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Biochemistry

The baculovirus antiapoptotic p35 gene also functions via an oxidant-dependent pathway

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ABSTRACT **Cellular imbalance in the levels of antioxidants and reactive oxygen species resulting in apoptosis is directly associated with a number of parasitic infections, aging, and several genetic and multifactorial diseases. The baculovirus *Ac*NPV-encoded antiapoptotic *p35* gene prevents apoptosis induced by a variety of apoptotic agents in different systems. We demonstrate the ability of the *p35* gene to inhibit oxidative stress-induced apoptosis. *In vitro* cultured *Spodoptera frugiperda (*Sf9) insect cells infected with wild-type *Ac*NPV carrying the antiapoptotic *p35* gene did not undergo apoptosis when subjected to oxidative stress generated by the exogenous application of oxidants or *in vivo* generation of reactive oxygen species or on direct exposure of cells to UV radiations. An *Ac*NPV mutant carrying a deletion of the *p35* gene failed to arrest cell death. Transfection of cells with a recombinant plasmid containing the *p35* gene under the transcriptional control of a stress promoter (*Drosophila hsp*70) was also able to rescue cells from oxidative stress-induced cell death, demonstrating the direct involvement of P35. ESR spin-trapping studies conducted *in vitro* and *in vivo* demonstrated that P35 functions directly as an antioxidant by mopping out free radicals and consequently prevents cell death by acting at an upstream step in the reactive oxygen species-mediated cell death pathway.**

Apoptosis or programmed cell death is a genetically encoded manifestation of the cell suicide program that occurs during development, immune cell proliferation, maintenance, and perpetuation of cellular integrity and tissue homeostasis. Inappropriate apoptosis is linked to a number of parasitic infections as well as origin and progression of several genetic and multifactorial disease states, neurodegenerative disorders, aging, and cancer (1–4). Cellular imbalance in the levels of antioxidants and reactive oxygen species (ROS) triggers cells to undergo apoptosis without DNA repair (5). Catalase, hydrogen peroxide scavenging enzyme, inhibits UV-B induced apoptosis, suggesting the direct involvement of ROS in UV-irradiated apoptosis (6). Thus, despite the extensive antioxidant defense mechanisms to counteract the deleterious effects of ROS, aerobic cells may face a state of oxidative siege under adverse environmental conditions leading to cell death (7). Although the exact pathway of ROS-mediated apoptosis is not known, it has been suggested that these ROS could act, among others, as the signaling molecules to destabilize the cellular redox state influencing the activity of several transcription factors, including the NF-κB and Fos/Jun (8–10). Nonetheless, the central and evolutionarily conserved role of aspartate-specific cysteine proteases (caspases) in the final execution of apoptosis is well established (11).

Anti-apoptotic genes such as *bcl-2, ced*-9, cowpox virus *crm*A, baculovirus inhibitor of apoptosis (*iap*), and *p35,* when overexpressed, prevent apoptosis induced by a variety of apoptotic agents in different systems. Bcl-2 is a well studied prototype of these anti-apoptotic proteins. The *bcl-2* family includes those that promote cell survival by inhibiting adaptors needed for activation of the caspases, while other members of this family promote apoptosis (12, 13). A crucial balance between these competing activities of the members of the *bcl-2* family determines the fate of the cell. Membrane-bound localization of Bcl-2 (mainly in mitochondria) helps to support the anti-apoptotic property of this protein as a free radical scavenger and/or its ability to interact with other proteins such as cytochrome *c* involved in apoptotic induction (14–18). The baculovirus inhibitor of apoptosis (*iap*) blocks apoptosis induced by diverse signals functioning at a central point or upstream of caspase activation (19).

