Title:	Interaction of chloramphenicol with titin I27 probed using single-molecule force spectroscopy
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Abstract:	Titin is a giant elastic protein which is responsible for passive muscle stiffness when muscle sarcomeres are stretched. Chloramphenicol, besides being a broad-spectrum antibiotic, also acts as a muscle relaxant. Therefore, it is important to study the interaction between titin I27 and chloramphenicol. We investigated the interaction of chloramphenicol with octamer of titin I27 using single-molecule force spectroscopy and fluorescence spectroscopy. The fluorescence data indicated that binding of chloramphenicol with I27 results in fluorescence quenching. Furthermore, it is observed that chloramphenicol binds to I27 at a particular concentration (~ 40 μM). Single-molecule force spectroscopy shows that, in the presence of 40 μM chloramphenicol concentration, the I27 monomers become mechanically stable, resulting in an increment of the unfolding force. The stability was further confirmed by chemical denaturation experiments on monomers of I27, which corroborate the evidence for enhanced mechanical stability at 40 μM drug concentration. The free energy of stabilization for I27 (wild type) was found to be 1.95 ± 0.93 kcal/mole and I27 with 40 μM drug was 3.25 ± 0.63 kcal/mole. The results show a direct effect of the broad-spectrum antibiotic chloramphenicol on the passive elasticity of muscle protein titin. The I27 is stabilized both mechanically and chemically by chloramphenicol.
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