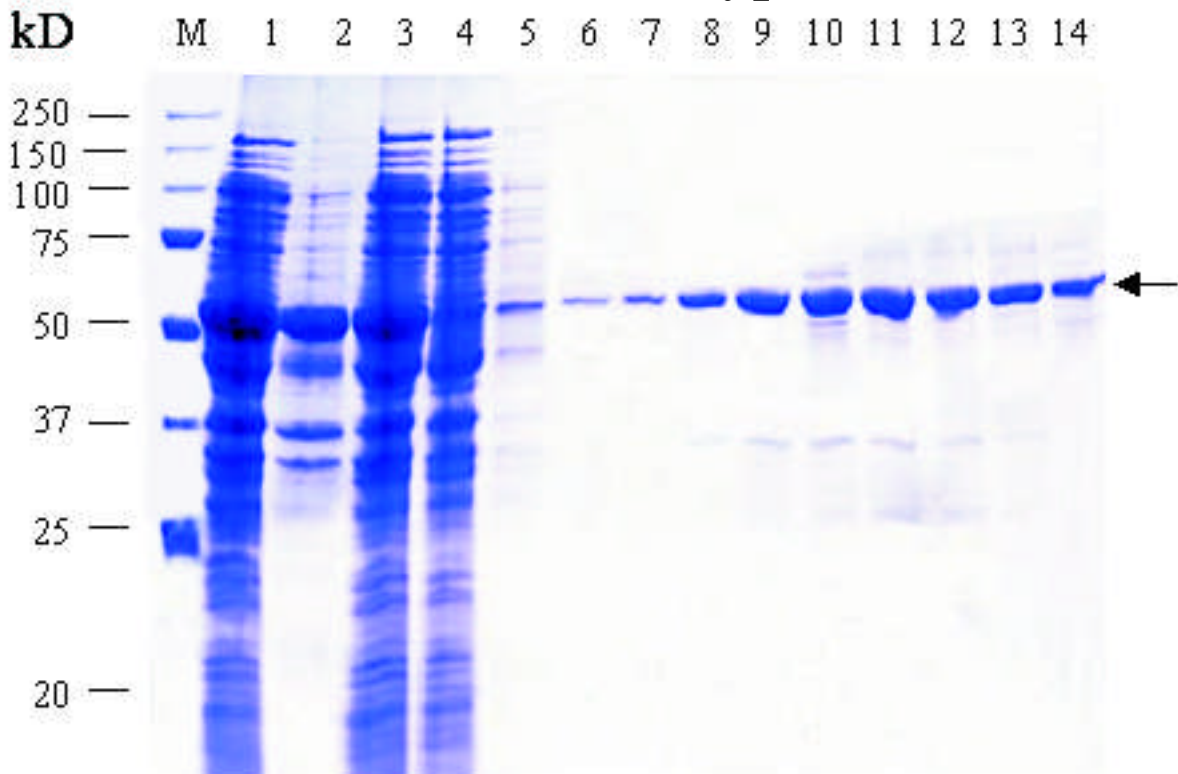
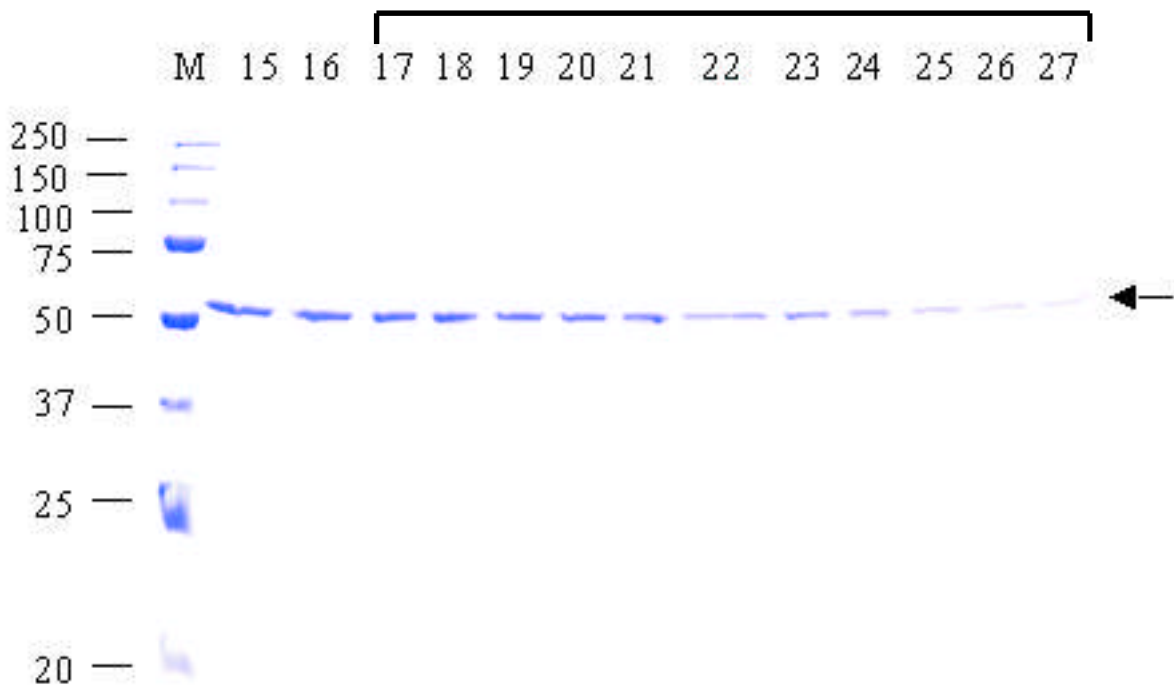
