USE OF PROTON NUCLEAR OVERHAUSER EFFECTS FOR THE DETERMINATION OF THE CONFORMATIONS OF AMINO ACID RESIDUES IN OLIGOPEPTIDES

S. J. Leach, George Némethy, and H. A. Scheraga
Department of Chemistry, Cornell University, Ithaca, New York 14853

Received December 27,1976

SUMMARY: The use of the Nuclear Overhauser Effect to determine backbone and side-chain conformations of oligopeptides is discussed. The distance between the H^{α} proton of a given residue and the amide proton of the following residue depends only on the dihedral angle ψ. A calibration curve is given for the determination of ψ from the Nuclear Overhauser Effect involving these protons. In amino acids with branched side chains, e.g., threonine, isoleucine, and valine, the Nuclear Overhauser Effect involving the H^{β} proton and the amide proton in either the same or the following residue gives limited information about both χ^1 and either ϕ or ψ. The Nuclear Overhauser Effect involving the H^{α} and H^{γ} protons in leucine gives information about χ^1 and χ^2.

We propose the use of the Nuclear Overhauser Effect as an aid in assigning backbone and side-chain dihedral angles of oligopeptides (1). Such data would supplement the use of proton vicinal coupling constants, which provide information about the dihedral angles ϕ and χ^1 (2,3), and of H^{α}-^{15}N vicinal coupling constants which have been suggested (4,5) for the study of ψ.

Nuclear Overhauser enhancement of nmr signals occurs for pairs of protons in close spatial proximity (6). Bell and Saunders showed (7) that the magnitude (in percent) of the Nuclear Overhauser Effect can be correlated with the internuclear distance d by means of the equation

$$\text{Nuclear Overhauser Effect} = 100/Ad^6 \qquad (1)$$

where the constant A has the value 1.8×10^{-2} Å^{-6} for H···H interactions.

Measurements of the Nuclear Overhauser Effect have been used qualitatively in a few conformational studies of oligopeptides. Howard et al. (8) used the Nuclear Overhauser Effect to investigate β-bends by showing the

---

Abbreviations: ECEPP, Empirical Conformational Energy Program for Peptides; nmr, nuclear magnetic resonance.

ISSN 0006-291X

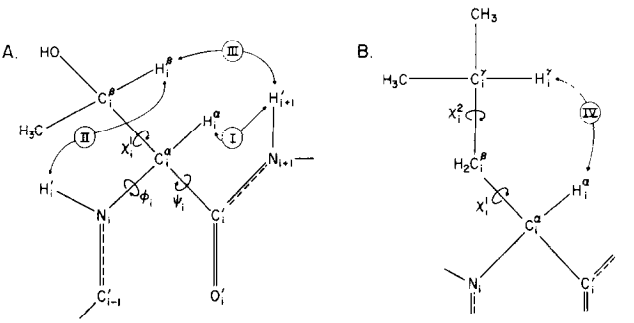

Fig. 1. A. A threonyl residue. B. A leucyl residue. The Roman numerals and the curved arrows indicate the pairs of protons for which Nuclear Overhauser Effects are discussed here. The subscripts refer to the standard nomenclature for residues in a polypeptide (1).

proximity of terminal methyl groups. Khaled and Urry (9) distinguished between type I and type II β-bends by showing that the distance, and hence the Nuclear Overhauser Effect, between the CH^{α} proton of the first residue and the NH proton of the second residue of the bend is different in the two types. Gibbons et al. (10) mentioned a similar use of the Nuclear Overhauser Effect to show close proximity of several protons in gramicidin S.

We present, first, a generalization of the findings of Khaled and Urry (9). Because of the planarity of the peptide group, the distance d_I between the α proton of residue i (H^{α}_i) and the amide proton of the next following residue i+1 (H'_{i+1}) in the sequence depends only on the dihedral angle ψ_i (Figure 1A). Thus, a general correlation can be derived between the value of the Nuclear Overhauser Effect involving these two protons and ψ_i (Figure 2).

