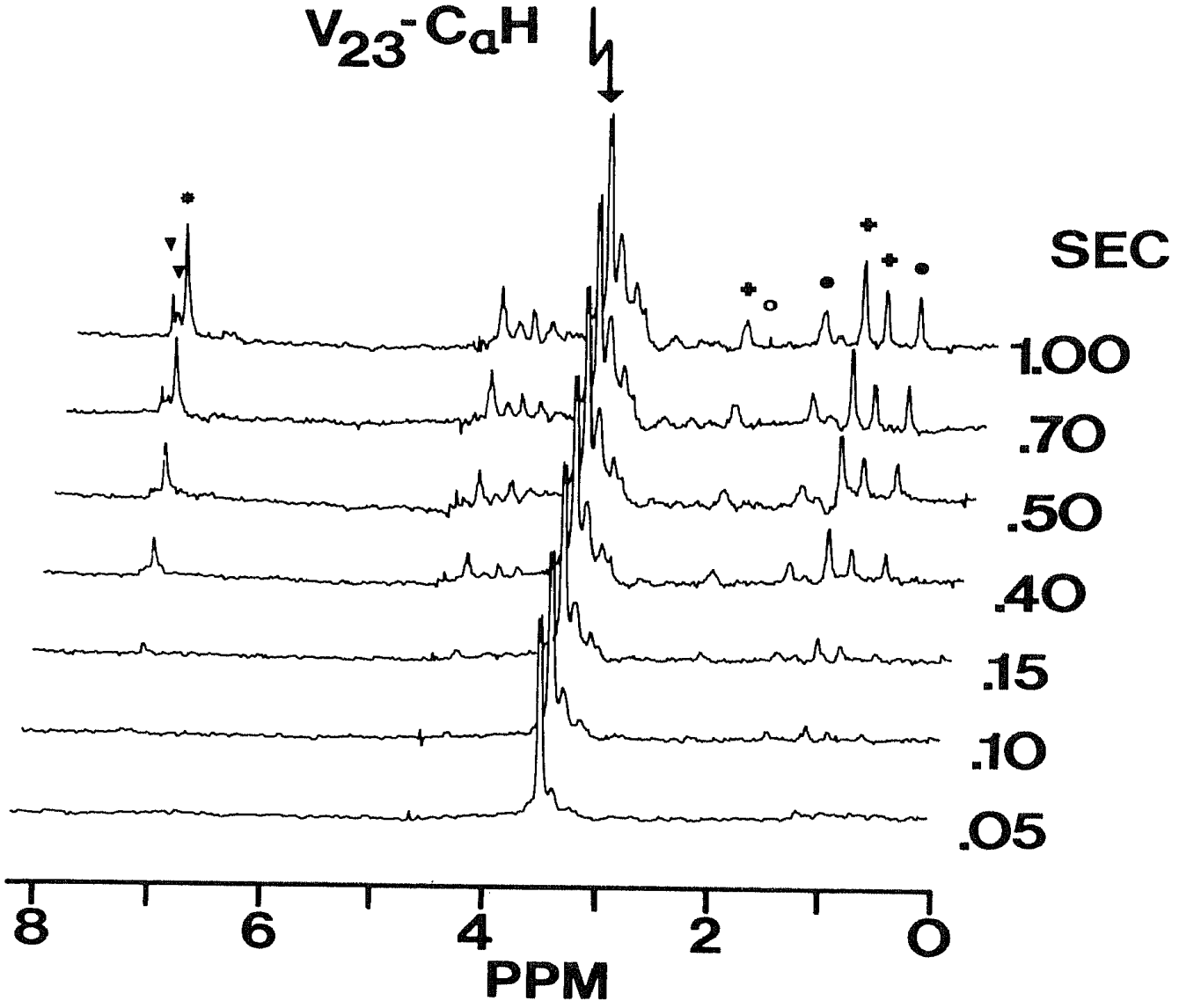

FIGURE 3. TOE (truncated driven nuclear Overhauser enhancement) difference spectra[20] of micelle-bound glucagon recorded with preirradiation of the α-proton resonance of Val-23 at 3.50ppm (arrow). The numbers on the right indicate the time used for preirradiation prior to data aquisition. In this presentation, positive peaks correspond to negative nuclear Overhauser effects. The individual peaks correspond to the following amino acid residues of glucagon: ▼ Phe-22, + Val-23, * Trp-25, ● Leu-26, o Met-27. (Reproduced with permission from ref.[21]).

