27. A Hen Enzyme ADP-ribosylates Normal Human and Mutated c-Ha-ras Oncogene Products Synthesized in *Escherichia coli*

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Introduction. *Ras* oncogene products are similar in biochemical properties to guanine nucleotide-binding (G regulatory) proteins of the transmembrane signaling system such as adenylate cyclase or cGMP phosphodiesterase,¹⁾ and have some amino acid sequences homology.^{2),3)} ADP-ribosylation of G regulatory proteins, Ns and Ni, by bacterial toxins and by cellular ADP-ribosyltransferase stimulates the adenylate cyclase system.^{4),5)}

Ras proteins were produced in *Escherichia coli* with plasmids bearing chemically synthesized *ras* genes. ADP-ribosylation of the *ras* proteins by a cellular enzyme *in vitro* was studied. We observed that normal human c-Ha-*ras* oncogene product as well as mutated c-Ha-*ras* oncogene products undergo ADP-ribosylation in the presence of hen liver enzyme.

Materials and methods. [Adenylate-³²P]NAD was purchased from New England Nuclear. ADP-ribosyltransferase was purified from hen liver nuclei and the biochemical characteristics have been previously reported.⁶⁾ Mutated c-Ha-*ras* protein with a valine at position 12 was produced in *Escherichia coli* and purified in a homogeneous state.⁷⁾ Similarly, normal c-Ha-*ras* protein and other mutated proteins were produced and purified (unpublished data).

The reaction mixture contained in 25 μ l, 1.2 μ g of purified c-Ha-*ras* oncogene product, 10 μ M [adenylate-³²P]NAD (2.5 μ Ci), 18 ng protein of hen liver ADP-ribosyltransferase, 50 mM Tris-HCl (pH 8.0) and 10 mM dithiothreitol. The reaction was for 30 min. at 25°C and was terminated by the addition of an equal volume of 2-fold concentrated sample buffer used for electrophoresis.⁸⁾ The incubation mixture was electrophoresed directly on 12.5% polyacrylamide gel containing 0.1% SDS and 0.38 M Tris-HCl (pH 8.8).⁸⁾ The gel was stained with 0.1% Coomassie brilliant blue R-250, dried and exposed to Kodak XRP film.

Results and discussion. Both normal human c-Ha-*ras* oncogene product, p21, and a mutated product with a valine at position 12 instead of a glycine were ADP-ribosylated by hen liver ADP-ribosyltransferase (Fig. 1B, lanes 3 and 4). ADP-ribosylation of the c-Ha-*ras* proteins was inhibited by the presence of 40 mM arginine methylester or 40 mM nicotinamide (data not shown). When the hen enzyme was heated for 5 min. at 100°C, the ADP-ribosylation of normal human and mutated c-Ha-*ras* products was not observed at all (data not shown).

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Fig. 1. ADP-ribosylation of normal human and mutated c-Ha-ras oncogene products by hen liver ADP-ribosyltransferase. Purified p21 was incubated with 10 μ M [32 P]NAD in the presence or absence of the hen liver enzyme and then electrophoresed on 12.5% polyacrylamide gel containing 0.1% SDS. (A) Stained with Coomassie brilliant blue. (B) Autoradiogram. Lanes: 1, normal c-Ha-ras p21 without hen enzyme; 2, mutated p21 (Val-12) without hen enzyme; 3, normal p21, plus hen enzyme; 4, mutated p21 (Val-12), plus hen enzyme; 5, hen enzyme without *ras* protein; 6, no *ras* protein or hen enzyme, [32 P]NAD alone. The standard protein markers are phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa) and cytochrome c (12 kDa).

Fig. 2. ADP-ribosylation of two kinds of mutated c-Ha-ras oncogene products by hen liver ADP-ribosyltransferase. (A) Stained gel. (B) Autoradiogram. Lanes: 1, mutated p21 (Leu-61); 2, mutated and truncated *ras* protein (Val-12 and deleted for 18 amino acid residues of carboxyl-terminus). The standard protein markers are the same as those in Fig. 1.

The minor band of about 33 kdalton, which was ADP-ribosylated, would be a protein in the enzyme preparation which was copurified through the enzyme purification procedure.

Other mutated c-Ha-*ras* oncogene products, p21 with a leucine at position 61 and carboxyl-terminal truncated derivative (deleted for 18 amino acid residues) of p21 with a valine at position 12, were also ADP-ribosylated by this enzyme (Fig. 2).

The hen liver enzyme which was used in the present experiment is mainly localized in nuclei but a portion of the enzyme exists in the extranuclear fraction. It is likely that this enzyme causes ADP-ribosylation of proteins in cell membrane such as *ras* oncogene products. Recently, Tsai *et al.* reported that a nontransforming v-Ha-*ras* oncogene product, synthesized in *E. coli*, was ADP-ribosylated by turkey erythrocyte ADP-ribosyltransferase.⁹⁾ The turkey erythrocyte enzyme and the hen liver enzyme are believed to ADP-ribosylate arginine residues.^{6),10)} Normal human c-Ha-*ras* oncogene product contains 12 arginine residues of the total 189 amino acid residues.¹¹⁾ One or more of these arginine residues might be ADP-ribosylated by the hen ADP-ribosyltransferase.

The mechanism of cell transformation by *ras* oncogene product is still unknown. Decreased GTPase activity or alteration of the GTP binding state of *ras* proteins is suggested to be related to the transforming activity.^{12),13)} Transducin is a G regulatory protein in the cGMP phosphodiesterase system. ADP-ribosylation of an arginine residue of the α subunit of transducin is associated with a decrease in its GTPase activity.¹⁴⁾ It has been reported that ADP-ribosylation of a nontransforming v-Ha-*ras* protein decreased both GTP binding activity and GTPase activity.⁹⁾ The effect of ADP-ribosylation of normal and mutated c-Ha-*ras* proteins on GTP binding and GTPase activities is currently under investigation.

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