In addition, we show that certain conformations can be differentiated by observing the Nuclear Overhauser Effect between various pairs of backbone and side-chain protons whose relative positions depend on the values of two adjacent dihedral angles (Figure 1). Because of the difficulty in measuring the Nuclear Overhauser Effect for protons in CH_2 groups, this part of our study is restricted to amino acids with branched side chains, i.e., to those containing a CH group.

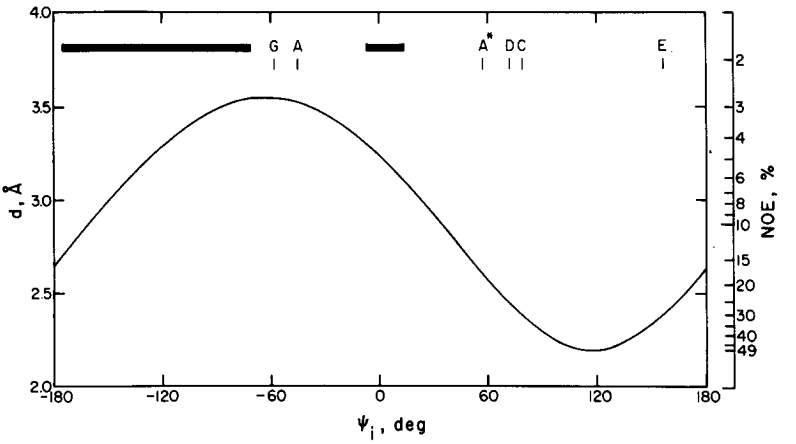

Fig. 2. The $H^{\alpha}_{i} \cdots H'_{i+1}$ distance, d_I, plotted as a function of ψ_i in an L-amino acid residue, for the backbone geometry used in the ECEPP computer program (11). The vertical scale on the right-hand side of the Figure shows the Nuclear Overhauser Effect (NOE) according to Eq. 1. The heavy horizontal bars at the top of the Figure show those ranges of ψ_i for which the energy of an alanyl residue exceeds ΔE = 3 kcal/mol for any ψ_i on a (ϕ,ψ) conformational map (12). The values of ψ_i for the minimum-energy conformations of N-acetyl-alanyl N'-methyl amide (12) are indicated by short vertical lines. Letters A to G refer to the short-hand notation for low-energy conformational states (12).

METHODS

Interatomic distances and peptide potential energies were computed by means of a computer program (ECEPP) developed in this laboratory for conformational energy calculations on peptides (11). The program is based on a standardized set of parameters for molecular geometry described in detail elsewhere (11).

RESULTS AND DISCUSSION

Figure 2 shows the $H^{\alpha}_{i} \cdots H'_{i+1}$ distance, d_I, and the corresponding Nuclear Overhauser Effect as a function of ψ_i for an L-amino acid. A sizable Nuclear Overhauser Effect (> about 10%, corresponding to d < 2.9 Å) is predicted for $35° < \psi < 180°$ and $-180° < \psi < -160°$. This range of ψ coincides almost exactly with the width of the large low-energy region, in which ΔE < 3 kcal/mol, in the (ϕ,ψ) conformational energy map for N-acetyl-alanyl-N'-methyl amide (Fig. 2 of ref. 12). This region encompasses the extended chain (E), the C_7^{eq} hydrogen-bonded ring (C), and other related conformations (12). The

Nuclear Overhauser Effect is also large for the α_L conformation (A*). By contrast, $d_I > 3.3$ Å, and hence the Nuclear Overhauser Effect is very small (<4%), over the entire range $-120° < \psi < -10°$. This range includes that low-energy region, within the $\Delta E < 3$ kcal contour, which contains the α_R conformation. Such a value for the Nuclear Overhauser Effect is only slightly above the experimental error of measurement for a rigid model system (9). Thus, the absence or presence of a sizable Nuclear Overhauser Effect involving the H^{α}_{i} and H'_{i+1} protons serves as a distinguishing tool between the right-handed α-helical and most of the other conformations of amino acid residues. In addition, Fig. 2 can be used to determine approximate values of ψ from observed Nuclear Overhauser Effect data. It is unlikely that conformations occur in the high-energy region $-170° < \psi < -70°$ in small peptides. If this region of ψ is disregarded in Fig. 2, ψ becomes a single-valued function of the Nuclear Overhauser Effect in the range $-70° < \psi < 50°$; it is double-valued only for $\psi > 50°$. In many cases, this would allow an unambiguous determination of ψ. When the Nuclear Overhauser Effect is small (d_I is large), the relative errors of the Nuclear Overhauser Effect are large, leading to some uncertainty in the determination of ψ. This uncertainty is increased by the flatness of the d_I vs. ψ curve around $\psi = -60°$.