(see figure caption 3 for the resonance assignments) and a highly selective TOE difference spectrum was obtained. In other experiments with the same glucagon sample and with micelle-bound melittin, highly selective NOE's were observed after preirradiation of different resonance lines for as long as 8 sec[21]. Using the qualitative considerations outlined in the following, studies of spin diffusion then allowed to delineate the overall shape of the three-dimensional structure of micelle-bound polypeptide chains[21].

Two essential requirements for efficient proton-proton spin diffusion are that the product of the nuclear Larmor frequency, ω, and the effective rotational correlation time, τ_c, is larger than 1, and that the preirradiated proton and the protons affected by direct dipolar coupling are not spatially separated from the bulk of the protons[19,20]. Since cross relaxation depends on the inverse sixth power of the proton-proton distance, internuclear distances of 4 to 5 Å or more constitute an effective barrier for the propagation of spin diffusion[20,22]. For most amino acid residues of the polypeptide chains of glucagon or melittin bound to deuterated detergent micelles, the condition $\omega\tau_c > 1$ prevails, as is clearly manifested in the negative NOE's between protons attached to the same amino acid residues[21]. Therefore, absence of cross relaxation between protons of two different amino acid residues indicates that these protons are located at a distance of more than 4 to 5 Å in the deuterated micelle[21]. With regard to the spatial arrangement of polypeptide chains in deuterated lipid micelles, one thus has that in an extended chain spin diffusion is restricted mainly to pathways along the covalent polypeptide structure (Fig. 4). On the

