Purification of the Mr 22,000 Calcium-binding Protein (Sorcin) Associated with Multidrug Resistance and Its Detection with Monoclonal Antibodies

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ABSTRACT

A low molecular weight cytoplasmic protein (Mr, 19,000–22,000) has been reported to be overexpressed in some multidrug-resistant cells. We have found that a cytoplasmic protein with a molecular weight of 22,000 is highly expressed in the human myelogenous leukemia K562 cells resistant to Adriamycin (K562/ADM). The Mr 22,000 protein was shown to be one of the major calcium-binding proteins in the cytoplasmic extract from K562/ADM cells. The protein was purified to apparent homogeneity from K562/ADM cells using a four-step procedure including ammonium sulfate fractionation, anion-exchange chromatography, and gel filtration. 1.5 mg of the Mr 22,000 protein was purified from 3.0 × 10⁹ of K562/ADM cells. The protein was acidic (pI 5.3) and exists as a homodimer (Mr, 44,000) as revealed by gel filtration and sucrose density-gradient centrifugation. The purified protein appeared as a single band (Mr, 22,000) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence or absence of reducing agents, suggesting that the homodimer was generated by noncovalent linkage. Monoclonal antibodies specific to the Mr 22,000 protein were raised by in vitro immunization with purified protein or by in vivo immunization with the crude membrane fraction of K562/ADM. These antibodies were used as probes for the detection of the protein. We have surveyed the expression of the Mr 22,000 protein in various multidrug-resistant and -sensitive cell lines, and found that the overexpression of the protein is not a sufficient nor a necessary condition for the acquisition of the multidrug-resistant phenotype.

INTRODUCTION

When cell lines are made resistant to naturally occurring anticancer agents such as Vinca alkaloids or anthracyclines, they usually show cross-resistance to certain other drugs to which the cells have not been previously exposed. The classes of agents to which this cross-resistance extends are diverse, but the drugs are all complex high molecular weight natural products. The precise spectrum of cross-resistance varies with the selecting drug, which suggests that drug resistance is due to a multicomponent system (1). The basis for this multidrug-resistant phenotype is at least in part a decreased intracellular accumulation of these drugs (2, 3). Compounds which affect cellular calcium, such as the calcium channel blocker verapamil and calmodulin inhibitors, have been found to interfere with the efflux of drugs from multidrug-resistant cells (4, 5), suggesting that the increased drug efflux might result from a Ca²⁺-dependent process.

In several experimental systems, multidrug resistance is connected with elevated levels of high molecular weight (Mr, 170,000–180,000) surface glycoprotein (P-glycoprotein) (6–14). Recent findings indicate that P-glycoprotein is directly involved in drug transport mechanisms in multidrug-resistant cells (for review see Ref. 15). Monoclonal antibodies that specifically recognize P-glycoprotein have been generated (12, 14), and have been used for the detection of P-glycoprotein in experimental cell lines and clinical samples. They have also served as useful tools for studies on the mechanisms of drug resistance.

Besides the overexpression of P-glycoprotein in the plasma membrane, a low molecular weight cytoplasmic protein (Mr, 19,000–22,000) has been reported to be overexpressed in some multidrug-resistant cells (8, 16–25). Koch et al. (22) have recently shown that the Mr, 22,000 protein (CP22) binds calcium with high affinity, suggesting a link with the ability of calcium-channel blockers to reverse multidrug resistance (4, 5). Van der Bliek et al. (23) have cloned cDNAs of the Mr, 22,000 protein from Chinese hamster cells, and showed that the protein has four “E-F hand” structures typical of calcium-binding sites. In spite of these findings, the roles, if any, of these low molecular weight cytoplasmic proteins in multidrug-resistance are still unknown (25).

In this report we have purified the Mr, 22,000 calcium-binding protein to apparent homogeneity from the Adriamycin-resistant human leukemia cell line, K562/ADM (13). The protein was acidic (pI 5.3) and exists as a homodimer (Mr, 44,000). Specific monoclonal antibodies were raised against the protein and used as probes for the detection of the protein.