The ability of the *Autographa californica* nuclear polyhedrosis virus (*Ac*NPV)-encoded *p35* gene to rescue cells that are programmed to die has been investigated in several test systems against a number of apoptotic stimuli such as growth factor withdrawal and treatment with actinomycin D, staurosporine, glucocorticoid, etc. (20, 21). The anti-apoptotic ability of P35 is attributed to its interaction with and inhibition of the members of the caspase family (11, 22, 23). Recently a protease, *Sf* caspase-1, has been identified from *Spodoptera frugiperda* 9 (Sf9) insect cells that is potently inhibited by P35 and can also cleave P35 (24). The ability of P35 to intercept oxidative stress-induced apoptosis is not known despite the more or less universal action of P35 in complementing Bcl-2 function (25, 26). It is also not clear whether P35 additionally acts on other intermediate targets in the pathway of apoptosis particularly induced by oxidants, and it is therefore believed that P35 can act only via an oxidant-independent pathway. Given the well established inhibitory action of P35 on the downstream executors of apoptosis, we investigated the ability of P35 to inhibit oxidative stress-induced cell death. Our results demonstrate that P35 is able to directly mop out free radicals and prevent cell death by also acting in an oxidant-dependent pathway at a very upstream step in the cascade of events associated with oxidative stress-induced apoptosis.

MATERIALS AND METHODS

Cell Culture. *S. frugiperda* (Sf9) cells were grown in TNMFH medium supplemented with 10% FCS and antibiotics (27).

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Abbreviations: *Ac*NPV, *Autographa californica* nuclear polyhedrosis virus; δ*p35Ac*NPV, *p35*-deletion mutant of *Ac*NPV; ESR, electron spin resonance; ROS, reactive oxygen species; moi, multiplicity of infection.

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Confluent cells with >95% viability (tested with trypan blue exclusion) were used in all experiments.

Plasmid Construction. The baculovirus *p35* gene was cloned in pGem7zf(+/−) vector under the *Drosophila* heat shock 70 gene promoter (*hsp*70). A 480-bp *hsp*70 gene fragment carrying the promoter was excised from the pAcDZ1 (28) by *Xba*I and *Eco*RI and cloned in the multiple cloning region of pGem7zf(+/−) to generate the construct pNU1. The baculovirus *p35* gene was PCR-amplified by using low error *Pfu* polymerase (Stratagene) and forward (5′-*Kpn*IGGGGTACCTTGTATTAAGTGAGCATTTTGAG) and reverse (AAATTTATTGCCTAATATTATTTTGGATCCCG*Bam*HI–3′) primers obtained from Rama Biotechnology, Secunderabad, India. PCR was carried out for 30 cycles comprising: denaturation 94°C, 1 min; annealing 50°C, 1 min; extension 72°C, 2 min. The amplified *p35* DNA was restricted with *Kpn*I and *Bam*HI and cloned in pNU1. The new construct possessing the *p35* gene downstream to the *hsp*70 promoter was sequence verified and designated as pNN1.

Induction and Rescue of Apoptosis in Sf9 Cells. Different H_2O_2 concentrations were used to induce apoptosis in Sf9 cells, as described earlier (29), and apoptosis was assayed by using cellular blebbing and nucleosomal DNA ladder formation as the major parameters, following a procedure adapted from Hershberger *et al.* (30). For P35-mediated inhibition of oxidative stress-induced apoptosis, 1.5×10^6 Sf9 cells were infected with wild-type or *p35* deletion mutant *Ac*NPV [≈10 multiplicity of infection (moi)] and incubated for 0–6 h before being treated with 1 mM H_2O_2. Cells were harvested at 24 h after treatment. A small aliquot (45 μl) of the cell suspension was used to score percentage of apoptotic cells, and the rest was subjected to oligonucleosomal DNA ladder analysis. For transfection of Sf9 cells with the recombinant plasmid, 1.5×10^6 cells were transfected in a 35-mm dish with 8–12 μg of pNN1 DNA by using lipofectin (GIBCO/BRL) as described (31). Six hours after transfection mix was added, cells were thoroughly washed with complete medium and incubated for 24 h at 27°C. Heat-shock treatment was given at 42°C for 30 min followed by incubation for 4 h at 27°C for the expression of *p35* gene. Cells were then subjected to H_2O_2 treatment and were monitored for apoptosis 24 h after treatment.

