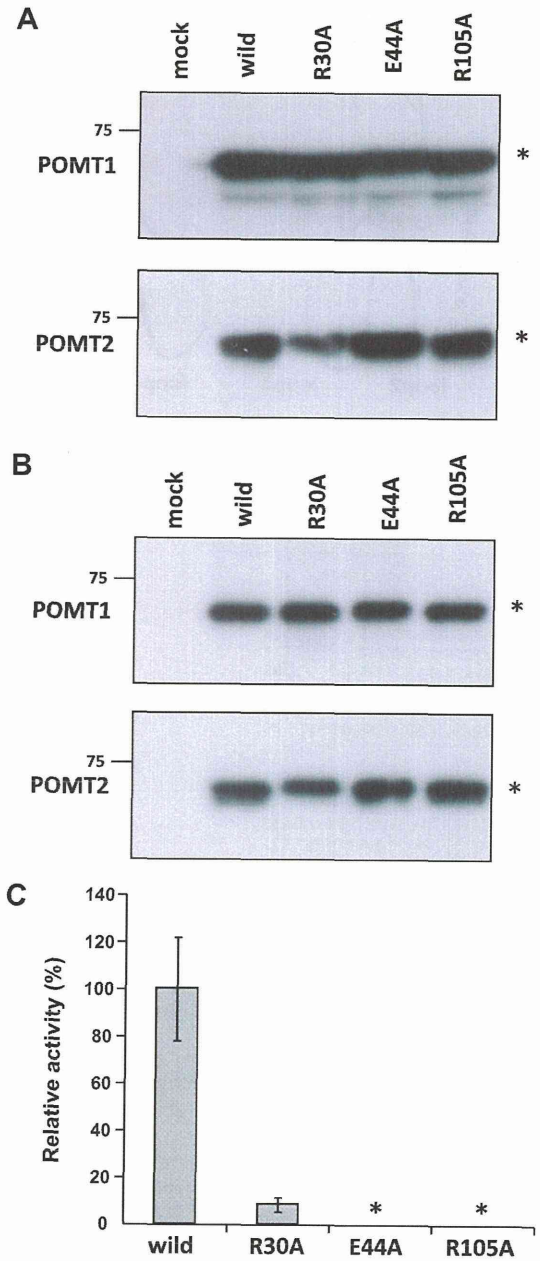

Fig. 2. Effects of amino acid substitutions in POMT1 on POMT activity and complex formation. (A), Western blot analyses of the microsomal fractions from cells co-transfected with POMT1-myc mutants and POMT2. (B), POMT1–POMT2 complex formation *in vivo*. POMT1-myc mutants and wild-type POMT2 were transfected into HEK293T cells and immunoprecipitated by anti-myc (9E10) antibody-conjugated agarose. The resulting precipitates were analyzed by immunoblotting with anti-myc antibody (A-14) and anti-POMT2 antibody. (C), POMT activities of the POMT1-myc mutants co-expressed with POMT2. wild (wild-type POMT1-myc and wild-type POMT2); R30A (R30A-mutant POMT1-myc and POMT2); E44A (E44A-mutant POMT1-myc and POMT2); R105A (R105A-mutant POMT1-myc and POMT2). Asterisks in (A) and (B) indicate the migration positions of each POMT1-myc protein and POMT2 protein. Molecular weight standards are shown on the left. POMT activity was based on the amount of mannose transferred to a GST-αDG. Average values of three independent experiments are shown. POMT activities were normalized by protein expression levels. Asterisks in (C), No activities were detected.