Figure 2 confirms and extends the conclusions of Khaled and Urry (9). The large Nuclear Overhauser Effect for the $C^{\alpha}H$ of the second residue in a tetrapeptide forming a type II β-bend can be used to distinguish the type II bend from type I, I', and II' bends.

The distances between the two pairs of protons, indicated by II and III in Fig. 1A, depend on two dihedral angles within a given residue, viz., ϕ or ψ, respectively, and χ^1. Therefore, a given value for the Nuclear Overhauser Effect might correspond to many pairs of such dihedral angles. However, it will be shown that a parallel consideration of conformational energies and of the Nuclear Overhauser Effect results strongly limits the number of conformations which are consistent with the Nuclear Overhauser Effect data. Even

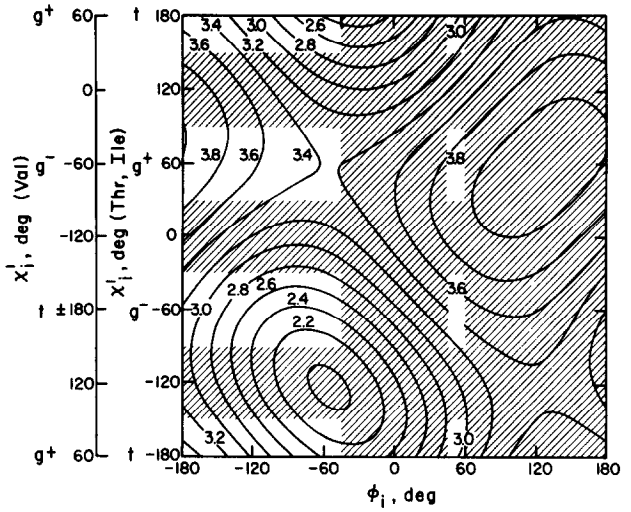

Fig. 3. The $H'_i \cdots H^{\beta}_i$ distance, d_{II}, plotted as a function of ϕ_i and χ^1_i for threonine and isoleucine. Contour lines connect points with equal values of d_{II}. Shading denotes regions in which $\Delta E > 3$ kcal/mol for every choice of ψ, and regions within ±30° of eclipsed side-chain conformations. Because of the convention for numbering branched side chains (1), χ^1 of valine differs from that of threonine by +120° when the hydrogens are oriented identically. The auxiliary ordinate scale to the left of the map should be used for valine to obtain χ^1_i in the standard convention.

qualitative information about the absence or presence, or order of magnitude of the Nuclear Overhauser Effect, is sufficient for this purpose.

Figure 3 shows contours of equal $H'_i \cdots H^{\beta}_i$ distance, d_{II}, as a function of ϕ_i and χ^1_i for the L-threonyl residue. The distance can be obtained from a value for the Nuclear Overhauser Effect using eq. 1. According to a (ϕ,ψ) energy map (Fig. 2 of ref. 12), conformations with $-45° < \phi < 45°$ and $60° < \phi < 180°$ are of high energy ($\Delta E > 3$ kcal/mol) for any choice of ψ, and it is unlikely that they occur in small peptides. Likewise, side-chain conformations within about ±30° of the eclipsed forms ($\chi^1 = 0°, \pm 120°$) have a relatively high energy and occur rarely (12). Therefore, these regions on the (ϕ,χ^1) map, indicated by shading in Fig. 3, usually can be disregarded. The unshaded regions of Fig. 3 can be divided into three groups, according to the predicted values for the Nuclear Overhauser Effect. Conformations in which the side chain is in a