MATERIALS AND METHODS

Cells and Culture Conditions

The human and murine multidrug-resistant and -sensitive cell lines used are summarized in Table 1 (26, 27). Relative resistance values of these cell lines were reassayed for the present work and may not agree exactly with previously reported values. The cells were cultured as described (26). P3-X63-Ag8-U1 plasmacytoma cells and hybridoma cells were cultured in RPMI 1640 supplemented with 1 mM sodium pyruvate, 50 μM mercaptoethanol, 18 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10% fetal bovine serum, and 100 μg/ml kanamycin.

Preparation of Cell Extracts and Membrane Fractions

Cells were washed with PBS,3 suspended at 4 × 10⁷ cells/ml in the extraction buffer [10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride], and incubated for 15 min in an ice bath. The swollen cells were disrupted with 15 to 20 strokes in a tightly fitting Dounce homogenizer. The nuclei were removed by centrifugation at 400 × g for 10 min through 5 ml of 25% sucrose in the extraction buffer. Enucleated cell homogenate was spun out at 22,000 × g for 30 min and the supernatant was used as a cytoplasmic extract. The resultant pellet was used as a crude membrane fraction.

Gel Electrophoresis and Protein Assay

SDS-PAGE was carried out according to Laemmli (28). Isoelectric focusing was done using the Phast system (Pharmacia) as recommended by the supplier. Two-dimensional gel electrophoresis was performed as described by O’Farrell (29). Gels were fixed and stained by Coomassie

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: PBS, 0.15 M NaCl/20 mM sodium phosphate (pH 7.4); TPS, PBS containing 0.05% Tween 20; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunoabsorbent assay.
Calcium Binding Assay

This assay was done as described by Maruyama et al. (31) using 
"CaCl₂ (10 mg/ml Ca) from Amersham. Cell extracts were prepared as described above, and 100 μg of cytoplasmic extract protein was subjected to electrophoresis on 10–20% polyacrylamide gels (28). Proteins were transferred to nitrocellulose (32). The sheets were washed, incubated with 45Ca²⁺, and prepared for autoradiography as described previously (28).

In Vitro Phosphorylation

Cells were washed with PBS and suspended at 3 × 10⁷ cells/ml in the phosphorylation buffer [10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 2 mM MgCl₂ in the presence or absence of 0.2 mM CaCl₂ or 1 mM EGTA. Cell extracts were prepared as described above. In vitro phosphorylation was done essentially as described previously (27). Phosphorylation reaction was started by adding 10 μl of [γ-³²P]ATP (0.1 mCi, final concentration of 10 μM) to 90 μl of cell extracts (approximately 1.0 mg protein). After 10 min of incubation at 30°C, the reaction was stopped by adding 25 μl of 5-fold concentrated Laemmli's sample buffer (28) and boiling for 3 min. The samples were subjected to SDS-PAGE analysis followed by autoradiography.

Purification of the M, 22,000 Protein from K562/ADM Cells

Fractions containing the M, 22,000 protein were determined by separation on 10–20% polyacrylamide gels and Coomassie blue staining as described above. The material was centrifuged at 22,000 × g for 1 h to remove the slight precipitate that remained.

Step 3: DEAE-Sepharose Chromatography. The sample was applied to a DEAE-Sepharose (Pharmacia Inc.) column (1.6 x 20 cm) which had been equilibrated with 20 mM Tris-HCl buffer (pH 7.4). The column was eluted with a linear gradient made up of 150 ml each of 0 and 0.5 M NaCl in the same 20 mM Tris buffer.

Step 4: Gel Filtration. The resulting solution was passed through a Sephadex G-200 (Pharmacia Inc.) column (2.6 x 100 cm) equilibrated with PBS. After gel filtration, peak fractions containing the M, 22,000 protein were pooled, and concentrated by ultrafiltration using Centricon-10 (Amicon). The sample was dialyzed overnight against 50 mM NH₄HCO₃ (pH 8.0), lyophilized, and stored at −70°C until use.