UV Irradiation of Sf9 Cells. Sf9 cells (1.5 million per 35-mm-diameter plate) were exposed to UV for 35 sec at room temperature by using a UV transilluminator from Ultraviolet Products equipped with 6-W (312-nm) bulbs. The exposure was given from the bottom of the dishes by placing them directly over the UV lamps fixed in the Ultraviolet Products transilluminator. After irradiation, cells were incubated at 27°C for 24 h under dark conditions before being processed for apoptosis.

ESR Monitoring of ROS Generation. *In vitro* generation of (O_2^-) was mediated by incubating cells with the xanthine/xanthine oxidase system (X + XO) at 37°C for 30 min in 50 mM phosphate buffer (pH 7.5) with or without purified recombinant P35 protein (a kind gift from Promega). *In vivo* generation of ROS (OH^-) was maintained through the Fenton reaction. For this, cells were loaded with iron by incubating them for 10 min with 10 μg/ml aqueous solution of iron–sorbitol obtained from Rallis India Pharmaceutical Division, Konnagar, India. The iron-loaded cells were treated with 1 mM H_2O_2 and immediately exposed to UV (≈312 nm, 1.8×10^{-6} watts per cm^2 at lamp-to-target distance) for 30 min and then subjected to ESR spectrometry at room temperature with Field 3280 ± 100 G, sweep time 4 min, frequency modulation 100 KHz 0.63 × 10 G, amplitude 5×10^3, power 20 mW, radio frequency 9.44 and response 0.3 sec. DMPO (5, 5 dimethyl-1-pyroline N-oxide) obtained from Sigma was used as a spin-trap in all these experiments. ESR-integrated absorption intensity (I) was calculated by the formula $I = KW^2h$, where K = line shape constant (6.5×10^{-10}), W = width of the lines, h = height of the peak lines.

RESULTS

Expression of the Baculovirus *p35* Gene Inhibits H_2O_2-Induced Apoptosis of Insect Cells. Having earlier demonstrated the utility of insect cells as a model to study oxidative stress-induced apoptosis (29), we investigated the role of the baculovirus-encoded *p35* gene in inhibiting H_2O_2-mediated cell death. *In vitro* cultured Sf9 cells were infected with wild-type *Ac*NPV at an moi of 10 and then treated with apoptotic dose of H_2O_2 (1 mM) at different time periods after infection. Apoptosis was scored by monitoring cellular blebbing (Fig. 1*A*) as well as the formation of nucleosomal ladder (Fig. 1*B*). Protection against H_2O_2-induced apoptosis was observed as a direct function of viral infection time. Treatment

