Mono(ADP-ribosyl)ation of DNA by apoptosis-inducing protein, pierisin

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ABSTRACT
The cabbage butterfly contains a potent cytotoxic protein, pierisin-1, and this protein is suggested to be an ADP-ribosylating toxin. Pierisin-1 effectively transfered an ADP-ribosyl group to DNA, but not to protein, as is the case with other bacteria-derived ADP-ribosylating toxins. Several spectral analyses and independent syntheses indicated that the acceptor site for ADP-ribosylation is N-2 of guanine base. Pierisin-1 induced apoptosis in mammalian cells accompanied by a release of cytochrome c and activation of a variety of caspases, and this apoptosis was inhibited by overexpression of Bcl-2. Pierisin-1 would be a novel DNA-damaging protein.

INTRODUCTION
We found the presence of a strong cytotoxic factor in larvae and pupae of the cabbage butterfly, Pieris rapae (1). The factor, pierisin-1, is an 850-amino acid protein consisting of a protease-cleavable 27-kDa N-terminal and a 71-kDa C-terminal region (2). These regions are suggested to be ADP-ribosyltransferase and receptor-binding domains, respectively (3). Although effective concentration varies from cell to cell, pierisin-1 induces apoptosis in most mammalian cells (4).

ADP-ribosyltransferase catalyzes the transfer of an ADP-ribosyl moiety of NAD to specific proteins. Several bacteria produce a so-called 'ADP-ribosylating toxin' that comprises a catalytic subunit and receptor-binding subunit(s). ADP-ribosylating toxins exhibit their own biological activity on the basis of which protein is a target for ADP-ribosylation. Diphtheria toxin ADP-ribosylates diphthamide of EF-2, and thereby protein synthesis of the attacked cells is inhibited. Pierisin-1 is also considered to be an ADP-ribosylating toxin, therefore, identification of the target for ADP-ribosylation was essential to understand the mechanisms of exertion of cytotoxicity and induction of apoptosis. We looked for the target molecule and found it to be DNA (5). Hence, pierisin-1 might be a DNA-modifying/damaging protein. The apoptotic pathway induced by pierisin-1 was also investigated (6).

RESULTS AND DISCUSSION
In efforts to identify a substrate protein for ADP-ribosyltransferase activity of pierisin-1 using [adenylate-32p]-NAD and fractionated cell extracts, we found a candidate factor by SDS/PAGE and autoradiography. This factor was of high molecular weight and heterogeneous. In addition, it was protease resistant but DNase sensitive. This observation was not found if pierisin-1 was omitted. An extensive study to find the protein substrate was unsuccessful. Thus we suspected that DNA might be the substrate for ADP-ribosylation by pierisin.

When a variety of oligonucleotides having different sequences were used for acceptors of the moiety from 32p-NAD and fractionated cell extracts, we found a candidate factor by SDS/PAGE and autoradiography. This result suggests that guanine base might be an acceptor. Indeed, incorporation occurred efficiently when GC-oligomer, but not AT-oligomer, was used. In addition, a decrease in the recovery of deoxyguanosine and the appearance of a new peak on HPLC was observed when calf-thymus DNA was used as an acceptor and then digested with nuclease mix and
alkaline phosphatase. These observations suggested that guanine base is an acceptor of the ADP-ribosyl moiety from NAD.

For structural determination of the reaction product by pierisin-1, 2'-deoxyguaninosine was used as an acceptor molecule. The UV spectrum and ESI-MS suggested the appearance of ADP-ribosylated dG. Several NMR analyses revealed the structure as α and β forms of \( N^2-(\text{ADP-ribos-1-yl})-2'-\text{deoxyguanosine} \) (Fig. 1). Finally, we independently synthesized chemically and confirmed the structural identity with the enzyme reaction product. From the above results, we concluded that pierisin-1 is a guanine-specific ADP-ribosyltransferase that transfers an ADP-ribosyl moiety from NAD to N-2 of guanine base.

The \( ^{32}\text{P}\)-postlabeling method for detection of ADP-ribosylated dG indicated that ADP-ribosylation of N-2 of guanine base also occurred in HeLa cells treated with pierisin-1.

Pierisin-1 is a strong apoptosis inducer. We constructed HeLa cells transfected with the human \( \text{bcl-2} \) gene to investigate whether this anti-apoptotic protein inhibits pierisin-1-induced apoptosis. When incubated with pierisin-1, the \( \text{bcl-2} \) transfected did not exhibit apoptotic cell death. The amount of cytochrome c in the cytosol of parent cells, but not the \( \text{bcl-2} \) transfecant, were increased by the incubation. Further, a variety of caspases, including caspase-2, 3, 6 and 9 were suggested to be activated by pierisin-1 in parental HeLa cells. These results indicate that pierisin-1-induced apoptosis is mediated primarily via a mitochondrial pathway involving Bcl-2 and caspases.

Incubation of the \( \text{bcl-2} \) transfecant with pierisin-1 for longer than 24 hours resulted in growth arrest, detachment and cell death without apoptotic morphological change. This result suggests that the death signal which is upstream of mitochondrial events was already triggered by the treatment. Such observations have also been reported in Bcl-2 expressed cells treated with DNA-damaging factors such as UV irradiation. Pierisin-1 is a DNA-modifying protein and this activity is most likely the cause for cell death. Thus, we concluded that pierisin-1 would be a novel DNA-damaging protein.

**REFERENCES**


Fig. 1. Chemical structure of \( N^2-(\text{ADP-ribos-1-yl})-2'-\text{deoxyguanisine} \)