Sucrose Density-Gradient Centrifugation

This was done as described previously (33) using bovine serum albumin (M, 67,000), ovalbumin (M, 43,000), and chymotrypsinogen A (M, 25,000) obtained from Pharmacia as standard molecular weight markers.

Generation of Monoclonal Antibodies

Monoclonal antibodies against the M, 22,000 protein were generated by two different methods.

Method I. Monoclonal antibodies to the protein were raised by using in vitro immunization techniques as described (34, 35). About 2 × 10⁶ spleen cells from 8–9 female BALB/c mice were immunized in vitro with 0.8 or 4.0 μg of the purified M, 22,000 protein in thymocyte-conditioned medium. The cells were cultured for 5 days and fused with P3-X63-Ag8-U1 myeloma cells as described (36), and hybridomas were selected in hypoxanthine/aminopterin/thymidine medium. The culture supernatants from growing hybridomas were tested by ELISA and the subclasses of the immunoglobulins were determined by two different methods.

Method II. BALB/c mice were immunized with i.p. injection of 10⁷ of intact K562/ADM cells. 30 weeks later, the mice were given a second i.p. injection of the crude membrane fraction from K562/ADM cells (10 μg of protein per mouse) with complete Freund's adjuvant. 11 days after the second immunization, the mice were boosted with i.v. injection of the crude membrane preparation from K562/ADM cells (10 μg of protein per mouse). 3 days after the booster, spleen cells from the...
immunized mice were fused with P3-X63-Ag8-U1 myeloma cells, and hybridomas were selected as described (14, 37). The culture supernatants from growing hybridomas were tested by ELISA.

**Immunoblotting**

After SDS-PAGE the proteins were transferred to nitrocellulose (Schleicher & Schuell, Dasse, W. Germany) by overnight electrophoresis at 30 V in 25 mM Tris, 0.192 M glycine (pH 8.3), containing 20% (v/v) methanol. The nitrocellulose was washed briefly with water and incubated for 1 h in PBS supplemented with 5% bovine serum albumin, and 1 h in TPBS containing 10 µg/ml of monoclonal antibody. The nitrocellulose was washed in diluted TPBS for 3 x 10 min and incubated and 1 h in TPBS containing 10 Me/ml of monoclonal antibody. The incubated for 1 h in PBS supplemented with 5% bovine serum albumin, and stained with Coomassie brilliant blue, demonstrating the steps of purification: lane 2 and lane 1. The protein was not clearly detected in parent K562 cells.

**Purification of the M, 22,000 Protein.** The M, 22,000 protein was purified from the K562/ADM cell extract by ammonium sulfate fractionation followed by DEAE-Sephacel chromatography and gel filtration. Fig. 1 (lanes 2–5) demonstrates SDS-PAGE analysis of the proteins in typical purification steps. The M, 22,000 protein was a rather major protein consisting of 1.5% of the total soluble cytoplasmic protein in K562/ADM cells. The M, 22,000 protein was precipitated with 40% saturation of ammonium sulfate, and eluted from the DEAE-Sephacel column at 125–150 mM NaCl. The protein was eluted from the Sephadex G-200 column at a Kav value of 0.528, and the apparent M, was calculated to be 44,000. By densitometric scanning of the Coomassie blue-stained polyacrylamide gel, the amount of the M, 22,000 protein was estimated. Table 2 summarizes the purification of the M, 22,000 protein. Isoelectric focusing analysis revealed that the protein was acidic (pI 5.3). Interestingly, the elution pattern of the purified M, 22,000 protein from Sephadex G-200 column suggested that the protein behaved as a larger molecule with M, 44,000. By sucrose density-gradient centrifugation analysis, the apparent molecular mass of the protein was 44,000. By SDS-PAGE analysis, the purified protein had the mobility of M, 22,000 (Fig. 1). No M, 44,000 band was detected by SDS-PAGE analysis whether in the presence or absence of reducing agents (data not shown). The results suggest that the M, 22,000 protein exists as a homodimer. The binding of the two molecules was via noncovalent linkage, and the binding was cleaved by the treatment with denaturing reagent such as SDS.