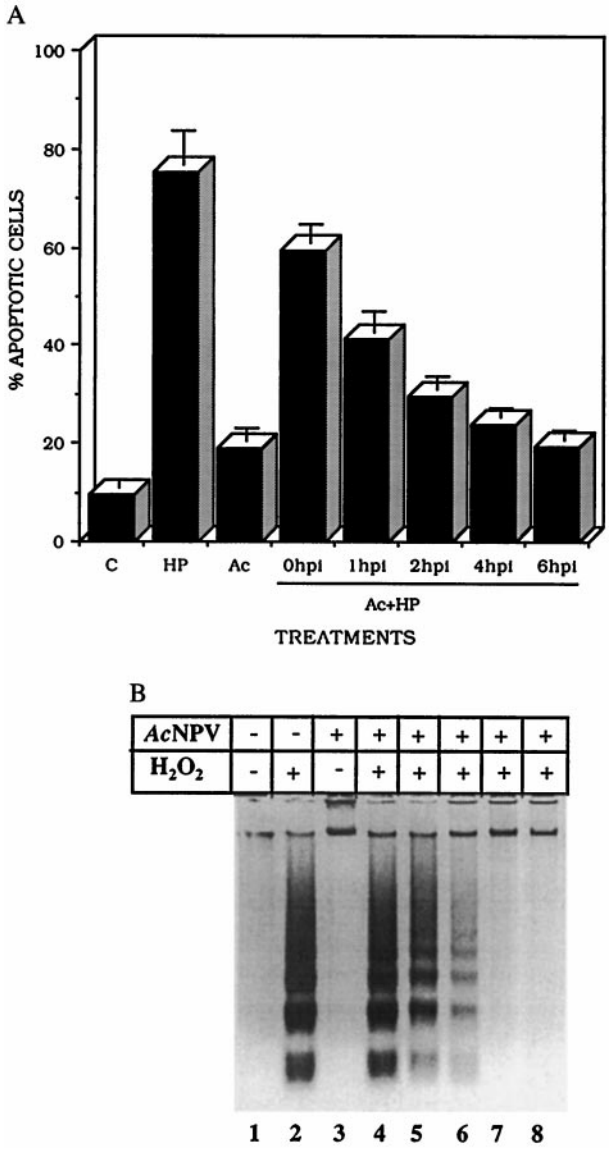


FIG. 1. (*A*) The *Ac*NPV *p35* gene inhibits H_2O_2 (HP)-induced apoptosis. Sf9 cells infected with wild-type baculovirus at 10 moi and then exposed to 1 mM concentration of HP at different time periods after infection (0–6 h). Percentage cells undergoing apoptosis was scored. (*B*) Nucleosomal ladder assay for the cells infected with wild-type virus before HP-exposure at different time periods after infection. Lane 1, uninfected cells; lane 2, 3, cells treated with HP and *Ac*NPV (Ac) alone; lanes 4, 5, 6, 7, 8, cells treated with HP at different h post infection (0, 1, 2, 4, 6, respectively).

of Sf9 cells immediately after viral infection (i.e., 0 h post-infection) yielded about 10% reduction in apoptosis as compared with uninfected cells. This decrease in the apoptotic response is possibly because of the presence of a minute quantity of P35 protein in the virions at the time of infection (30). Exposure of cells to H_2O_2 at increasing times after infection caused reduced apoptosis that could be caused by the early expression of the *p35* gene in the cells. The presence of the nucleosomal ladder complemented (Fig. 1*B*) the microscopic cellular blebbing observations.

To convincingly document the role of P35 in arresting oxidative stress-induced cell death, a *p35*-deletion mutant of *Ac*NPV (δ*p35 Ac*NPV) was used. Infection of Sf9 cells with mutant *Ac*NPV (at moi of 10) followed by apoptotic induction with H_2O_2 failed to protect cells against apoptotic onslaught, pointing to the direct involvement of P35 in this process (Fig. 2*A*). Expectedly, the mutant virus alone induced about 79% apoptosis at 24 h in Sf9 cells that was a reflection of the apoptotic response mounted by the insect cells in response to viral infection. Cells treated with H_2O_2 at 0, 1, 2, 4, and 6 h after infection with δ*p35 Ac*NPV registered about 68, 80, 88, 91, and 94% apoptosis, respectively. The microscopy observations were complemented by the appearance of a DNA ladder (data not shown). It was apparent that the *p35*-deletion mutant of *Ac*NPV could not arrest the apoptotic response mounted by the host Sf9 cells. In complementary experiments, Sf9 cells were exposed to varying doses of UV irradiation and monitored for apoptosis after 24 h. The dose (35 sec) showing 70–80% apoptosis was selected for further experiments. Our results show that wild-type *Ac*NPV-infected cells could completely abrogate apoptosis induced by UV irradiation, whereas the mutant virus (δ*p35 Ac*NPV) failed to do so (Fig. 2*B*), thereby complementing the oxidant-dependent mode of action of the P35 protein.

