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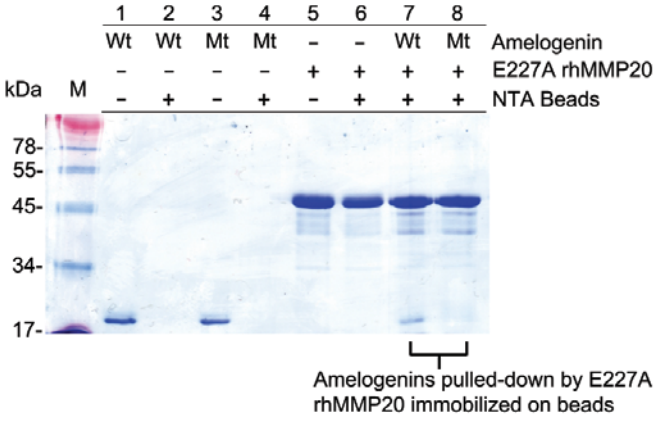
Reduced Amelogenin-MMP20 Interactions in Amelogenesis Imperfecta

APPENDIX

Pull-down Assays to Compare the Binding Affinities of the E227A rhMMP20 for rh174 Wild-type and P41T Mutant Amelogenins

Thirty microliters of ProBond Ni-NTA beads (Invitrogen, Carlsbad, CA, USA) were washed and mixed with 200 μL of binding buffer (20 mM Tris-HCl, pH 7.5) containing E227A rhMMP20 with a poly-His tag (1 mM) in 1.5-mL tubes. After 1 hr of incubation at room temperature, the beads were thoroughly washed with binding buffer and re-suspended in 200 μL of assay buffer. The rh174 or P41T amelogenin (40 μg each) was added to the tubes and incubated for 1 hr at room temperature under constant shaking. The beads were then washed with the binding buffer to remove any unbound amelogenin, and boiled in 40 μL of SDS sample buffer to elute the proteins for SDS-PAGE. All experiments were performed in triplicate, and the signal intensities of the bands of rh174 and P41T mutant amelogenins pulled down by E227A rhMMP20 were compared by NIH Image 1.63 software (NIH, Bethesda, MD, USA). The control groups included: supernatants from reactions containing amelogenin without Ni-NTA beads and E227A rhMMP20 (lane 1 for rh174 and lane 3 for P41T mutant in Appendix Fig.); amelogenin with beads without E227A rhMMP20 (lane 2 for rh174 wild-type and lane 4 for P41T mutant); supernatants containing E227A rhMMP20 without beads and amelogenin (lane 5); and E227A rhMMP20 with beads without amelogenin (lane 6).

Both rh174 and P41T amelogenins did not bind to the Ni-NTA beads and could not be detected by SDS-PAGE (lanes 2 and 4) in the negative controls without E227A rhMMP20 (Appendix Fig.). In the reactions containing E227A rhMMP20, all inactive enzymes showed strong and identical bands at 55 kDa (lanes 5 to 8). The rh174 amelogenin pulled down by MMP20 showed a stronger band (lane 7) than did P41T mutant amelogenin (lane 8) at the 25-kDa size. The signal intensities of the bands of the rh174 amelogenin (1.00 ± 0.19, n = 3) pulled down by the inactive E227A rhMMP20 were significantly higher than those of the P41T mutant amelogenin (0.48 ± 0.07, n = 3) ($P < 0.01$). M, molecular-weight marker; Wt, rh174 wild-type amelogenin; Mt, P41T mutated amelogenin.



Appendix Figure. SDS-PAGE analysis of the pull-down assays to compare the binding affinities of the E227A rhMMP20 for rh174 wild-type and P41T mutant amelogenins.