Conformational Analysis of Melittin in Solution Phase: Vibrational Circular Dichroism Study

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Abstract: *The vibrational absorption and vibrational circular dichroism (VCD) spectra of melittin in D_2O solutions at different pH values, different salt concentrations, or different 2,2,2-trifluoroethanol (TFE) concentrations are recorded in the amide I′ (1850–1600 cm^{-1}) region. Two models are used to simulate this peptide in different conditions, and a coupled oscillator program is used to obtain the calculated absorption and VCD spectra. This study indicates that melittin adopts a mixed structure in D_2O solution at low pH, low salt concentration, or low TFE concentration. With an increase in pH, salt concentration, or TFE concentration, the structure changes to α-helix and further increases lead to aggregation. These results demonstrate the versatility of VCD in probing the conformations of peptides under different environmental perturbations.* © 2003 Wiley Periodicals, Inc. Biopolymers (Biospectroscopy) 70: 614–619, 2003

Keywords: *conformational analysis; melittin; solution phase; vibrational circular dichroism*

INTRODUCTION

Melittin, a 26-residue (H_2N-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2) water-soluble peptide, is the major component of honey bee venom.[1] It integrates into membranes, causes cell lysis, and can also form voltage-gated channels in planar membranes.[2] Depending on the peptide concentration, pH, ionic strength, and the nature of the negative counterion, melittin is either monomeric or associated as tetrameric aggregates in aqueous solution.[3]

A large number of studies have been undertaken to determine the conformational properties of melittin in order to understand the molecular mechanism of melittin's interaction with membranes. The structure of melittin has been extensively studied by various techniques, such as X-ray diffraction,[3a] IR,[4] NMR,[5] circular dichroism (CD),[5a,5b,6] and so forth. These results showed that melittin is very flexible and can change from mixed to α-helix structures in different environments. Melittin adopts a mixed structure (sometimes referred to as random coil) in aqueous solution at low pH and forms a stable α-helical structure in aqueous solution at basic pH or in the presence of a high salt concentration.[3b,7] Addition of fluoroalcohols such as 2,2,2-trifluoroethanol (TFE) and hexafluoroacetone hydrate[5a,d] to aqueous solutions of peptides also induces a structural transition from

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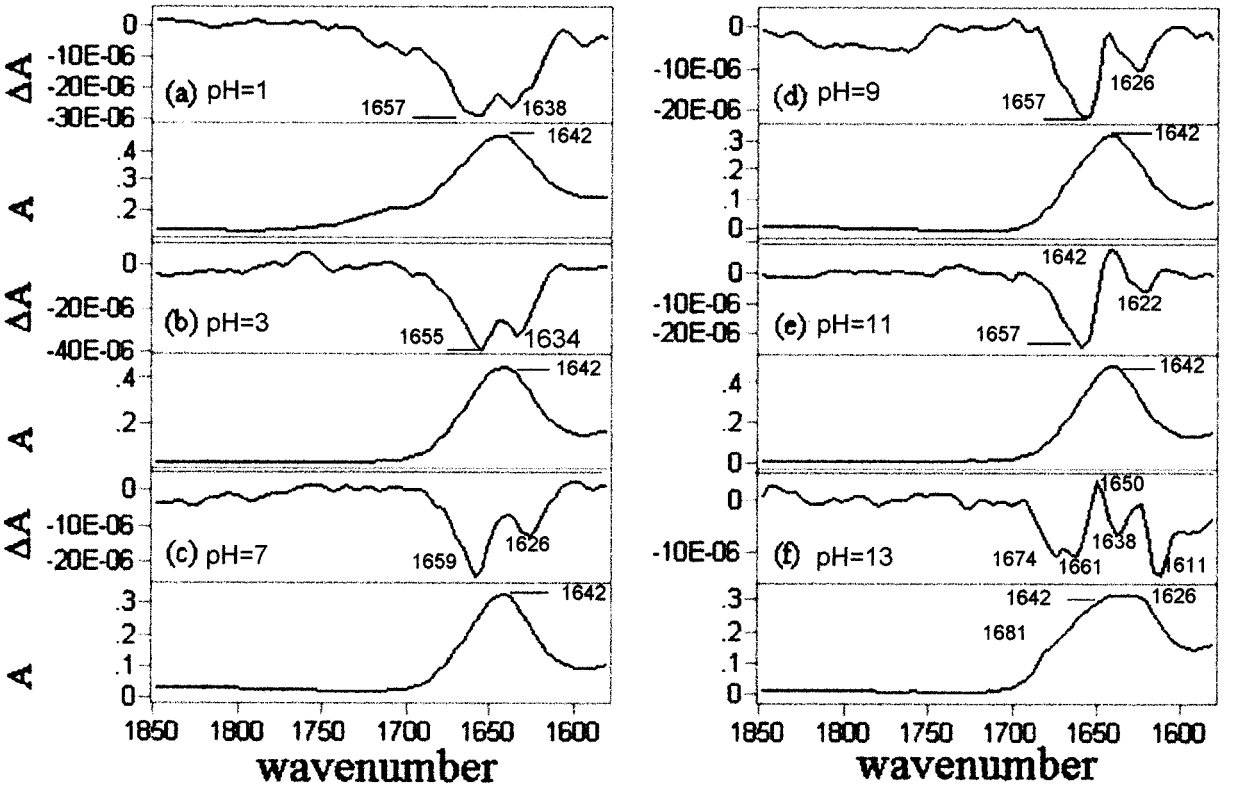