The ability of P35 to similarly provide protection against cell death induced by intracellular accumulation of ROS was also confirmed. Sf9 cells were treated with hydroxylamine, a known inhibitor of catalase (32, 33), for endogenous generation of ROS. Sf9 cells were infected with wild-type *Ac*NPV carrying the *p35* gene and 6 h later were treated with hydroxylamine (2.5 mM). The apoptotic response was scored as usual. As expected, 2.5 mM hydroxylamine caused about 60% apoptosis. Infection of cells with wild-type *Ac*NPV before the treatment with hydroxylamine resulted in significant reduction of apoptosis (Fig. 3). These results demonstrate the ability of the *p35* gene to inhibit apoptosis induced by oxidative stress irrespective of the source of the same.

A Cloned Copy of the p35 Gene Can Also Intercept H_2O_2-Induced Apoptosis. To investigate the possible involvement of other viral genes besides *p35* in arresting H_2O_2-induced apoptosis, a recombinant plasmid construct (pNN1) carrying the *p35* gene placed under the transcription control of the *Drosophila hsp*70 gene promoter was used. Ability of the *hsp*70 promoter to be induced as a function of oxidative stress was first confirmed (data not shown). Sf9 cells were transfected with 8–12 μg of pNN1, incubated for 24 h, and then treated with H_2O_2. The expression of the *p35* gene was confirmed by Northern blot analysis 24 h after H_2O_2 treatment by using radiolabeled *p35* DNA as probe (data not shown). Cells transfected with the *p35* gene construct registered nearly 40% protection against H_2O_2-induced apoptosis (Fig. 4). The reduced levels of protection observed against H_2O_2-induced apoptosis in *p35* transfected cells compared with those obtained with wild-type *Ac*NPV may be attributed to the involvement of other viral genes. The *iap* and *sod* genes are potential candidate genes that may have a role(s) in this process (19, 20, 34–36). It is nonetheless evident that a cloned copy of the *p35* gene, outside the viral context, is able to significantly intercept oxidative stress-induced apoptosis.

