STUDIES ON THE MODE OF SELF-ASSOCIATION OF TYROCIDIN B

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Summary. Experiments on the self-association of dialyzed solutions of tyrocidine B have been performed in the ultracentrifuge. The data were analyzed by the two-species plot technique, and give evidence for the hypothesis that tyrocidine B associates to form both n-mers and larger aggregates. n was found to depend on temperature, with n = 8 at 20° and 25°.

Tyrocidines A, B, and C are cyclic decapeptides of known primary structure (1). These peptides may undergo extensive self-association via non-covalent interactions (2,3). Clearly, the small size of the tyrocidines renders them ideal models for the study of these interactions, which probably are similar to those stabilizing the tertiary and quaternary structures of proteins in solution. A necessary first step in such a study is to define the nature of the self-association. Williams et al. (3), in a preliminary investigation of the mode of association, proposed that tyrocidine B undergoes association to form a micelle-like n-mer, followed by association of the n-mers to yield larger particles. In this communication, we report new and stronger evidence for this mode of association.

Materials and Methods. Tyrocidine B was purified via countercurrent distribution (7). Dialysis in 30% acetic acid-.1M NaCl was performed in a thin-film dialyzer. The membrane was acetylated for 5 hours with 25% acetic anhydride in pyridine in order to render it impermeable to the monomeric peptide. Within experimental error, the results were independent of the temperature of dialysis. The partial specific volume was taken as .746 cc/gm (3).

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Low speed sedimentation equilibrium experiments were performed on a Model E ultracentrifuge equipped with a photoelectric speed control and interference optics. Cells of the external loading type were employed in all of the experiments (4). No base fluids were used. Loading concentrations were determined with a differential refractometer. The interference plates were read on a Gaertner comparator with digitized output. The data was processed by a computer program which gave weight average molecular weights (M_w) to better than 1% accuracy (5). Number average molecular weights (M_n) were obtained by the procedure of Adams (6).

Results. In Figure 1 are shown plots of M_n and M_w vs. concentration at three different temperatures in 30% acetic acid-.1M NaCl. Two features of the data are immediately apparent: (i) For a given concentration, the molecular weights are lower at higher temperatures. (ii) Up until a certain "critical" concentration, the molecular weights are constant and equal to 1350 ± 100, in good agreement with the known monomer molecular weight of 1346. The data of Figure 1 may be replotted in the form of "two-species" plots (8). In such plots, linear regions indicate concentrations at which ideal monomer-n-mer association occurs. Negative deviations from the line denote contributions from a positive second virial coefficient, while positive deviations indicate further association, above the n-mer level (8). As shown in Figure 2, the lower concentration data at 32°, 25°, and 20°, lie on the theoretical lines for n = 7, n = 8, and n = 8, respectively. Since linearity for the low concentration points is found at three temperatures, it seems unlikely that a fortuitous cancellation of nonideality and additional association occurs, especially since the second virial coefficient should be quite small under these conditions. Thus, the data indicate the occurrence of a cooperative monomer-n-mer association, as well as the presence of additional association above the n-mer level. A distribution of aggregate sizes about n cannot be excluded. However, the linearity found in the two-species plots suggests that any such distribution would be rather narrow. The additional association, while it clearly is present, is difficult to characterize at the