FIGURE 1 The vibrational absorption (bottom traces) and VCD (top traces) spectra of melittin in D_2O solutions at pH (a) 1, (b) 3, (c) 7, (d) 9, (e) 11, and (f) 13.

largely mixed conformations to an α-helical state. The structure of monomeric peptides consists of two α-helical segments. These segments are connected by a hinge[5c] to form a bent helical rod with the hydrophilic and hydrophobic sides facing in opposite directions. The α helix is distinctly amphiphilic because of the asymmetric distribution of the polar side chains.[8] This is different from gramicidin A, a hydrophobic linear polypeptide, whose amphiphilicity is largely provided by backbone carbonyls.

In this work, vibrational CD (VCD)[9–15] is used to investigate the secondary structures of melittin. Numerous VCD studies have been conducted on peptides and proteins to establish the relationship between VCD patterns and secondary structures.[11] The secondary structures of gramicidin D have been studied using VCD spectroscopy.[15]

To understand the conformational behavior of melittin, we investigated the following three factors: the conformational change as a function of the pH; the conformational change in the presence of varying concentrations of KCl salt; and the conformational change in the presence of varying concentrations of a fluoroalcohol, TFE.

MATERIALS AND METHODS

Melittin was purchased from Sigma Chemical Co., D_2O was purchased from Cambridge Isotope Labs, and TFE was purchased from Aldrich Chemical Co.

The IR and VCD spectra were recorded on a commercial Fourier transform VCD spectrometer (ChiralIr, Bomem–Biotools). The spectra were measured at 6–10 mg/mL in D_2O and in other cases. The sample was held in a fixed pathlength (50 μm) cell with BaF_2 windows. All spectra were collected at a resolution of 8 cm^{-1} at room temperature. The data collection time was 3 h for each VCD spectrum using 6075 ac scans and 675 dc scans collected in two sets. The solvent absorption was subtracted out in the absorption spectra. In the VCD spectra the raw VCD spectrum of the solvent was subtracted.

Two models were used to simulate the structures of melittin under different conditions. One of the models was built and geometry optimized using the Chemsite 95 program.[16] The other model was obtained from the Protein Data Bank.[17] The vibrational frequencies, absorption, and VCD intensities for melittin were calculated using a coupled oscillator model[18] program provided by Dr. M. Diem of Hunter College. The theoretical absorption and VCD spectra were simulated with Lorentzian bandshapes and an 8 cm^{-1} full width at half-height.