Calcium-binding Capacity of the M, 22,000 Protein. Calcium-binding assay was carried out with 44Ca2+ on nitrocellulose paper (30). The M, 22,000 protein was one of the major calcium-binding proteins in the extracts from K562/ADM cells (Fig. 2). No comparable activity was evident in the parent K562 cells, confirming that the binding was to the resistance-associated protein. The purified protein preserved calcium-binding capacity (Fig. 2, lane 3). The results indicate that the M, 22,000 protein (pI 5.3) purified from the human drug-resistant cell line corresponds to the M, 22,000 calcium-binding protein designated CP22 or sorcin which was previously reported in some multidrug-resistant cell lines (22, 23, 25).

**In Vitro Phosphorylation Assay.** Fig. 3 shows the in vitro phosphorylation patterns of the soluble proteins from K562 and K562/ADM cells in the presence or absence of Ca2+. The M, 22,000 protein was not clearly detected in K562/ADM cells by this 32P-labeling technique. The presence or absence of Ca2+ in the assay conditions did not cause any difference in the 32P-labeling of the M, 22,000 protein. The results suggest that the M, 22,000 protein overexpressed in K562/ADM cells is rarely phosphorylated, if at all.

**Generation of Monoclonal Antibodies.** Monoclonal antibodies were raised against the purified M, 22,000 protein using in vitro immunization technique (34, 35). After selection by ELISA and limiting-dilution clonings, two hybridoma lines producing monoclonal antibodies designated HOT12 and HOT22 were established. The immunoglobulin class of HOT12 and HOT22 were IgM.

We also obtained two monoclonal antibodies by an in vivo immunization procedure. The two monoclonal antibodies (termed HOT104 and HOT111) recognized the M, 22,000 protein in K562/ADM cells as revealed by ELISA analysis.

**Table 2 Purification of the M, 22,000 protein**

All the data are from the same purification experiment, which is one of three such experiments done. The data are typical of the average results that are obtained for any given step. The amounts of the M, 22,000 protein were estimated by densitometric scanning of the Coomassie blue-stained polyacrylamide gels.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein</th>
<th>Recovery (%)</th>
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<tr>
<td>Cell extract [a]</td>
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</tr>
<tr>
<td>Soluble fraction</td>
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<td>6.6</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sephadex G-200</td>
<td>1.47</td>
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</tbody>
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[a] Cell extract was prepared from 3.0 x 10^6 K562/ADM cells.

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**Fig. 1. Detection and purification of the M, 22,000 protein from K562/ADM cells.** Cytoplasmic extracts from K562 (lane 1) and K562/ADM cells (lane 2) were electrophoresed on a 10–20% linear gradient polyacrylamide gel and stained with Coomassie brilliant blue. Arrowhead, position of the M, 22,000 calcium-binding protein designated CP22 or sorcin which was previously reported in some multidrug-resistant cell lines (22, 23, 25).
The immunoglobulin subclasses of HOT104 and HOT111 were IgG1 and IgG2a, respectively.

Antigen Specificity of Monoclonal Antibody. Immunoblotting analysis showed that HOT12 specifically recognized the M, 22,000 protein in the K562/ADM cell extract (Fig. 4). No comparable protein band was evident in the parent K562, even by longer exposure of the autoradiogram (Fig. 4B). Three other monoclonal antibodies were also specific to the M, 22,000 protein (data not shown).

Detection of the M, 22,000 Protein in Various Cell Lines by Immunoblotting. Using the monoclonal antibody HOT12 as a probe for the detection of the M, 22,000 protein, we studied the cytoplasmic extracts from various drug-resistant and -sensitive cell lines (Fig. 5). The M, 22,000 protein was highly expressed also in K562/VCR, a rather weakly resistant cell line.