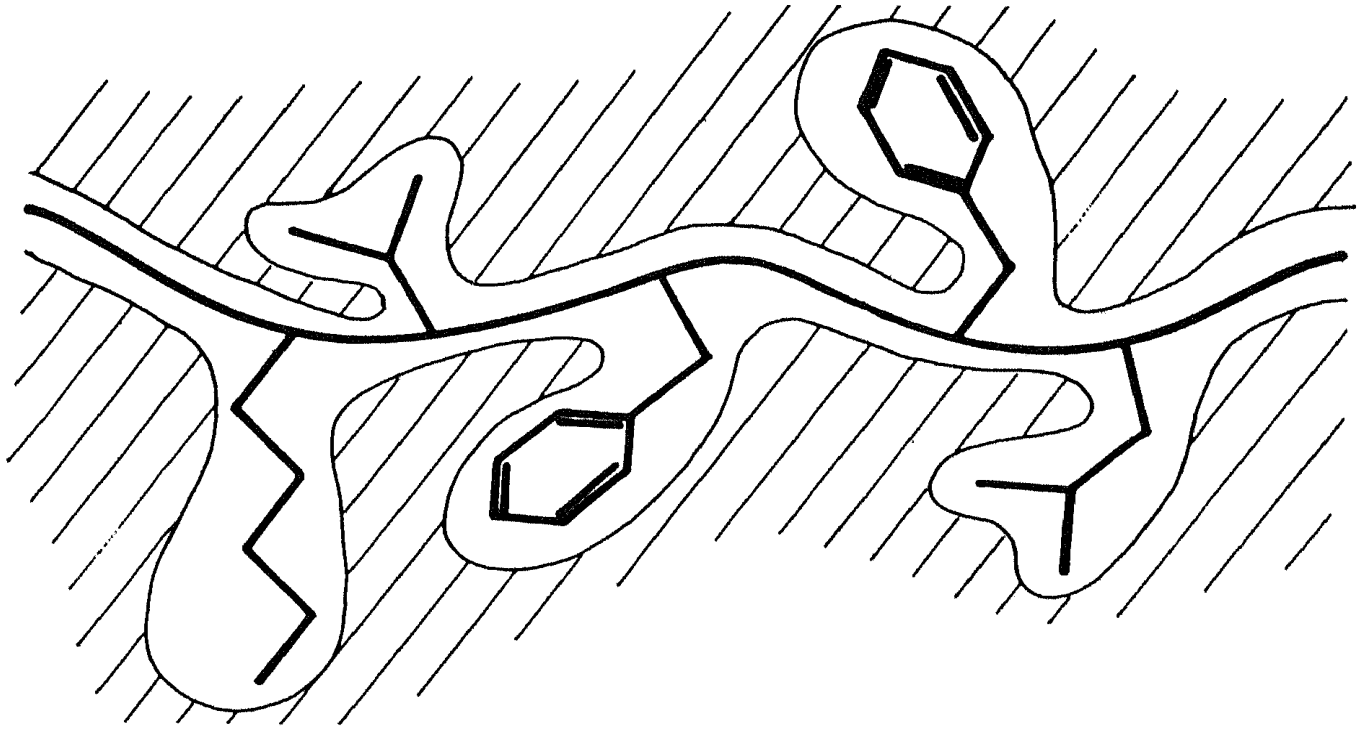

FIGURE 4. Schematic drawing of an extended polypeptide chain incorporated into a lipid micelle. The dashed areas represent the space occupied by the deuterated lipid. (Reproduced with permission from ref.[21]).

other hand, when the polypeptide forms a compact spatial structure in the interior of a deuterated lipid micelle, cross relaxation is to be expected also between protons of different amino acid side chains, in much the same way as in globular proteins. On this basis studies of spin diffusion represent a quite straightforward method to investigate whether micelle-bound polypeptide chains adopt a predominantly extended conformation, or a more compact, globular structure.

LOW RESOLUTION CONFORMATION FOR MICELLE-BOUND MELITTIN OBTAINED FROM COMBINED USE OF PROTON-PROTON OVERHAUSER ENHANCEMENTS AND A DISTANCE GEOMETRY ALGORITHM.

In spite of the relatively low molecular weight, individual resonance assignments for micelle-bound melittin were so far obtained only for a limited number of amino acid residues in the C-terminal segment of the polypeptide chain[23,24]. Nuclear Overhauser enhancement measurements showed that upon binding of melittin to the detergent micelles the entire polypeptide chain adopts a quite rigid conformation, and that the amino-terminal and carboxy-terminal halves of the primary structure constitute separate domains within the conformation of micelle-bound melittin[23]. Using the distance constraints from NOE's as the input for a distance geometry algorithm[22], the conformation of the polypeptide segment 16-24, which is near the C-terminus, was determined in more detail[24]. Fig. 5 shows a group of 4 structures obtained in four different computer runs using the same experimental data. The principal common features of the structures (a) - (d) in Fig. 5 are two successive, irregular right-handed turns formed, respectively, by the backbone atoms of residues 16-20 and 20-24.

With regard to the structural basis of the functional properties of melittin, it was further of interest to investigate the location of the polypeptide chain in the micelle. Selective line broadening of melittin resonances by different spin labels attached to the lipid chains in the micelle was used to locate different portions of the melittin amino acid sequence relative to the micelle surface[25]. From this data it appeared unlikely that melittin penetrates through the micelle interior. Rather, it appeared that extended portions of the N-terminal as well as the C-terminal domains of the melittin conformation are near the micelle surface. A more specific location and orientation could be determined for residues 16-24 of micelle-bound melittin. The paramagnetic line-broadening effects indicated that the peripheral side chain protons of residues 21-24 are approximately equidistant from the center of the micelle and near the level of the phosphate group of the detergent molecules in the dodecylphosphocholine micelles[24]. This then pointed to a quite unexpected structural feature, i.e. from the conformations in Fig. 5 it appeared that the axis of the turn formed by residues 16-20 must lie roughly perpendicular to the micelle surface. The residues 16-20 would thus penetrate 6-8 Å into the apolar interior of the micelle. Since on the other hand evidence was obtained that both the C-terminal and the N-terminal domains of melittin are near

