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Carboxyl group hydrogen bonding in X-ray protein structures analysed using neutron studies on amino acids

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A method is proposed to make a distinction between ionized and neutral carboxyl groups in X-ray protein structures. This is based on an analysis of the relative hydrogen bonding populations and bond-length bond-valence correlations in high-precision neutron studies of amino acids and small peptides. With the help of this method, four amino acid residues containing carboxyl groups in the refined structure of triclinic hen egg-white lysozyme have been analysed. Two of these, Glu-35 and Asp-52, are involved in lysozyme function, while the other two, Glu-7 and Asp-101, form a protein–protein inter-molecular contact in the triclinic structure.

Hydrogen bonding in protein structure; Neutron study on amino acid; Triclinic hen egg-white lysozyme; Carboxyl group hydrogen bonding

1. INTRODUCTION

An accurate and unambiguous analysis of the hydrogen bonding network in an X-ray protein structure is an essential aspect of the interpretation of biological function in terms of the molecular structure. The inherent inability of the X-ray method to provide precise hydrogen atom positions, however, makes this task extremely difficult, although commendable attempts have been made to analyse hydrogen bonding in X-ray protein structures with the help of scatter plots of donor and acceptor groups [1], and positions of hydrogen atoms generated using stereochemical criteria [2]. On the other hand, neutron studies of protein structures can, in principle, provide reliable hydrogen atom positions, but such studies have been limited due to various practical difficulties, as well as due to the need to locate and refine almost twice as many atoms, compared to an X-ray experiment, since the hydrogen atoms are now visible [3]! Accurate information on the hydrogen atom stereochemistry and systematics of hydrogen bonding can be obtained by analysing high-precision neutron studies on amino acids and small peptides [4]. This information, when combined with the X-ray protein structural data, can provide a complete description of hydrogen bond interactions in a protein structure, however, carboxyl groups pose a special problem even in this approach because a hydrogen atom cannot be assigned to a -COOH group in an X-ray protein structure unless its identity and the exact choice of -OH between the two oxygen atom positions are established in advance. Recently, using the neutron data, we have shown [5] that -COOH and $-COO^-$ groups have distinct hydrogen bonding characteristics, different from each other, that can be identified on the basis of hydrogen bond geometry and relative hydrogen bond populations, and interpreted with the help of bond-length, bond-valence correlations. A successful application of this method to the analysis of four amino acid residues containing carboxyl groups in the refined X-ray structure of triclinic hen egg-white (HEW) lysozyme [6,7] is reported here.

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2. MATERIALS AND METHODS

Forty carboxyl groups (18 -COOH and 22 $-COO^-$) from 36 crystal structures of amino acids and small peptides, studied by the neutron diffraction technique (for references, see [5]) were analysed. A majority of these studies were carried out at Brookhaven National Laboratory, USA, and BARC, India. Average values, sample standard deviations and ranges for various structural and conformational parameters were computed separately for -COOH and $-COO^-$ groups. Hydrogen bonds, X–H– – –Y involving carboxyl and other groups in these structures were identified, and hydrogen bond parameters, viz. X–H, H– – –Y and X. . Y distances, and bending angles HXY (angle between X–H and X. . .Y) were analysed. Orientations of carboxyl group hydrogen bonds with respect to these groups were also examined. Bond strengths for both covalent and hydrogen bonds involving oxygen atoms were computed using empirical bond-length, bond-valence correlations [8,9]. Analysis of carboxyl groups in a typical X-ray structure, initiated by using the model of triclinic HEW lysozyme, refined at 2 Å resolution [7] by the method of stereochemically restrained least squares [10], has subsequently been replaced by the model, that is currently being refined at 1.5 Å resolution (Ramanadham, M., Sieker, L.C. and Jensen, L.H., to be published). The *R*-value for this model, consisting of 1,001 protein and 269 solvent atoms, is 0.144.

3. RESULTS AND DISCUSSION

Significant differences are observed between the corresponding structural parameters of -COOH and -COO^- groups. In all the cases but one, the hydrogen atom of a -COOH group is in cyn conformation with respect to the carbonyl oxygen. In most cases, the angular deviation of the hydrogen atom from the COO plane is within 5°, and rarely exceeds 10°. The average value of the COH angle is 112(2)°.