Fig. 2. Binding of Ca** to the M, 22,000 protein in multidrug-resistant cells. Detection of calcium-binding proteins by 45Ca autoradiography on nitrocellulose membrane after SDS-PAGE was carried out according to Maruyama et al. (31). The figure represents calcium-binding assay analyzing cytoplasmic extract from K562 (lane 1) and K562/ADM cells (lane 2), and the M, 22,000 protein purified from K562/ADM cells (lane 3).

Fig. 3. In vitro phosphorylation of K562 and K562/ADM cytoplasmic extracts. Cytoplasmic extracts were labeled with [γ-32P]ATP and analyzed by SDS-PAGE in a 4–20% linear gradient gel. The figure represents an autoradiogram of the resolved phosphoproteins from K562 (A) and K562/ADM (B) cytoplasmic extracts which were labeled in the presence of 0.2 mM Ca** (lane 1) or 1 mM EGTA (lane 2). Arrow, position of the M, 22,000 protein.

Fig. 4. Antigen specificity of the monoclonal antibody HOT12. Cytoplasmic extracts from K562 and K562/ADM cells (40 μg of protein per lane) and the purified M, 22,000 protein (2 μg per lane) were subjected to SDS-PAGE in a 10–20% polyacrylamide slab gel and the fractionated proteins were subsequently transferred to nitrocellulose paper. The blot was then reacted with HOT12 and the immune complex was illuminated with radiiodinated antimouse immunoglobulin followed by autoradiography. Lane 1, immunoblot analysis of the purified protein; lane 2, cytoplasmic extracts from K562, lane 3, K562/ADM cells. B, a 5-fold longer exposure than in A. Arrow, position of the M, 22,000 protein.

Fig. 5. Immunoblot analysis of cytoplasmic extracts from various cell lines. Cytoplasmic extracts from various cell lines (40 μg of protein per lane) were prepared and subjected to immunoblot analysis with HOT12. B, a 3-fold longer exposure than in A. Arrows, position of the M, 22,000 protein.
The 22,000 protein was also preserved in R1-3 and R1-5, which were revertant cell lines derived from K562/ADM cells (Table 1). The protein was not clearly detected in the human drug-resistant sublines, CEM/VLB100, 2780AD, and KB-C4 (Fig. 5A). However, when the film was exposed for a longer period, the M, 22,000 protein was detected in 2780AD and KB-C4 (Fig. 5B). Even by this longer exposure of the film, the protein was not detected in CEM/VLB100, or in the drug-sensitive parent cell lines (K562, CCRF-CEM, A2780, and KB3-1) (Fig. 5B). The M, 22,000 protein was highly expressed in the Adriamycin-resistant human cell line K562/ADM. The M, 22,000 protein was not detected in the parent K562/ADM cells. The protein was an acidic (pI 5.3), calcium-binding protein (Fig. 2). The results indicate that the M, 22,000 protein overexpressed in K562/ADM cells is the same as the M, 22,000 protein (sorcin) reported by other authors (22, 23, 25). In order to conclusively rule out homology with the M, 22,000 protein overexpressed in K562/ADM cells, it would be necessary to test reactivity of the monoclonal antibody HOT 12.

Previously, an increased expression of a soluble cytoplasmic protein with a molecular weight of about 20,000 (M, 19,000~22,000) was reported in several drug-resistant cell lines (8, 16~25). The overexpression of the protein appeared associated with amplified genes such as double-minute chromosomes and/or homogeneously staining regions (17~20, 23, 25). The protein was found to be a calcium-binding protein (22, 23, 25), and designated as CP22 (22) or sorcin (soluble resistance-related calcium-binding protein) (25). We have found that a M, 22,000 protein is highly expressed in the Adriamycin-resistant human cell line K562/ADM. The M, 22,000 protein was not detected in the parent K562/ADM cells. The protein was an acidic (pI 5.3), calcium-binding protein (Fig. 2). The results indicate that the M, 22,000 protein overexpressed in K562/ADM cells is the same as the M, 22,000 protein (sorcin) reported by other authors (22, 23, 25). In contrast to this, Fine et al. (38, 39) reported an increased phosphorylation of a protein with a molecular weight of 20,000 in pleiotropic drug-resistant MCF-7 human breast cancer lines. This protein kinase activity was Ca^2+ dependent, cyclic nucleotide independent, membrane bound, and inhibited by calmodulin antagonists (38). Our in vitro phosphorylation assay (Fig. 3) showed that the M, 22,000 protein overexpressed in K562/ADM cells was not a major phosphorylated protein, suggesting that the M, 22,000 protein was a different one from the M, 20,000 protein with increased phosphorylation, which had been reported by Fine et al. (38, 39). In order to conclusively rule out homology with the M, 22,000 protein, it would be necessary to test reactivity of the monoclonal antibodies versus the M, 20,000 protein of the MCF-7/ADR cells.