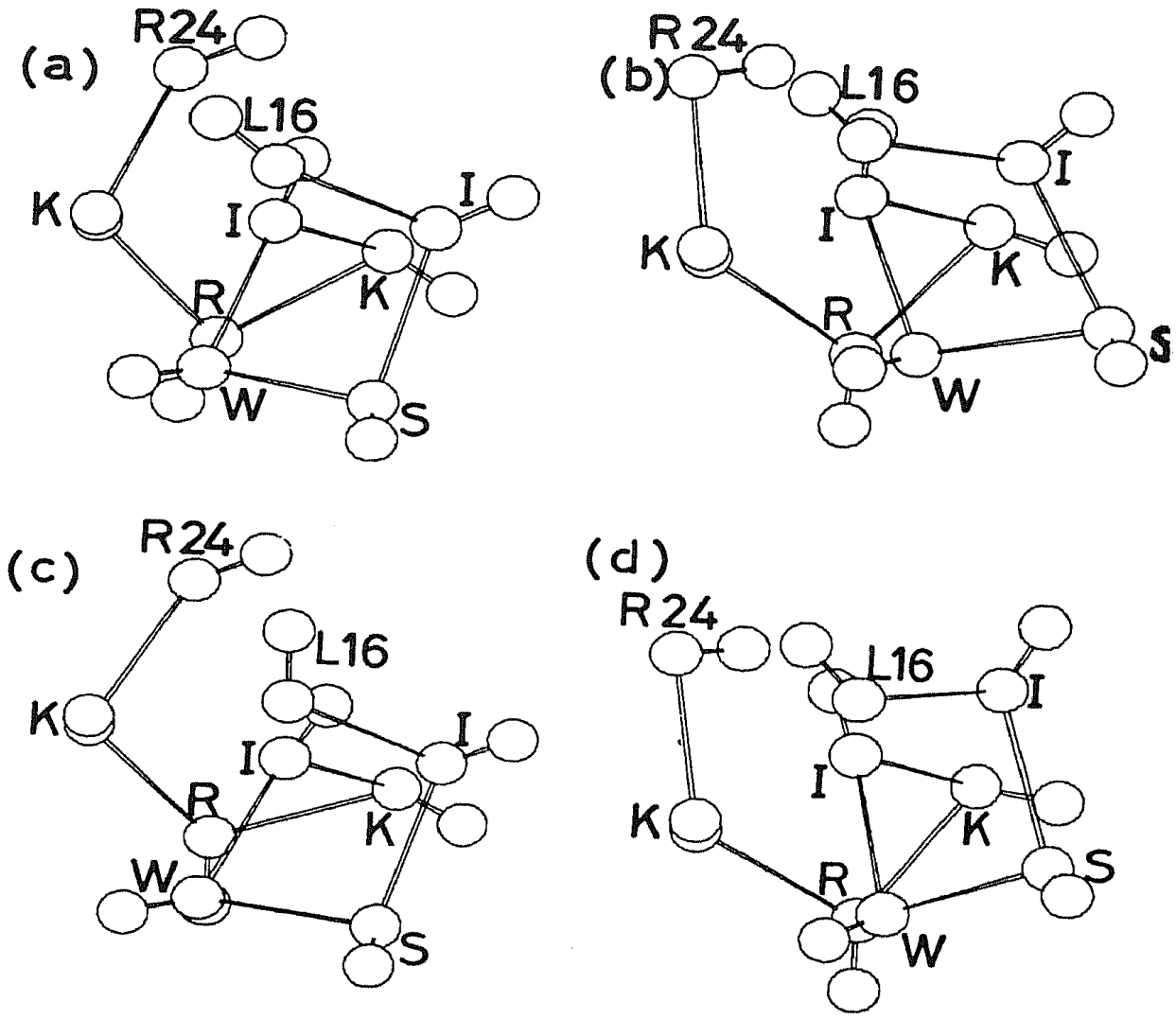

FIGURE 5. Computer drawing of four structures for the segment 16-24 of micelle-bound melittin which were obtained from four different computer runs using the same experimentally determined distance constraints as input for the distance geometry algorithm[22]. Root mean square distances between these structures are of the order of 1.0 Å. Only the positions of the α- and β-carbon atoms are shown. All four structures were oriented in the same way: First, L 16, I 20 and K 21 were placed in a horizontal plane and then, to avoid overlapping of too many atoms, the structures were rotated by 10° about the horizontal axis through the centroid. (Reproduced with permission from ref.[24]).

the micelle surface, the central region -Thr-10-Thr-11-Gly-12-Leu-13-Pro-14- must form a turn in the apolar micelle interior, causing a reversal of the backbone direction. As was argued in more detail elsewhere[24], it appears likely that this structural arrangement of melittin would cause major perturbations of ordered lipid layers.

LOW RESOLUTION CONFORMATION FOR MICELLE-BOUND GLUCAGON.