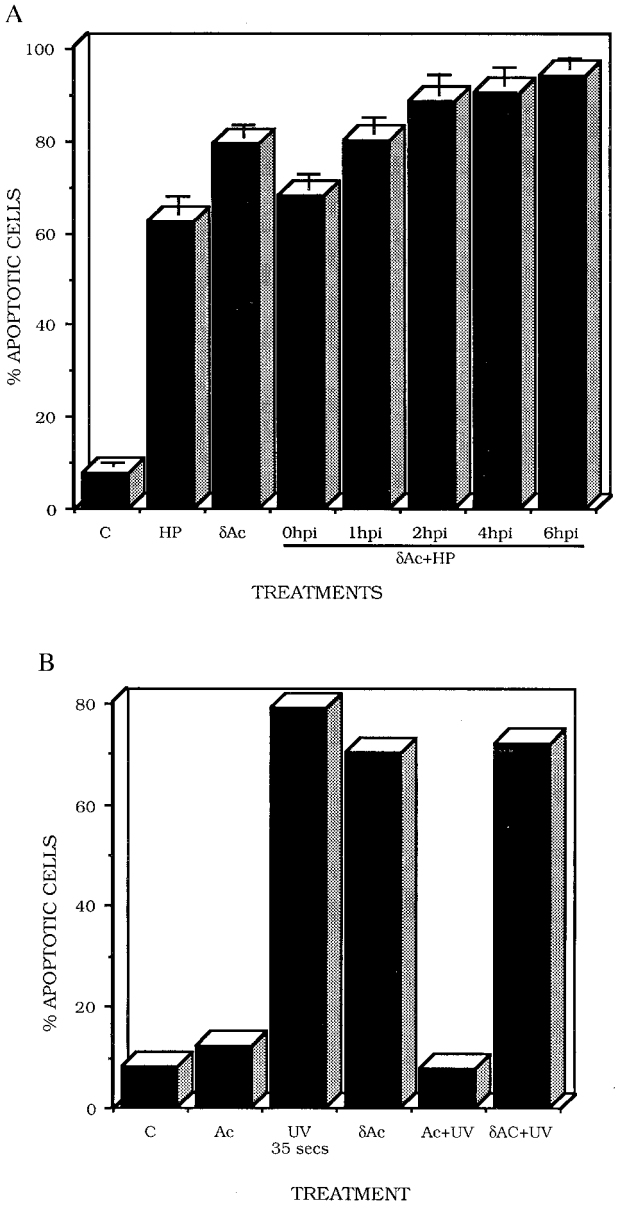


FIG. 2. (*A*) Infection with *p35* deletion mutant *Ac*NPV (δ*p35Ac*NPV) fails to inhibit H_2O_2 (HP)-induced apoptosis. Sf9 cells were infected with 10 moi of mutant virus (δ*Ac*) and then exposed to 1 mM concentration of HP for different time periods. Percentage of cells undergoing apoptosis was scored morphologically by cellular blebbing. (*B*) Infection of cells with wild-type *Ac*NPV but not the *p35* deletion mutant *Ac*NPV (δ*p35 Ac*NPV) inhibits UV-B-induced apoptosis. Sf9 cells were infected with 10 moi of wild-type or *p35* mutant virus and then exposed to UV-B light for 35 sec. Percentage of cells undergoing apoptosis was scored morphologically by cellular blebbing. The different lanes are: lane 1, control cells (C); lane 2, cells infected with wild-type *Ac*NPV (Ac); lane 3, cells treated with UV, and lane 4, cells infected with mutant virus (δAc); lanes 5 and 6, cells infected with *Ac*NPV and δ*p35 Ac*NPV (δAc) before UV-B irradiation.

P35 Acts Very Upstream in the Pathway of Oxidative Stress-Induced Apoptosis. To determine the stage at which P35 actually arrests H_2O_2-induced apoptosis, cells were first exposed to oxidative stress followed by interception with P35. At different time intervals after H_2O_2 exposure, cells were infected with *Ac*NPV (Fig. 5*A* and *B*) or transfected with *hsp*70 promoter-driven *p35* gene construct (data not shown). It was apparent that P35 was unable to intercept oxidative stress-induced apoptosis in Sf9 cells once the same has already begun. These results point to the action of P35 very upstream in the pathway of oxidative stress-induced apoptosis.

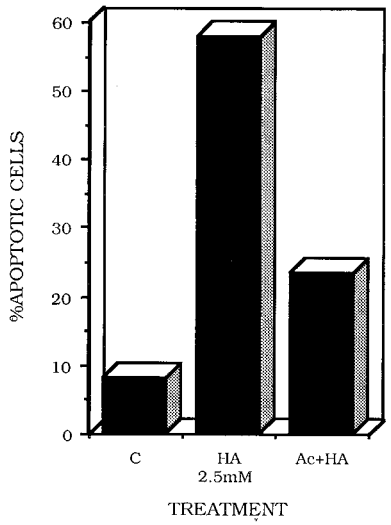


FIG. 3. *p35* can also inhibit cell death induced by intracellularly generated ROS. Sf9 cells were infected with wild-type *Ac*NPV (Ac) before being treated with 2.5 mM hydroxylamine (HA) and scored for apoptosis after 24 h. C, control cells.