The monoclonal antibody HOT 12 was raised against the M, 22,000 protein purified from the human leukemia cell line K562/ADM. HOT 12 was also reactive with the M, 22,000 protein from Chinese hamster ovary cells (CH^3C5), suggesting that the protein is conserved among species. Although the M, 22,000 protein was found to be hyperepressed in drug-resistant cell lines, the amounts of the protein expressed in drug-resistant and -sensitive cell lines appear not necessarily in direct proportion to the degree of drug resistance. For example, the weakly resistant cell line K562/VCR was shown to express a lower amount of the protein compared with the highly resistant cell lines such as 2780AD and KB-C4 (Fig. 5). The protein remained highly expressed in revertant cell lines, indicating that overexpression of the protein was not sufficient to maintain the multidrug-resistant phenotype (Fig. 5). In some drug-resistant cell lines such as CEM/VLB100, the amount of the M, 22,000 protein appeared as low as that in sensitive cell lines, suggesting that the multidrug-resistant phenotype could be acquired without accompanying the overexpression of the M, 22,000 protein. In summary, the overexpression of the M, 22,000 protein is not a sufficient or a necessary condition for the acquisition of the multidrug-resistant phenotype.

As the M, 22,000 protein associated with multidrug-resistance is a potential calcium-binding protein, it is tempting to relate this property to the ability of some calcium-channel blockers and calmodulin inhibitors to partially reverse the multidrug-resistant phenotype (4, 5). Our preliminary results suggested that the protein was not a specific acceptor protein of calmodulin inhibitors (e.g., W-7 or trifluoperazine) or calcium antagonists (e.g., verapamil). The protein was not a binding protein of anticancer drugs (e.g., vincristine or Adriamycin) as revealed by affinity chromatography using columns bound with chemotherapeutic drugs.4 Our preliminary results showed that the M, 22,000 protein was not a binding protein for the P-glycoprotein.5 The P-glycoprotein was reported to have an ATPase activity (40). The ATPase activity of the P-glycoprotein was not affected by the M, 22,000 protein. Thus, no evidence was obtained suggesting that the M, 22,000 protein might be a cofactor regulating the function of the P-glycoprotein in multidrug-resistant cells.

From the data now available, the role of the M, 22,000 protein in multidrug resistance is not clear. It is possible that the amplification of the gene for the M, 22,000 protein and overproduction of the protein would be merely the passive consequences of the amplification of P-glycoprotein genes, as suggested by Van der Blik et al. (23). Even if the overexpression of the M, 22,000 protein might be the passive consequence of the amplification of P-glycoprotein gene, a change in intracellular calcium environment would occur when such a large amount of calcium binding protein happened to exist in the cell. It was previously reported that some multidrug-resistant cell lines including K562/ADM had a higher Ca^2+ content than did the drug-sensitive cells (41). The overexpression of the M, 22,000 calcium-binding protein in some drug-resistant cells might explain this higher Ca^2+ content in some but not all resistant cell lines. Recently, Yamashita et al. reported that voltage-gated sodium channel current was enhanced in K562/ADM cells (42), although the biological meaning of this enhanced sodium channel current was not clear. These results imply that changes in intracellular ion environment might be involved in the mechanisms of drug resistance.

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REFERENCES

4 T. Yamori and T. Tsuruo, unpublished results.
5 H. Hamada and T. Tsuruo, unpublished results.

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