As proton donors, -COOH groups participate in hydrogen bonds that are relatively shorter and straighter than those having other donor groups. For example, average O. . .O distance and HOO angle for 13 hydrogen bonds having -COOH as the proton donor are, respectively, 2.57(6) Å and 4(3)°. The corresponding values for 32 O–H– – –O hydrogen bonds, having donor groups other than -COOH, are 2.79(11) Å and 7(4)°. Interestingly, while -OH in the side chains of Ser, Thr and Tyr is a proton donor as well as an acceptor in hydrogen bonds, in a -COOH group, whether it is in the main chain or the side chain, it is never found to be a proton acceptor in any hydrogen bond. This experimental observation can be readily interpreted in terms of the bond-length, bond-strength correlations. The combined bond strength of C–O and O–H bonds, averaged over -OH groups is 1.68 valence units (vu) in Ser en Thr side chains, and 1.78 vu in Tyr side chains. In both these cases, the computed bond strength is significantly smaller than the valency of 2 vu expected for an oxygen atom. This value, averaged over 18 -COOH groups, turns out to be 1.94 vu. Even in individual -COOH groups, it is observed that a stretching of the O–H bond on the formation of a short hydrogen bond is accompanyed by a shortening of the C–O bond, thus keeping the total valency of the -OH oxygen close to 2 vu.

Both the oxygen atoms of -COO^-, and the carbonyl oxygen of -COOH participate in hydrogen bonding only as proton acceptors. Generally, three to four hydrogen bonds per -COO^- group are observed with an average donor–acceptor distance of 2.80(9) Å and bending angle of 10(5)°. The average bond strength for either of the two C–O bonds is 1.5 vu. Only 10 carbonyl oxygen atoms out of 18 -COOH groups are involved in hydrogen bonds. Average values for the donor–acceptor distance and the bending angle for 8 of these hydrogen bonds, with a nitrogen atom as the donor are, respectively, 2.92(7) Å and 18(9)°. The average bond strength associated with the C=O bond is 1.75 vu.

X-ray protein structures suffer from errors somewhat larger than the level at which the preceeding analysis has been carried out. In addition, using the same set of ideal values as restraints for both -COOH and -COO^- groups, because prior distinction cannot be made, tends to smear out differences, thus introducing more uncertainty in the structural parameters of carboxyl groups.

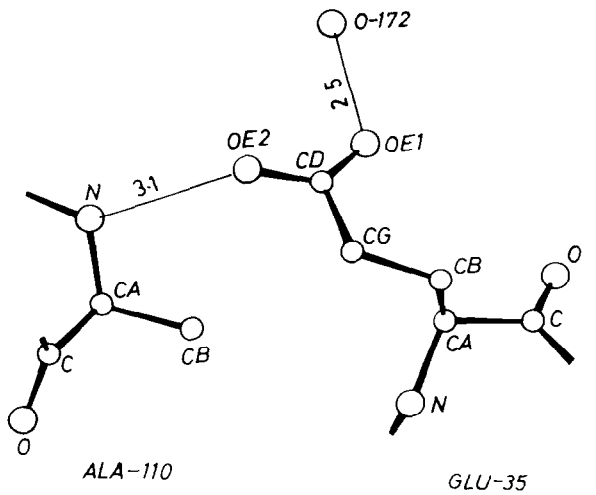

Fig. 1. Glu-35 hydrogen bonding in the X-ray structure of triclinic HEW lysozyme. Interaction with the amide nitrogen of Ala-110 is an intra-molecular hydrogen bond. O-172, a solvent oxygen atom, is the proton acceptor for the other hydrogen bond.

Therefore, the use of bond lengths, angles, etc., to ascertain the type of a carboxyl group may not be practical. On the other hand, the criterion based on hydrogen bonding can be more effective because it relies on multiple indicators, the individual numerical values of which may suffer from uncertainties, but the collective trends of which are more definitive. A set of working rules for this purpose are listed below.

(a) The -OH oxygen atom of a -COOH group will have only one hydrogen bonding atom; Y in its vicinity such that (i) the O. . .Y distance is not more than about 2.75 Å, (ii) the COY angle is less than about 130°, and (iii) the angular deviation of Y from the carboxyl plane is less than about 15°.

(b) The number of hydrogen bond donors, X_i within the hydrogen bonding distance is usually 2 (occasionally 3) for each of the two oxygen atoms of a -COO^-, and usually 1 (occasionally 2) for the carbonyl oxygen of a -COOH. Unless the donor is a -COOH group, X_i. . .O distances are usually larger than 2.7 Å. Also, the restrictions (ii) and (iii) listed in (a) do not generally apply to O. . .X_i vectors.

In the triclinic crystal structure of HEW lysozyme, Glu-35 (Fig. 1) has only two hydrogen bonding atoms near the side chain carboxyl group. The first one is the main chain amide nitrogen, a hydrogen bond donor, at a distance of 3.12 Å from the oxygen, OE2. A water molecule, O-172, is at a short distance of 2.54 Å from the second oxygen, OE1. The angular deviation of O-172 from the carboxyl plane is 6°, and the CD–OE1–O172 angle is 128°. Thus, the carboxyl group in the Glu-35 side chain is identified as -COOH, with OE1 as the hydroxyl position.