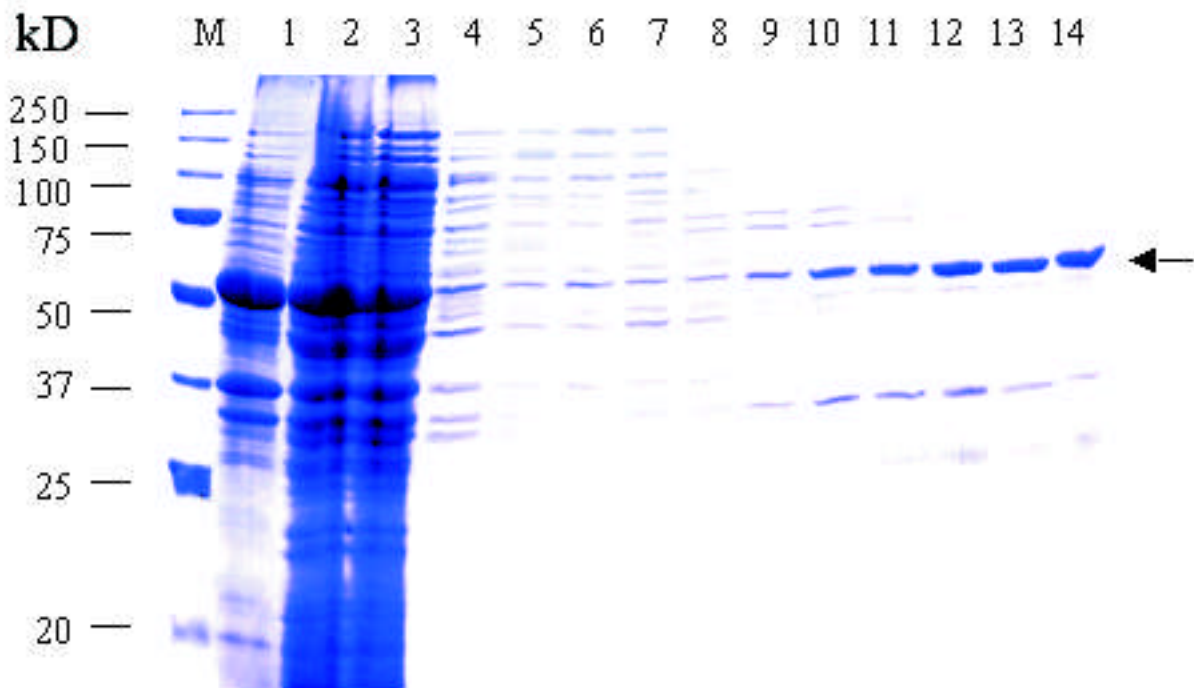