In glucagon individual assignments of amino acid side chains spin systems[18] were initially obtained for a limited number of residues which were, however, distributed throughout the amino acid sequence[14]. Studies of spin diffusion pathways as described above[21] then revealed that glucagon adopted a predominantly extended, quite rigid conformation, with a cluster of amino acid side chains formed by the residues 19-27 near the C-terminus. Based on the distance constraints between a limited number of assigned protons, more detailed information was obtained via the distance geometry algorithm for this portion of the glucagon molecule (Fig. 6)[22]. The following common features of the three structures in Fig. 6 are readily apparent: The molecule has a roughly cylindrical shape. The backbone is in the form of a distorted S, which consists of two loops from residues 19-22 and 23-27 connected by a nick at residue 22. In all three structures the same amino acid side chains are in close proximity to one another (Fig. 6). The aromatic rings of Phe-22 and Trp-25 are on the same side of the backbone, forming a hydrophobic "patch". Leu-26 is not part of this patch, and Val-23 is not in the immediate neighbourhood of the side chain of Trp-25.

The structure of micelle-bound glucagon is undoubtedly very different from that of monomeric glucagon in aqueous solution[5]. Further refinement of the glucagon conformation in the lipid micelles (see following section) seems to be needed to determine whether it is a different structure from that in glucagon single crystals[4]. While the structures in Fig. 6 contain features which are characteristically different from the originally proposed crystal structure[4], all these differences would also be compatible with the 2.8 Å resolution X-ray data (T.L. Blundell, private communication).

OUTLOOK: SEQUENTIAL RESONANCE ASSIGNMENTS AS A BASIS FOR DETERMINATION OF THE CONFORMATION OF MICELLE-BOUND GLUCAGON AT HIGHER RESOLUTION.

If in addition to the interatomic distance constraints imposed by the covalent structure all other interatomic distances in a polypeptide chain were known and the chirality of the structure independently determined, the corresponding three-dimensional structure could be unambiguously generated with the presently used distance geometry algorithm[22,26]. For the melittin peptide segment 16-24 as well as for the glucagon segment 19-27, however, only a limited number of constraints had originally[22-24] been obtained from the NOE experiments, since individual assignments were obtained only for a small number of side chain resonances. It is readily apparent that the reliability of the spatial polypeptide structures determined with the presently described methodology depends critically on the number of distance constraints between individually assigned protons[22,27]. We have therefore mainly concentrated on improvement of techniques for resonance assignments, including the amide protons of the polypeptide backbone[28-32]. With the use of proton-proton connectivity maps obtained from two-dimensional NMR experiments (Figs. 2 and 7)[33,34], complete individual assignments for all but very few hydrogen atoms in micelle-bound