There is a short inter-molecular protein–protein contact (Fig. 2) between the side chain carboxyl groups of

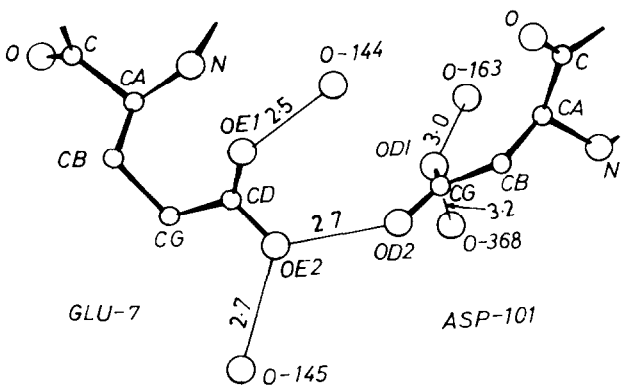

Fig. 2. Inter-molecular hydrogen bonding between the side chain carboxyl groups of Glu-7 and Asp-101 in the crystal structure of triclinic HEW lysozyme. Solvent oxygen atoms, O-144, O-145, O-163 and O-368, hydrogen bonded to these groups, are also shown.

Glu-7 and Asp-101 in the triclinic structure, which is possible only when at least one of them is unionized. The line connecting OE2 of Glu-7 and OD2 of Asp-101 is 2.72 Å long, and is almost co-planar with both the carboxyl groups, although the carboxyl groups themselves are not co-planar with each other. The angles CD–OE2. . .OD2 and CG–OD2. . .OE2 are, respectively 117° and 123°. While OD2 is involved in only one hydrogen bond, there is a second hydrogen bond between OE2 and a solvent molecule O-145, implying that OE2 cannot be a hydroxyl group. Thus, it can be concluded that OD2 is the -OH position of an unionized carboxyl group in the side chain of Asp-101. There is another water molecule, O-144 at a short distance of 2.55 Å from the second oxygen atom, OE1 of Glu-7.

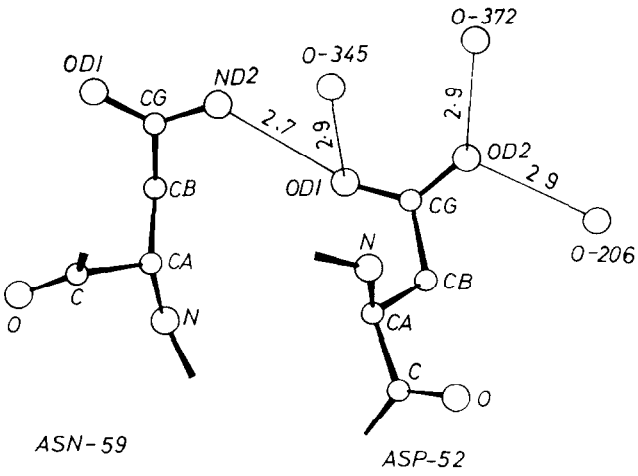

Fig. 3. Hydrogen bonds involving the side chain carboxyl group of Asp-52 in triclinic HEW lysozyme structure. Amide nitrogen from the side chain of Asn-59 belonging to the same protein molecule is a proton donor in one of these hydrogen bonds. The remaining three hydrogen bonds involve solvent oxygen atoms O-206, O-345 and O-372.

The angular deviation of O-144 from the carboxyl plane is 20°, and the angle, CD–OE1–O144, is 146°, both of which are quite large, however, a short donor–acceptor distance, and the absence of another hydrogen bond involving OE1 seem to imply that the Glu-7 side chain also contains a -COOH group, and OE1 is its -OH site.

Asp-52 (Fig. 3) in this structure is a clear-cut example of a side chain containing an ionized carboxyl group. Both the oxygen atoms of the carboxyl group are involved in two hydrogen bonds each. None of these hydrogen bonds is very short, and ranges of C–O. . .X_i angles and angular deviations of X_i from the carboxyl plane are, respectively, 113–150° and 23–87°.

It may be noted that, in the accepted model of the catalytic activity of HEW lysozyme [11,12], the unionized carboxyl group of Glu-35 acts as a general acid catalyst, while the ionized Asp-52 side chain stabilizes the oxocarbonium ion intermediate through electrostatic interaction. An abnormally high pK_a value, 6.1, due to which the side chain carboxyl group of Glu-35 remains unionized, has been attributed to the hydrophobicity of the adjacent Trp-108 residue, as well as the influence of Asp-52 on it [13]. A neutron study, carried out by Mason et al. [14] on a deuterated crystal of triclinic HEW lysozyme, clearly reveals the hydrogen atom position in the side chain carboxyl group of Glu-35, while no H-atom is seen in the side chain carboxyl group of Asp-52. The present analysis, based on hydrogen bonding patterns, thus, leads to conclusions, which are quite consistent with these studies.

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