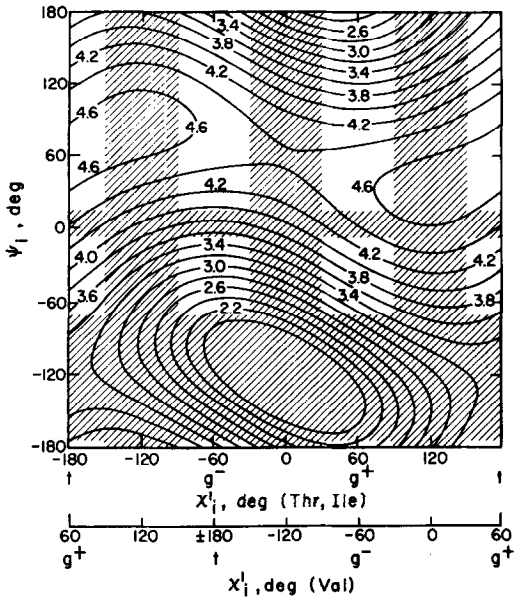

Fig. 4. The $H^{\beta}{}_i \cdots H'_{i+1}$ distance, d_{III}, plotted as a function of $\chi^1{}_i$ and ψ_i for threonine and isoleucine. See the legend of Fig. 3 for the explanation of the shading and of the auxiliary abscissa scale for valine.

---

g^+ position, i.e., $\chi^1 = 60° \pm 30°$ for threonine and isoleucine (in a g^- position for valine; cf. legend of Fig. 3) have a negligible Nuclear Overhauser Effect (<5%). A large Nuclear Overhauser Effect (>10%) can occur for the t or g^- side-chain conformations (g^+ or t for valine), but only when ϕ is negative and larger than about -130°, i.e., for the backbone conformations denoted as A, C, or F (12). A small Nuclear Overhauser Effect occurs for side-chain conformations t and g^- when ϕ is very negative (backbone conformations G, D, or E) or for the t state in the left-handed α-helical conformation (A*) for which ϕ is around 50°; however, some of these are of high energy.

Figure 4 shows contours of equal $H^{\beta}{}_i \cdots H'_{i+1}$ distance, d_{III}, as a function of $\chi^1{}_i$ and ψ_i, with the peptide bond fixed in the trans state (ω = 180°), for the threonyl residue. In a manner analogous to that of Fig. 3, high-energy regions for the two dihedral angles ordinarily can be disregarded, as shown by the shading in Fig. 4. A Nuclear Overhauser Effect of over 5% can occur only

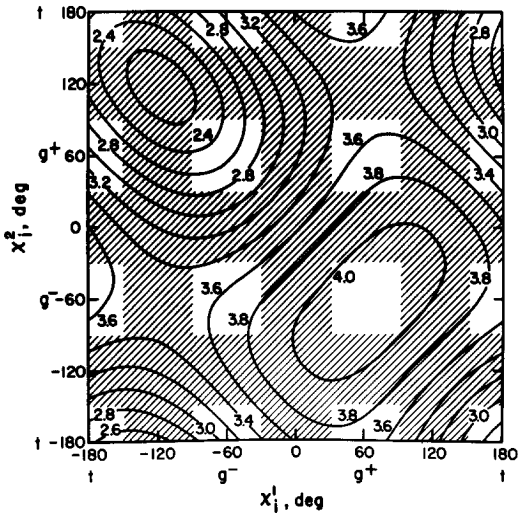

Fig. 5. The $H^{\alpha}_i \cdots H^{\gamma}_i$ distance, d_{IV}, plotted as a function of χ^1_i and χ^2_i. Contour lines connect points with equal values of d_{IV}. Shading denotes regions within ±30° of the eclipsed (high-energy) conformations for each dihedral angle.

when the side chain is in conformation g^- (t for valine) and $\psi < -20°$ (backbone conformations A and G), or when the side chain is in conformation g^+ (g^- for valine) and $\psi > 130°$ (backbone conformations E and F).