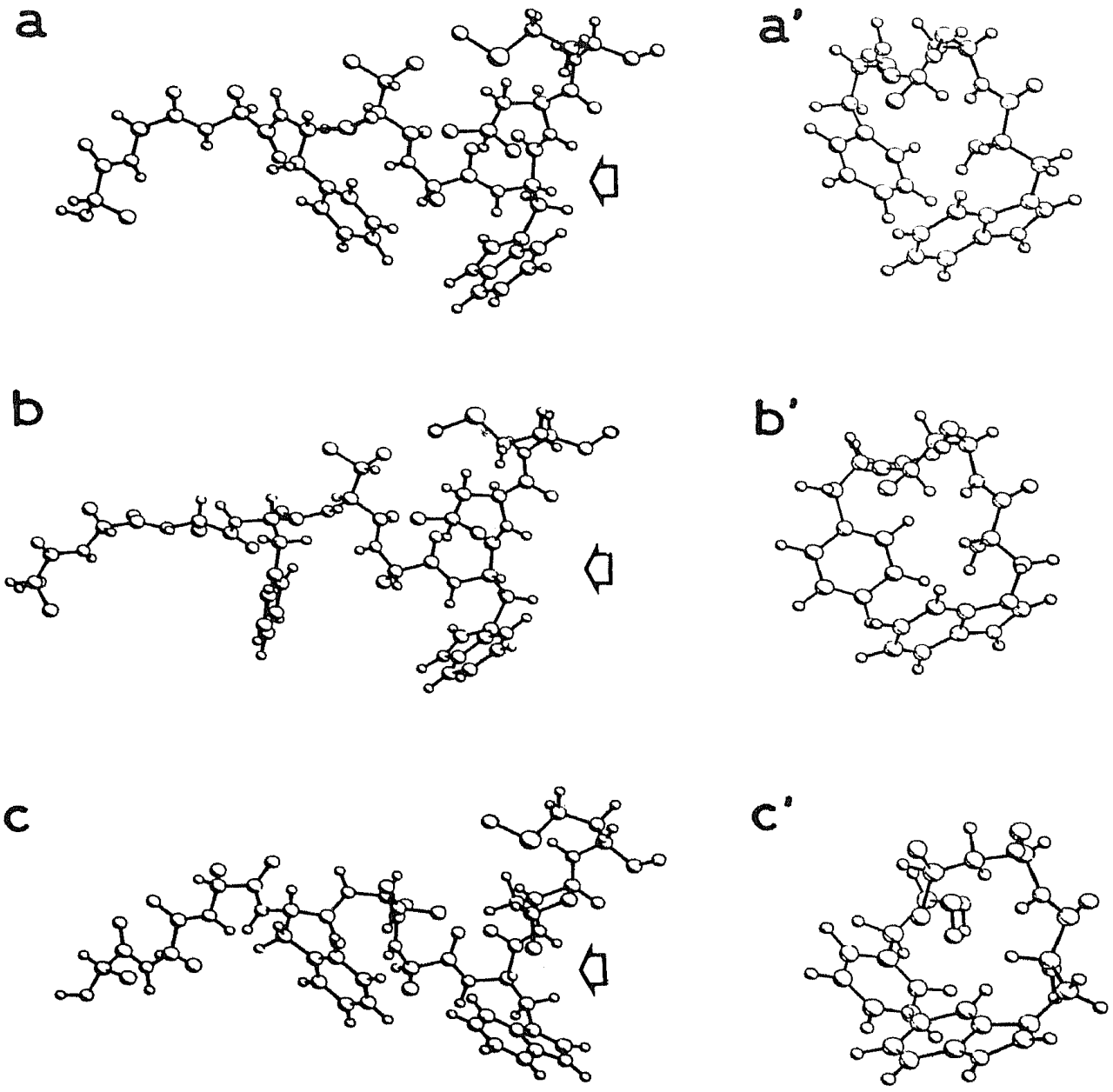

FIGURE 6 a-c. Computer drawings of three molecular geometries of the glucagon region 19-27 which satisfy the distance constraints imposed by the experimental nuclear Overhauser data. The drawings show the sequence —Ala-19 —Gln-20 —Asp-21 —Phe-22 —Val-23 —Gln-24 —Trp-25 —Leu-26 —Met-27 —, with Ala-19 in the lower left and Met-27 in the upper right in each figure. The complete molecular structure including the hydrogen atoms is shown, with the following exceptions: The residues Gln-20, Asp-21 and Gln-24, for which no Overhauser enhancements with side chain hydrogen atoms were observed, were replaced by glycine; the methyl groups of Ala-19, Val-23, Leu-26 and Met-27 are represented by the carbon atom only. The arrows indicate the direction along which the same structures are viewed in the drawings a'-c'. These show only the residues Phe-22 and the dipeptide fragment Trp-25 —Leu-26. In this presentation the locations of the hydrophobic rings of Phe-22 and Trp-25 and the orientation of the side chain of Leu-26 relative to the aromatic rings are nicely illustrated. (Reproduced with permission from ref.[22]).