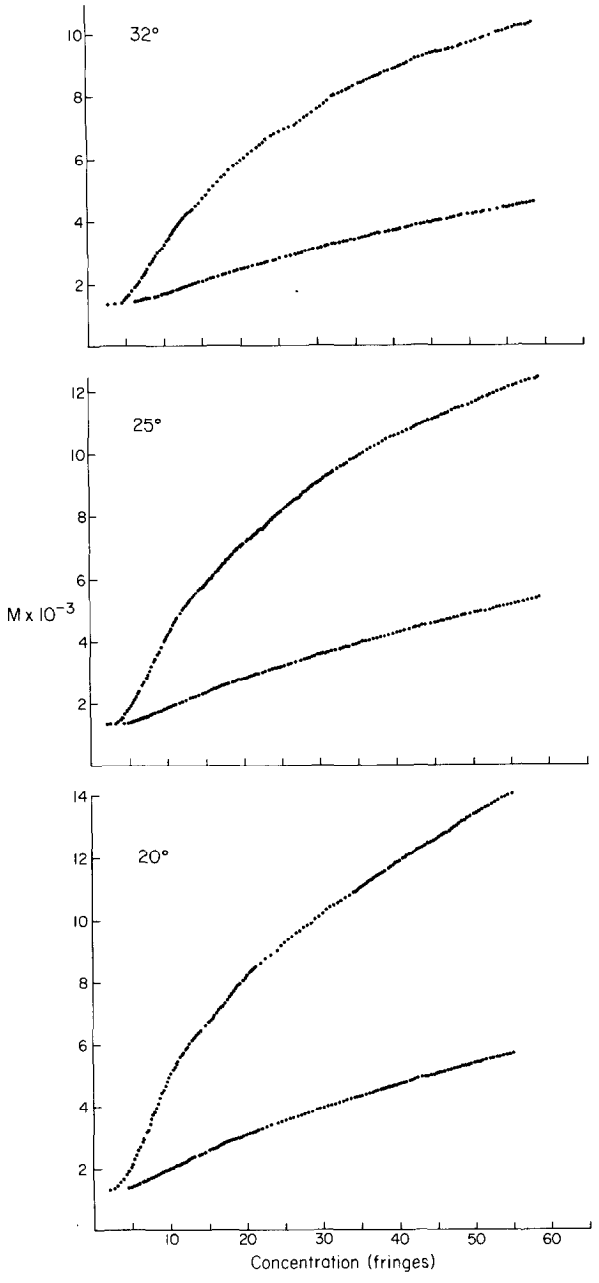

Figure 1. Molecular weight versus concentration data for tyrocidine B. At each temperature, the upper curve gives M_w, and the lower, M_n.

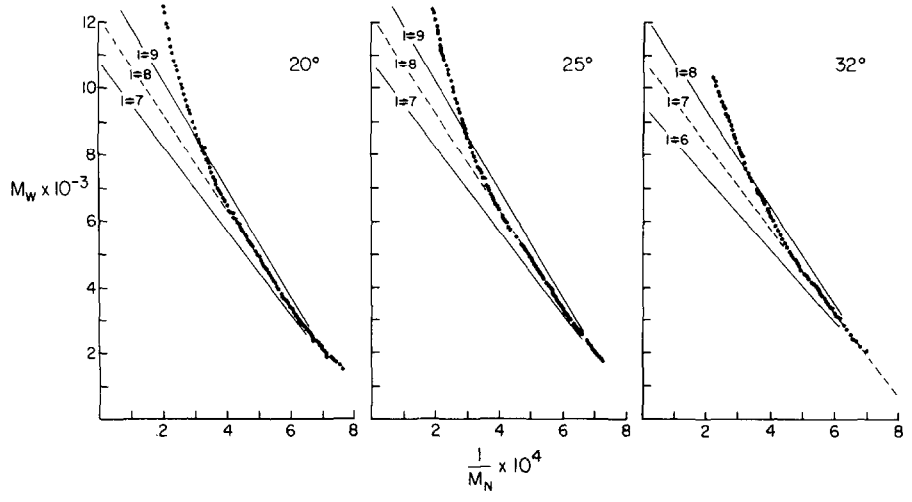

Figure 2. Two-species plots for tyrocidine B. At each temperature, the dashed, theoretical line indicates the equilibrium which best describes the data. The solid lines represent less satisfactory stoichiometries.

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relatively low concentrations employed in these experiments.

Discussion. Williams et. al. (3) have shown the necessity of using 30% acetic acid - .1M NaCl, to avoid time dependent changes in molecular weight and apparent heterogeneity in solutions of tyrocidine B. It has been established by Casassa and Eisenberg that dialysis is a necessity if molecular weight measurements are to be carried out in mixed solvents (9). Therefore, it is important to dialyze the peptide against the solvent, as we have done here. The technical obstacle of the small size of the peptide was overcome by using acetylated membranes. It should be noted that this technique is of general applicability and may be used to assure that many small molecules follow the Casassa-Eisenberg conventions.

Williams et al., from their experiments with undialyzed material, proposed that tyrocidine B undergoes a monomer-n-mer association, as well as association to yield larger particles. Experiments carried out between 4.5° and 35° indicated that n varied between 8 and 11, with n=9 at 25° (3). Our data, obtained with dialyzed solutions and a totally different method of analysis, gave very similar results. The approach taken here gives strong

evidence for the proposed mode of association since: (i) Dialyzed solutions were used. (ii) The two-species plot method of analysis is independent of the accuracy of values assumed for the partial specific volume, and does not require curve-fitting. (iii) The fact that the peptide remains monomeric until its concentration exceeds a certain "critical" value clearly rules out the occurrence of an indefinite association.

It is likely that the monomer-n-mer reaction occurs in aqueous salt solutions as well. Williams et al. (3) have detected a point analogous to a critical micelle concentration in such solutions, and the nuclear magnetic resonance data of Stern (10) indicates that the mode of interaction of the peptide with water is the same in the presence or absence of acetic acid.

The self association of tyrocidine B most closely resembles that of detergents. Detergents are known to form micelles by a monomer-n-mer reaction, as well as larger types of aggregates (11). The tyrocidine micelles are most likely closed, possibly spherical particles since they prefer to assume a definite size at a given temperature. A "stack of coins" type of aggregate would be expected to exhibit an indefinite association rather than the monomer-n-mer association actually found. Hence, it is unlikely that tyrocidine B affects bacterial membranes by transfer of metallic ions through such a "stack" of peptide molecules, as proposed by Eisenman (12). Indeed, there is evidence which demonstrates that the bacteriacidal activity of tyrocidine is similar to that of cationic detergents (13).

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