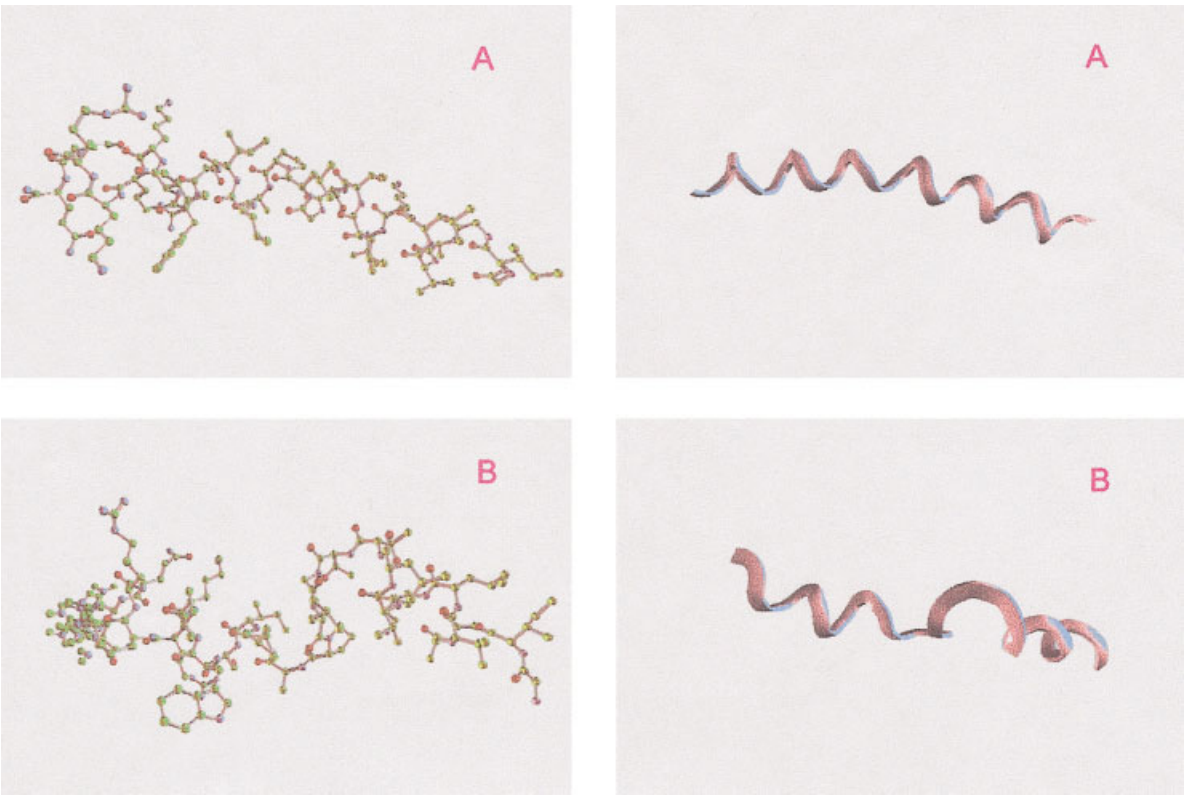

FIGURE 2 (A) Melittin with a stable right-handed α-helical structure (structure built with ChemSite) and (B) D-Pro melittin with a mixed structure (structure from Protein Data Bank).

RESULTS AND DISCUSSION

The absorption and VCD spectra of melittin in D_2O solutions at different pH values are shown in Figure 1. The absorption spectrum of melittin in D_2O at low pH [Fig. 1(a), bottom trace] shows a broad amide carbonyl band at 1642 cm^{-1}. This band remains at the same position as the pH value increases from 1 to 11, and no structural changes can be elucidated based on the raw absorption spectra. The VCD spectrum for the low pH solution has two significant negative bands centered at 1657 and 1638 cm^{-1} [Fig. 1(a), top trace]. As the pH increases, the negative band at 1638 cm^{-1}decreases. At pH 11 the VCD pattern is shown as a negative–positive couplet with a negative band at 1657 $cm^{-1}$and a positive band at 1642 cm^{-1}, which is a characteristic VCD pattern for right-handed α-helical structures.[11] The pattern at pH 13 is totally different from the patterns at other pH values, which can be attributed to aggregation (as reflected by the turbidity of the solution).