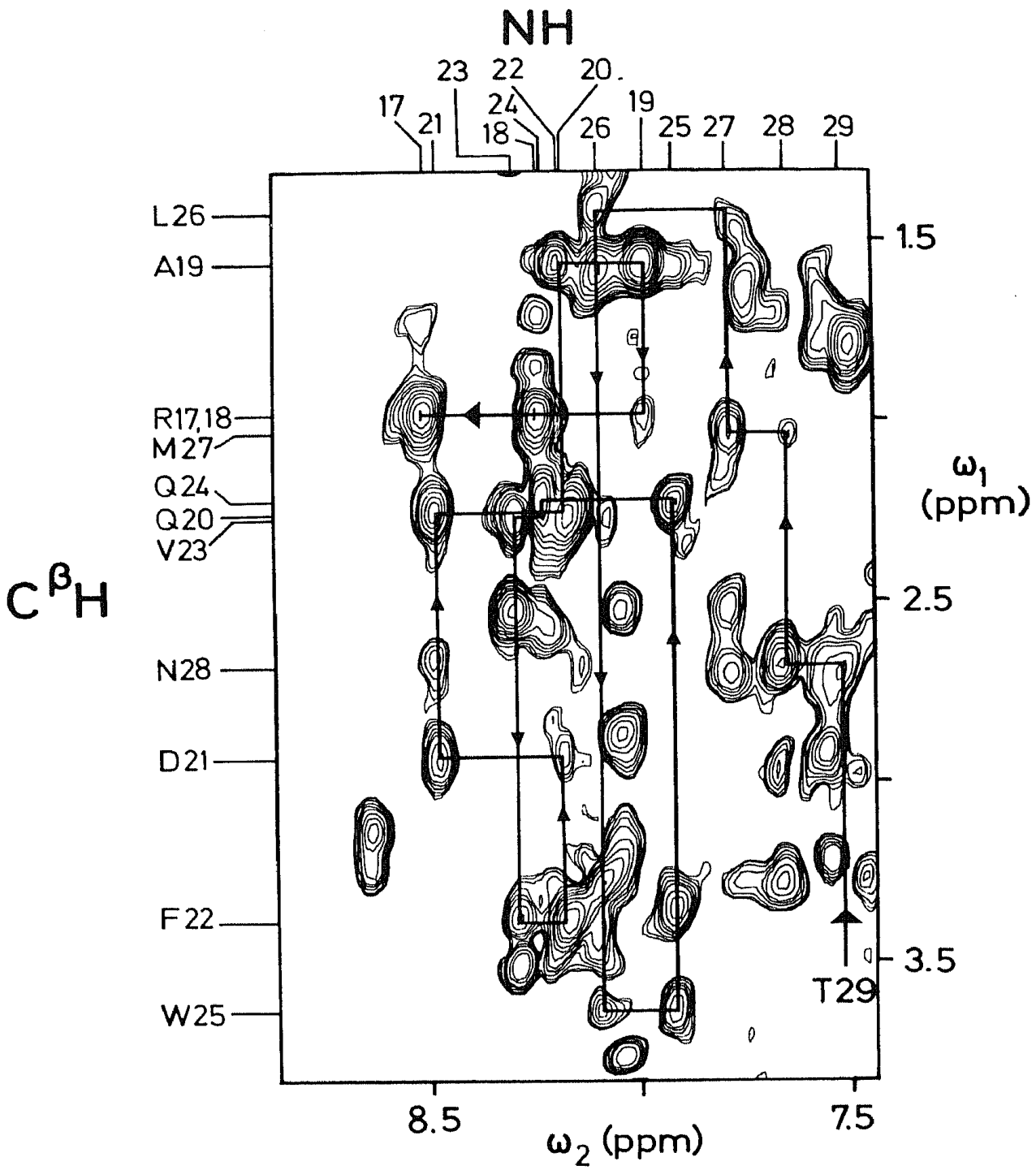

FIGURE 7. Contour plot of the spectral region (1.3 to 3.8ppm) x (7.4 to 8.8ppm) of the 360 MHz ^{1}H NOESY (two-dimensional nuclear Overhauser enhancement spectroscopy) spectrum of micelle-bound glucagon recorded in the same sample as Fig. 2. The solid lines with arrows indicate the sequential assignments for the polypeptide segment 17-29, which were obtained from NOE's between amide protons and the C^{β} protons of the preceding residues [31]. The numbers at the top of the figure indicate the amide proton chemical shifts of the corresponding residues, those on the left margin indicate the chemical shifts of one C^{β} proton for each residue. NOESY spectra represent through-space proton-proton connectivity maps, and combined with COSY spectra recorded under similar conditions they lead to sequential resonance assignments in polypeptide chains [30,31]. A detailed dicussion of the interpretation of this spectrum is being published elsewhere[17]. (Reproduced with permission from ref. [17]).

glucagon were obtained[17]. On this basis a very large number of distance constraints between distinct groups of protons can now experimentally be determined[35]. With this data as the input, the distance geometry algorithm should provide a much more precise structure for the glucagon segment 19-27,and the structure determination will be extended to the entire amino acid sequence of micelle-bound glucagon.

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