CIRCULAR DICHROISM AND OPTICAL ROTATORY DISPERSION OF GRAMICIDIN S IN AQUEOUS SOLUTION

Franco Quadrifoglio and Dan W. Urry

Institute for Biomedical Research
Education and Research Foundation
American Medical Association
Chicago, Illinois 60610

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Gramicidin S is a cyclic decapeptide whose primary structure consists of two identical pentapeptides closed into a ring.

$$\ulcorner\text{(L-Val - L-Orn - L-Leu - D-Phe - L-Pro)}_2\urcorner$$

Preliminary X-ray data (Schmidt et al., 1957) showed that the molecule in the crystal has a twofold symmetry axis and that the atoms are concentrated in layers 4.8 A apart along the c axis of the crystal, which is at right angles to the molecular symmetry axis. Previous infrared studies (Abbott and Ambrose, 1953) on crystals and optical rotatory dispersion measurements in aqueous solutions of gramicidin S (Ruttenberg et al., 1966) suggested that the molecule contains residues in α-helical conformation. Due to the cyclic nature of this antibiotic and to the presence of a twofold symmetry axis, conformational analysis calculations have been applied to this compound in an attempt to determine the lowest energy conformation. Two published results of such calculations show structures which are substantially different (Liquori et al., 1966; Vanderkooi et al., 1966).

In order to obtain information of the structure of Gramicidin S in aqueous solution and to assess the effect of chain length, circular dichroism (CD) and optical rotatory dispersion (ORD) measurements in the range 260-185 mμ have been carried out.

1966). If only one of the C-5' hydrogens of the coenzyme can participate in the exchange, the specific activity of the coenzyme (cpm/μmole) will be equal to the specific activity of the α-hydrogen of the propionaldehyde (cpm/μatom). If both hydrogens participate, the specific activity of the coenzyme will be twice that of the α hydrogen of propionaldehyde. An experiment in which propionaldehyde (α-^{3}H) was equilibrated with DBCC in the presence of dioldehydrase is shown in Table I. Since the specific activity of the 85% of the DBCC actually complexed with the enzyme is nearly twice that of the α-hydrogen of propionaldehyde, both hydrogens of the coenzyme must participate in the exchange reaction.

Transfer of Tritium from DBCC-C-5'(^{3}H) to Reaction Product - To demonstrate that both C-5' hydrogens of DBCC-C-5'(^{3}H) can be transferred to the reaction product, one must demonstrate essentially quantitative tritium transfer from DBCC-C-5'(^{3}H), which is known to be randomly labeled, or devise a synthesis which produces the two possible isomers and demonstrate transfer from each of the isomers to product. If the synthesis which we have previously used (Frey et al., 1967) was non-stereospecific, then participation of both hydrogens is already established. If it was stereospecific and only one of the two possible isomers was produced, then the other isomer must be synthesized and its ability to transfer tritium to the reaction tested. A synthesis was therefore designed to produce the other isomer. The starting material for the synthesis was 2',3'-isopropylidene-DBCC-C-5'(^{3}H) synthesized by the method previously employed. DBCC-C-5'(^{3}H) so obtained

will be called isomer A.[4] Derivatives with the same configuration at C-5' as DBCC-C-5'(^{3}H) isomer A will also be referred to as isomer A. The following procedure was used to obtain the other isomer (isomer B).

$$\text{2',3'-isopropylidene-DBCC-C-5'}(^3\text{H}) \xrightarrow[O_2]{h\nu} \text{2',3'-isopropylidene-adenosine aldehyde-C-5'}(^3\text{H}) \xrightarrow{NaBH_4}$$

$$\text{2',3'-isopropylidene-adenosine-C-5'}(^3\text{H}) \longrightarrow \text{DBCC-C-5'}(^3\text{H})$$

(isomer B)

The coenzyme so prepared (isomer B) contained a total of 29×10^3 cpm. It was added to dl-1,2-propanediol and diol-dehydrase. The resulting propionaldehyde contained 27×10^3 cpm. A similar experiment was carried out in which D-1,2-propanediol was used instead of dl-1,2-propanediol. In this experiment, propionaldehyde contained 26×10^3 cpm. Therefore, essentailly all of the tritium of DBCC-^{3}H (isomer B) can be transferred to the product. It is therefore conclusively established that both hydrogens at C-5' of the coenzyme can participate in the hydrogen transfer process.

Participation of both C-5' Hydrogens of DBCC in the Reaction Catalyzed by Ribonucleotide Reductase - Evidence has been obtained which suggests that both hydrogens of the coenzyme participate in the reaction catalyzed by ribonucleotide reductase (Abeles and Beck, 1967). When the reaction was

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[4] The term isomer A and B is used primarily to facilitate description of the procedures used. If the synthesis was non stereospecific, separate preparations of the two isomers would not have been obtained, and the preparations of DBCC-C-5'(^{3}H) would be mixtures of the two isomers.

carried out in H_2O (^3H), the specific activity of the coenzyme was approximately twice that of the solvent hydrogen. However, clear cut conclusions cannot be drawn since contribution of isotope effects cannot be evaluated. If the reaction is carried out in pure D_2O, isotope effects need not be considered. At equilibrium, the coenzyme will contain either one or two atoms of deuterium. Even if equilibrium is not reached, the incorporation of more than one deuterium atom establishes that both hydrogens at the C-5' position are subject to exchange. Deuterium incorporation from D_2O into DBCC was determined under the following conditions: potassium phosphate buffer, pD 7.5, 0.16 M; GTP, 0.008 M; dithiothreitol, 0.16 M; EDTA, 0.001 M; DBCC, 4.8×10^{-4} M; 32 mg of ribonucleotide reductase;[5] total volume, 50 ml. The final deuterium content of the reaction mixture was at least 95%. The reaction mixture was incubated under nitrogen at 37° for 12 hours. At the end of the reaction, the pH of the reaction mixture was adjusted to 3 with 6 N HCl and the coenzyme isolated by chromatography on Dowex 50 (Barker et al., 1960). After Dowex 50 chromatography, coenzyme was concentrated by phenol extraction and then transferred to an aqueous medium. The aqueous solution of the coenzyme was deaerated and then photolyzed. After photolysis, 5',8-cyclic adenosine was isolated (Weissbach et al., 1960). The cycloadenosine so

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[5] Ribonucleotide reductase was prepared by a modification of the procedure of Goulian and Beck (1966). The specific activity was 30 μmoles of dGTP/mg protein/hour under the following assay conditions: DBCC, 15 x 10-6 M; GTP, 5.0×10^{-3} M; potassium phosphate buffer, pH 7.5, 0.10 M; reduced lipoic acid, 50×10^{-3} M. The reaction, in a total volume of 0.5 ml, was allowed to proceed for 5 min. at 37°C. dGTP was assayed colorometrically (Blakely, 1966).

obtained was diluted with carrier and analyzed for deuterium. The cycloadenosine contained 1.6 atoms deuterium per molecule. These results show that both hydrogens at the C-5' position of the coenzyme can participate in the exchange reaction.

The experiments reported here conclusively establish that in at least two reactions involving DBCC coenzyme, both C-5' hydrogens can participate. Therefore, at some stage in the reaction an intermediate must exist in which these two hydrogens can become equivalent. Such equilibration could be brought about by a chemical modification of a carbon-cobalt bond, as for instance a cleavage of this bond, or through the formation of an intermediate containing an unusual valence at the C-5' position of the coenzyme, such as a pentavalent carbon atom.

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