Mechanism of Action of P35: P35 Protein Acts as a "Sink" for ROS. Experiments were initially designed to confirm the involvement of ROS in H_2O_2-mediated apoptosis. Cells were pretreated with DMSO, a known scavenger of hydroxyl radical (37), and then exposed to H_2O_2. Significant reduction in apoptosis was observed in cells pretreated with DMSO as opposed to untreated cells (29), thereby confirming the direct involvement of ROS in H_2O_2-mediated apoptosis. To investigate the mechanism of action of P35 in inhibiting ROS-mediated apoptosis, *in vitro* and *in vivo* experiments involving ESR spin-trapping were carried out. ESR spectrophotometry, which records specific signals for a given paramagnetic molecule (free radical), was exploited to determine whether P35 acts as a "sink" to sequester ROS. It was argued that the ESR spectrum generated by free radical should disappear in the presence of P35. *In vitro* studies were performed by using purified recombinant P35 protein, whereas *in vivo* experiments were carried out on Sf9 cells transfected with the plasmid pNN1. The spectra generated by the free radicals in the presence and the absence of P35 were analyzed. The generation of ESR signals by the superoxides was standardized by using the *in vitro* xanthine/xanthine oxidase system. Results clearly showed that the specific peaks obtained for the superoxide generated by the xanthine/xanthine oxidase system (Fig. 6*A Middle*) was greatly reduced in intensity in the presence of purified P35 protein. ESR integrated absorption intensity (*I*) (integrated line intensity of the first derivation signal) was determined (Table 1). Attenuation of *I* was found out to be almost 100% by the purified P35 protein (Fig. 6*A Bottom*).

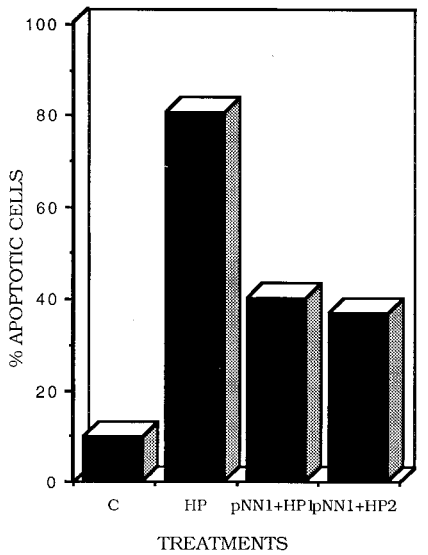
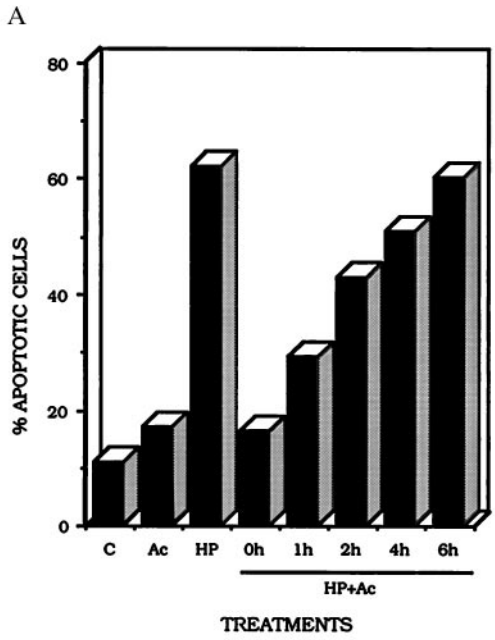


FIG. 4. Transfection of Sf9 cells with *p35* gene construct significantly inhibits H_2O_2 (HP)-induced cell death. Sf9 cells were transfected with 8–12 μg of pNN1, carrying the *p35* gene under the *hsp*70 transcriptional control, and were incubated for 24 h before being subjected to HP treatment. Cells were scored microscopically for apoptosis 24 h after HP treatment.