Purification of wild type mcSKD1



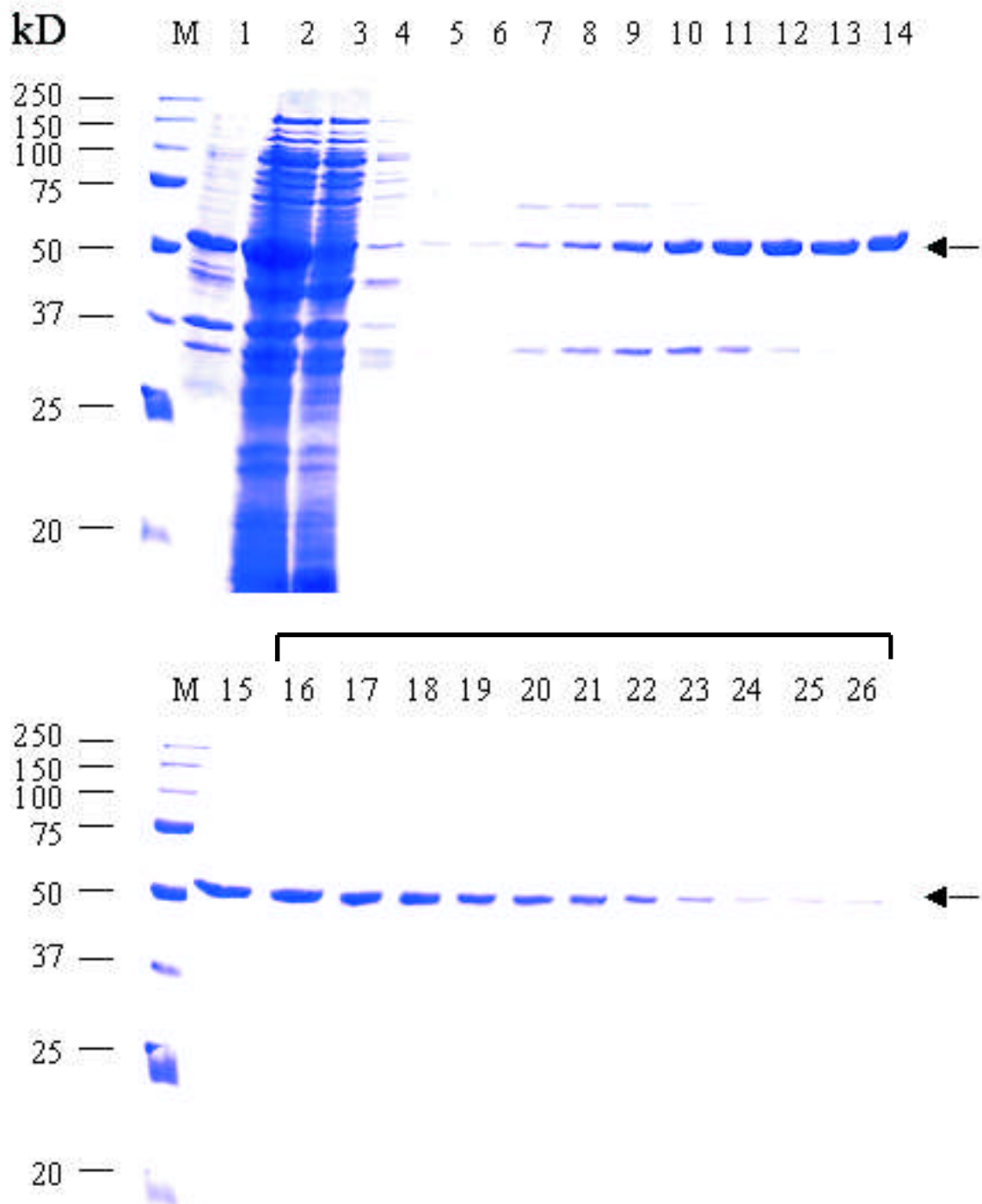
Purification of wild type mcSKD1 protein. Induced mcSKD1-(His)₆ protein was over-expressed and purified by Talon affinity column. Proteins were separated by SDS-PAGE and stained with coomassie blue. M: protein marker; 1: total protein; 2: pellet fraction; 3: soluble fraction; 4: flow-through fraction; 5: wash fraction; 6-28: elute fraction (continuant 20-100 mM imidazole). Arrows indicate the position of over-expressed protein. Elute fractions 18 to 28 were combined and concentrated by an Amicon Ultra PL-30 (Millpore, USA) column. This sample is described as “affinity-purified” mcSKD1 in the text.

Purification of mutant mcSKD1^{K177A} protein



Purification of mcSKD1^{K177A} protein. Induced mcSKD1^{K177A}-(His)₆ protein was over-expressed and purified by Talon affinity column. Proteins were separated by SDS-PAGE and stained with coomassie blue. M: protein marker; 1: pellet fraction; 2: soluble fraction; 3: flow-through fraction; 4: wash fraction; 5-27: elute fraction (continuant 20-100 mM imidazole). Arrows indicate the position of over-expressed protein. Elute fractions 17 to 27 were combined and concentrated by an Amicon Ultra PL-30 (Millipore, USA) column. This sample is used for ATPase assay described in the text.

Purification of mutant mcSKD1^{E231Q} protein



Purification of mcSKD1^{E231Q} protein. Induced mcSKD1^{E231Q}-(His)₆ protein was over-expressed and purified by Talon affinity column. Proteins were separated by SDS-PAGE and stained with coomassie blue. M: protein marker; 1: pellet fraction; 2: soluble fraction; 3: flow-through fraction; 4: wash fraction; 5-26: elute fraction (continuant 20-100 mM imidazole). Arrows indicate the position of over-expressed protein. Elute fractions 16 to 26 were combined and concentrated by an Amicon Ultra PL-30 (Millipore, USA) column. This sample is used for ATPase assay described in the text.

HPLC profiles of N-terminal amino acid sequencing of mcSKD1 protein