To understand the structural changes of melittin, two models are used to simulate the structures of melittin under different conditions (see Fig. 2). Conformation **A** was built and optimized using ChemSite.[16] This conformation has a right-handed α-helical structure with a hinge at Thr_{10}-Thr_{11}-Gly_{12}, as deduced from the NMR data.[5c] Conformation **B** (code 1bh1) was obtained from the Protein Data Bank.[17] This conformation is a kind of mixed structure with two local helical segments, and the segment in the middle (Thr_{10}-Leu_{16}) is unstructured. The frequencies, absorption, and VCD intensities of these models were calculated and their simulated absorption and VCD spectra are shown in Figure 3. Because of the inaccuracies in the calculations resulting from empirical programs (ChemSite 95 and a coupled oscillator model) compared to those obtainable with *ab initio* methods, the calculated band positions and relative intensities (Fig. 3) do not match those in the experimental spectra (Fig. 1) very well. Thus, we need to focus only on the overall spectral patterns. It can be seen, within these limitations, that the calculated absorption and VCD spectra of conformation **A** are consistent with those collected for melittin at pH 11 [Fig. 3, trace A and Fig. 1(e)], with a positive couplet (negative–positive couplet with a negative band on the high frequency side and a positive band on the low frequency side) for the amide carbonyls in the VCD spectrum. The calculated absorption and VCD spectra

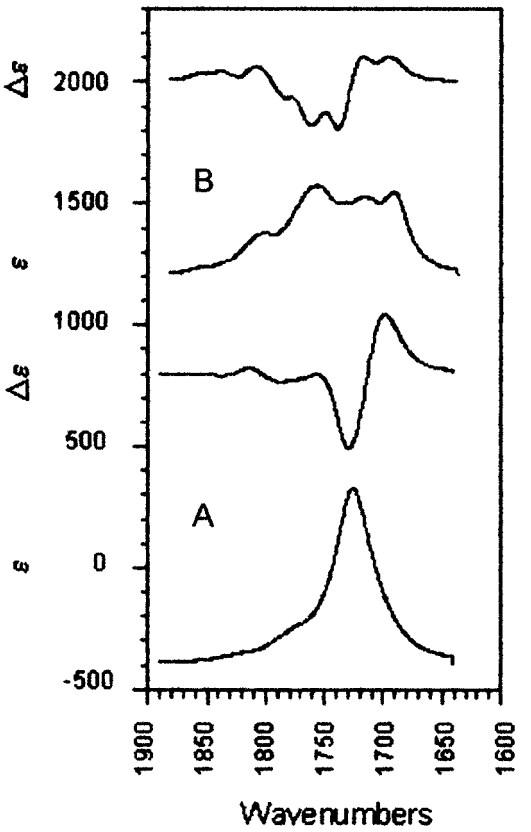

FIGURE 3 The predicted vibrational absorption (bottom traces) and VCD (top traces) spectra of (A) melittin with a stable right-handed α-helical structure (structure built with ChemSite) and (B) D-Pro melittin with a mixed structure (structure from Protein Data Bank).

of conformation **B** are in agreement with those recorded for melittin at pH 1, with two negative bands evident in the amide I′ region [Fig. 3, trace B and Fig. 1(a)]. We also used another model containing two parallel α helices from the Protein Data Bank (code 2mlt[3a,c]) to evaluate the aggregated structure, but that structure was predicted (not shown) to have essentially the same VCD as that of conformer **A.** Aggregation normally comes from extended β-sheet type structures, so *ab initio* simulations of the type carried out by Kubelka and Keiderling[19] might be appropriate for aggregated structures. However, such simulations are not feasible for melittin at the present time.

The influence of the ionic strength and TFE on the structure of melittin is shown in Figures 4 and 5, respectively. The absorption and VCD spectra of melittin in D_2O solutions in the presence of different concentrations of KCl are shown in Figure 4. The absorption spectrum of melittin in ion-free D_2O [Fig. 4(a), bottom trace] shows a broad amide carbonyl band at 1642 cm^{-1}. This band remains at approximately the same position as the concentration of KCl increases from 0 to ~2.3*M*. The VCD spectrum for the ion-free solution has two significant negative bands centered at 1659 and 1626 cm^{-1} [Fig. 4(a), top trace]. As the concentration of KCl increases, the negative band at 1626 cm^{-1} decreases. In the presence of ~0.06–0.58*M* KCl, melittin has the VCD pattern [a negative–positive couplet with a negative band at 1662 cm^{-1} and a positive band at 1643 cm^{-1}; Fig. 4(c,e), top trace] that is characteristic for right-handed α-helical structures.[11] Based on the predicted VCD results (Fig. 3), the structure of melittin in ion-free D_2O solution can be seen to be unstructured or mixed and a high salt concentration induces an α-helical structure. Turbidity of the solution is observed in solutions at very high salt concentrations [~2.3*M*; Fig. 4(f)]. The large negative and almost zero positive VCD [Fig. 4(f)] observed for solutions with very high salt concentrations may be attributed to the aggregation of melittin.