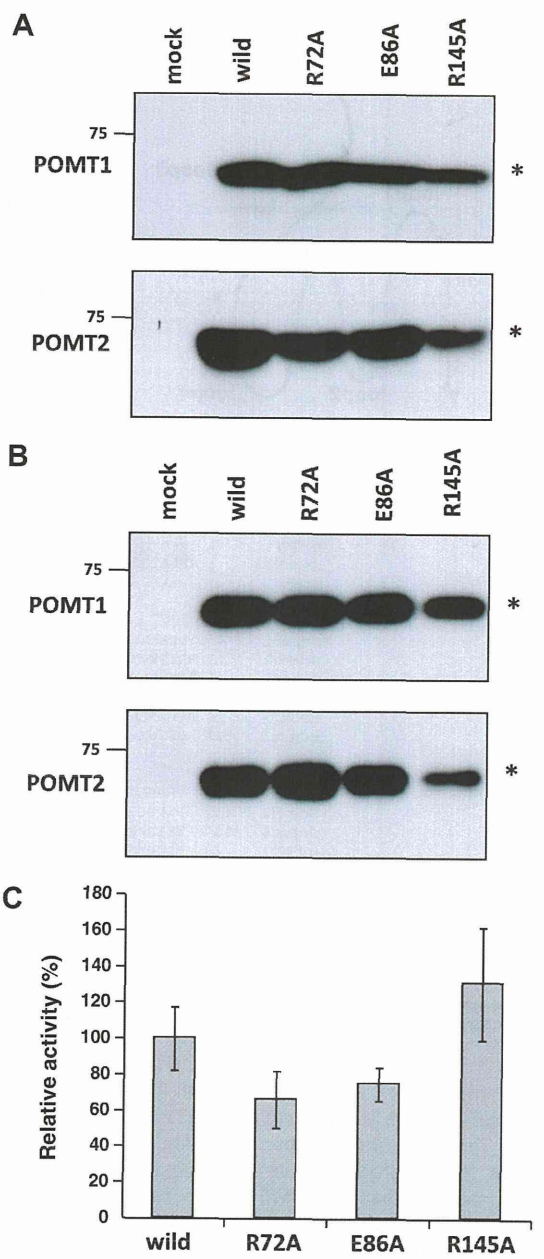

Fig. 3. Effects of amino acid substitutions in POMT2 on POMT activity and complex formation. (A) Western blot analyses of the microsomal fractions from the cells co-transfected with POMT1-myc and POMT2 mutants. (B) POMT1–POMT2 complex formation *in vivo*. POMT1-myc and POMT2 mutants were transfected into HEK293T cells and immunoprecipitated by anti-myc (9E10) antibody-conjugated agarose. The resulting precipitates were analyzed by immunoblotting with anti-myc antibody (A-14) and anti-POMT2 antibody. (C) POMT activities of the POMT1-myc co-expressed with POMT2 mutants. wild (wild-type POMT1-myc and wild-type POMT2); R72A (POMT1-myc and R72A-mutant POMT2); E86A (POMT1-myc and E86A-mutant POMT2); R145A (POMT1-myc and R145A-mutant POMT2). Asterisks indicate the migration positions of each POMT1-myc protein and POMT2 protein. Molecular weight standards are shown on the left. POMT activity was based on the amount of mannose transferred to a GST-αDG. Average values of three independent experiments are shown. POMT activities were normalized by protein expression levels.

suggests that loop 1 of human POMT2 is required for enzymatic activity but its crucial amino acid(s) may be different from those in POMT1.

Mycobacterium tuberculosis has a PMT homolog (Mtpmt) in which the three amino acids corresponding to Arg^{64}, Glu^{78} and Arg^{138} of ScPmt1p (boxed in Fig. 1C) are conserved [11]. Mtpmt catalyzed the initiation of protein mannosylation and Glu^{56} residue

(corresponding to Glu^{78} in ScPmt1p, Fig. 1C) was necessary for enzymatic activity [11]. Since there is not another PMT homolog in *M. tuberculosis*, Mtpmt may function as a homomeric complex or form a complex with other components to show enzymatic activity. The sequence similarity and the fact that a Glu residue in loop 1 is required for enzymatic activity suggest that protein *O*-mannosylation is conserved from bacteria to eukaryotes including human.

In the present study, we found that some of the amino acids that are critical for activity and complex formation are different in human POMT1 and POMT2. To better understand the mechanism of protein *O*-mannosylation, it will be necessary to study the structure of the POMT1–POMT2 complex. Such studies will help to determine the spatial relation among these critical amino acids.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research (20370053) from the Japan Society for the Promotion of Science, Intramural Research Grant (23–5) for Neurological and Psychiatric Disorders of NCNP, a Comprehensive Research on Disability Health and Welfare from the Ministry of Health (H22-021), a Grant from The Naito Foundation, and a Grant from The Research Foundation for Pharmaceutical Sciences.

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