The distance contour maps for isoleucine and valine are identical to those for threonine to within a few degrees (except for the shift of the χ^1-axis by 120° for valine; cf. the legend to Figs. 3 and 4).

The distance, d_{IV}, for the $C^{\alpha}H$ and $C^{\gamma}H$ protons of leucine (IV in Fig. 1B) depends on the two side-chain dihedral angles χ^1 and χ^2. A contour map of the $H^{\alpha}_i \cdots H^{\gamma}_i$ distance, d_{IV}, is shown in Fig. 5. If one disregards the ranges of the dihedral angles within ±30° of the eclipsed conformations, only the unshaded regions in the Figure remain. Four of them (tt, tg^+, g^-t, and g^-g^+) should show a sizable Nuclear Overhauser Effect.

CONCLUSIONS

The use of Fig. 2 permits the approximate evaluation of ψ for any amino acid residue except glycine. For threonine, isoleucine, and valine, a combina-

tion of the three Nuclear Overhauser Effect measurements which give d_I, d_{II}, and d_{III} limits the number of backbone and side-chain conformational states to 1 to 5, which are consistent with the Nuclear Overhauser Effect data. It should be noted that not all three side-chain conformations (t, g^+, or g^-) can occur in combination with any one backbone conformational state A to G (12).

If highly precise Nuclear Overhauser Effect data are available, the contour lines of Figs. 2-5 can be used to specify the conformation further.

The limitations of the method proposed are those inherent to Nuclear Overhauser Effect measurements. If the oligopeptide molecule is flexible, the observed Nuclear Overhauser Effect is a weighted average over various conformations. The constant A in Eq. 1 may alter somewhat for different classes of compounds (6). However, if future work leads to modification of the value of A, this can be accommodated by a change in the scale on the side of Fig. 2, without changing the usefulness of the rest of the Figures. The maximal Nuclear Overhauser Effect for a given distance d, as given in Eq. 1, may be attenuated if the correlation time is long because of slow tumbling of large molecules or if there are several nearby protons contributing to relaxation (6)

ACKNOWLEDGMENTS

This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases (AM-13743), the National Institute of Aging (AG-00322), the National Science Foundation (PCM75-08691), and the Australian Wool Corporation.

REFERENCES

1. IUPAC-IUB Commission on Biochemical Nomenclature (1970) Biochemistry 9, 3471-3479.
2. Karplus, M. (1963) J. Amer. Chem. Soc. 85, 2870-2871.
3. Bystrov, V. V., Portnova, S. L., Tsetlin, V. I., Ivanov, V. T., and Ovchinnikov, Yu. A. (1969) Tetrahedron 25, 493-515.
4. Gibbons, W. A., Némethy, G., Stern, A., and Craig, L. C. (1970) Proc. Natl. Acad. Sci. U.S. 67, 239-246.
5. Karplus, S., and Karplus, M. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3204-3206.
6. Noggle, J. H., and Schirmer, R. E. (1971) The Nuclear Overhauser Effect, Academic Press, New York.

7. Bell, R. A., and Saunders, J. K. (1970) Can. J. Chem. 48, 1114-1122.
8. Howard, J. C., Ali, A., Scheraga, H. A., and Momany, F. A. (1975) Macromolecules 8, 607-622.
9. Khaled, M. A., and Urry, D. W. (1976) Biochem. Biophys. Res. Commun. 70, 485-491.
10. Gibbons, W. A., Crepaux, D., Delayre, J., Dunand, J., Hajdukovic, G., and Wyssbrod, H. A. (1975) Peptides: Chemistry, Structure and Biology (Walter, R., and Meienhofer, J., eds.), pp. 127-136, Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.
11. Momany, F. A., McGuire, R. F., Burgess, W. A., and Scheraga, H. A. (1975) J. Phys. Chem. 79, 2361-2381. The FORTRAN computer program for ECEPP is available from the Quantum Chemistry Program Exchange as Program No. QCPE 286.
12. Zimmermann, S. S., Pottle, M. S., Némethy, G., and Scheraga, H. A. (1977) Macromolecules 10, 1-10.