The structural change from a mixed to an α-helical structure is also observed as the concentration of TFE increases in the solution (Fig. 5). In the D_2O solution in the presence of a 0.1% (v/v) TFE concentration, the VCD spectrum is similar to that predicted for mixed structures (Fig. 3, trace B). As the TFE concentration increases to 25% (v/v), the VCD spectrum changes to a characteristic pattern for an α-helical structure. In pure TFE solution the VCD spectral pattern changes further to one negative band [see Fig. 5(f)], which is attributed to the formation of tetramers because of aggregation.

CONCLUSIONS

Our VCD studies of melittin in D_2O solution at different pH conditions and in the presence of different concentrations of KCl or different concentrations of TFE show the following:

1. Melittin adopts a mixed structure in D_2O solutions at low pH, low salt concentrations, or low TFE concentrations.
2. Melittin adopts an α-helical structure in D_2O solutions at a pH of ~11, an ~0.06–0.6*M* KCl concentration, or an ~25% (v/v) TFE concentration.
3. The gradual structural changes in peptides as a function of environmental perturbation can be successfully monitored using VCD.

Thus, VCD provides a powerful approach to elucidate the conformational changes in peptides under different environmental perturbations.

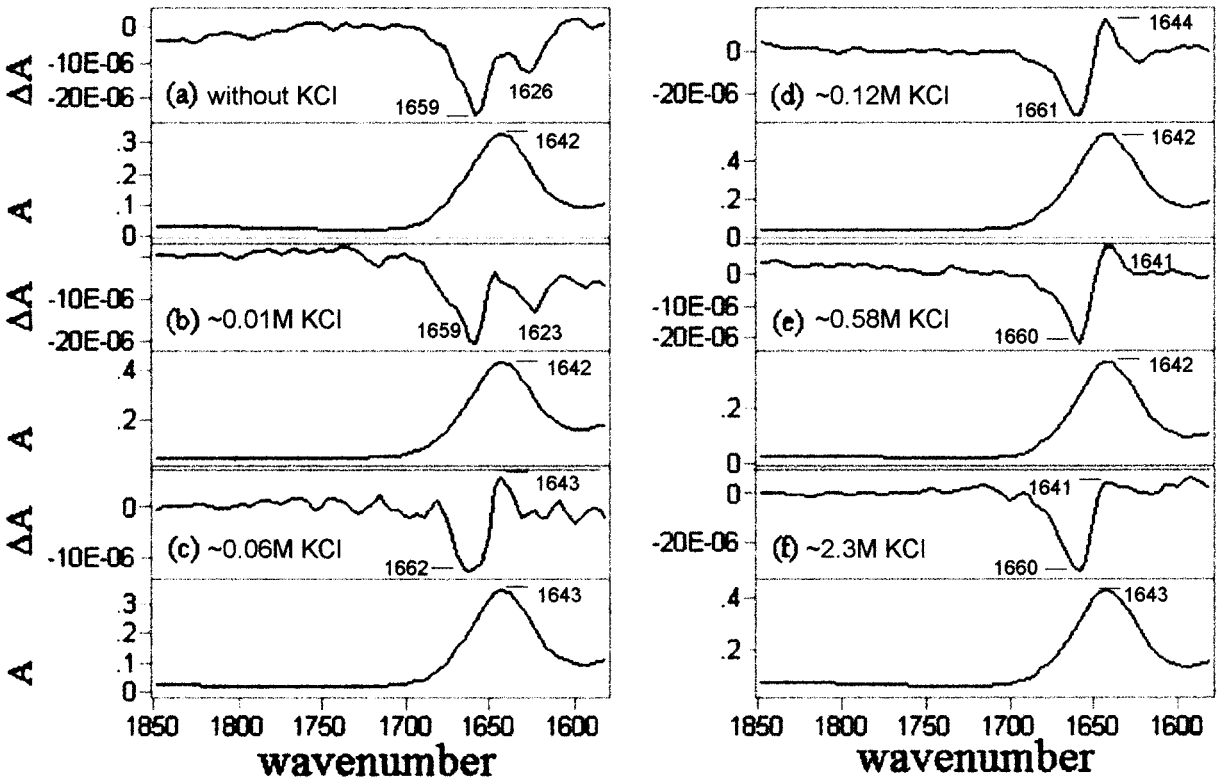

FIGURE 4 The vibrational absorption (bottom traces) and VCD (top traces) spectra of melittin in D_2O solutions with (a) no KCl or KCl salt concentrations of (b) ~0.01, (c) ~0.06, (d) ~0.12, (e) ~0.58, and (f) ~2.3*M*.

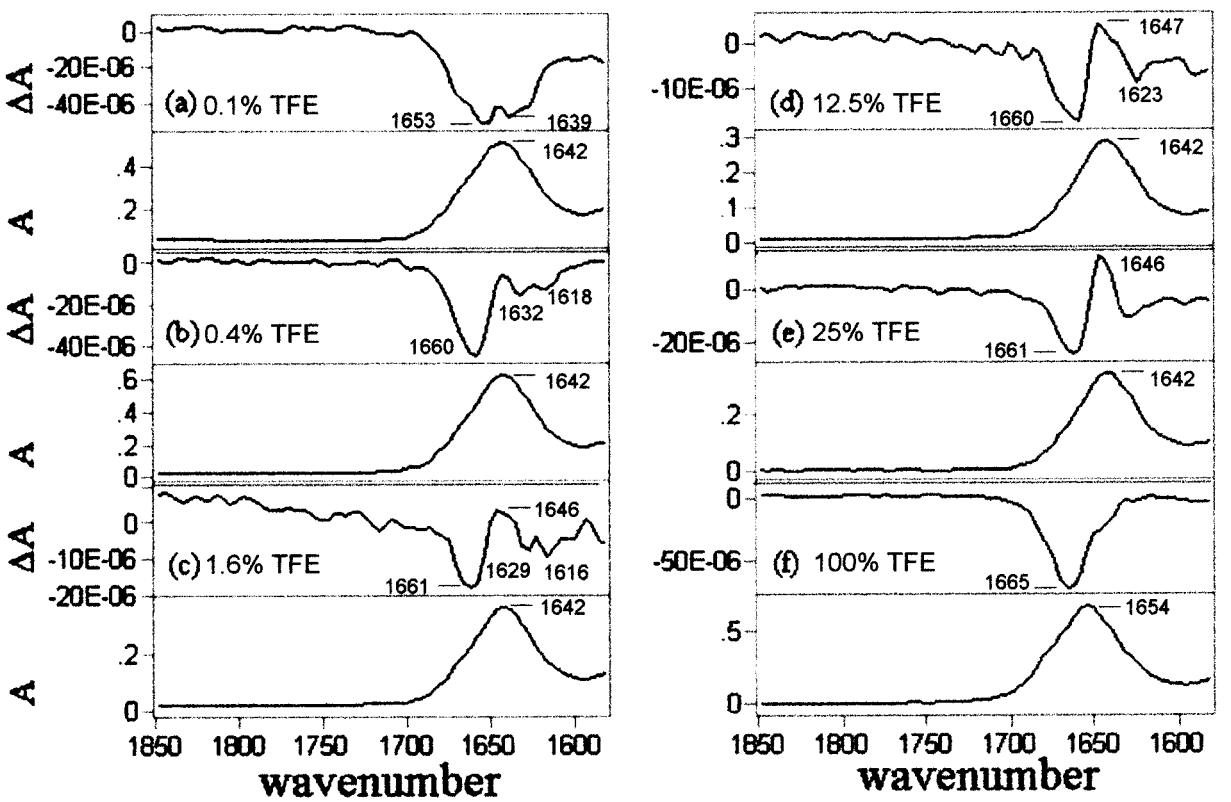

FIGURE 5 The vibrational absorption (bottom traces) and VCD (top traces) spectra of melittin in D_2O solutions with TFE concentrations (% v/v) of (a) 0.1, (b) 0.4, (c) 1.6, (d) 12.5, (e) 25, and (f) 100%.

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