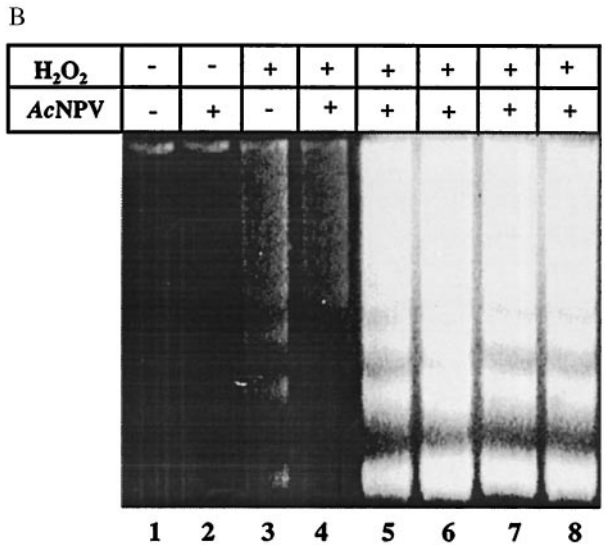


FIG. 5. P35 acts very upstream in preventing oxidative stress-induced apoptosis. (*A*) *p35* is unable to rescue cells from apoptosis after pretreatment with H_2O_2 (HP) Cells were infected with wild-type *Ac*NPV after different time periods of HP treatment and then assayed for apoptosis 24 h after viral infection. The number of apoptosed cells is scored microscopically. (*B*) Apoptosis was assayed by the generation of nucleosomal DNA ladder formation in cells infected with wild-type *Ac*NPV (Ac) at different time periods after HP treatment. Lane 1, C (control cells); lane 2, cells infected with *Ac* alone; lane 3, cells treated with HP alone; lanes 4, 5, 6, 7, 8, cells were infected with virus at different time periods (0, 1, 2, 4, 6 h) after HP treatment.

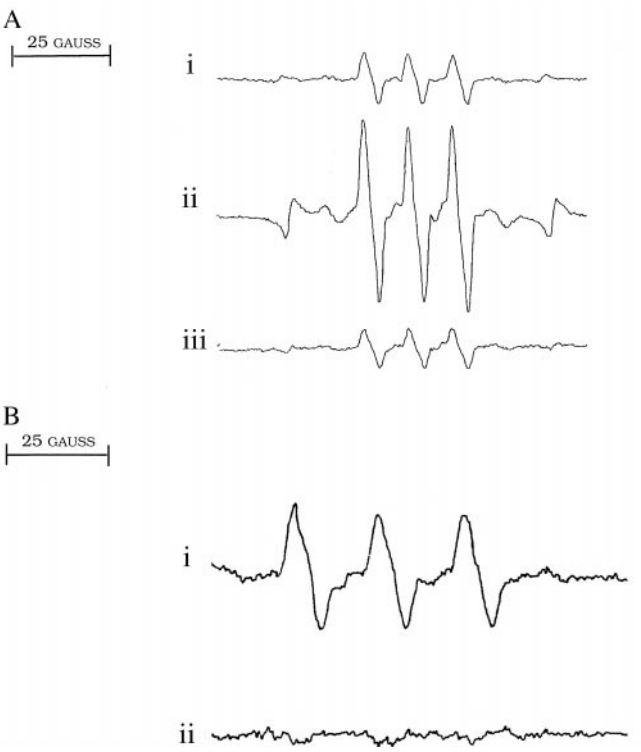


FIG. 6. Mechanism of action of P35. (*A*) P35 acts to sequester ROS. ESR spectrum of the superoxides generated by using *in vitro* xanthine/xanthine oxidase system. The basal level of the spectra generated by the xanthine oxidase (*i*) or those by the superoxides generated by the xanthine/xanthine oxidase system in the absence (*ii*) and the presence (*iii*) of the P35 protein are displayed. (*B*) ESR spectrum of ROS generated *in vivo* through the Fenton reaction in Sf9 cells. pNN1 transfected cells were subjected to intracellular ROS generation through Fenton reaction and monitored for the peaks generated by ESR spectrum. These spectra generated by hydroxyl radical in the absence of P35 (*i*) or after heat shock induction of pNN1 transfected cells (*ii*) are presented.

Similarly, the ESR spectrum of ROS generated *in vivo* was analyzed in the absence and presence of transiently expressed *p35.* Sf9 cells transfected with pNN1 were subjected to intracellular generation of hydroxyl radicals through the Fenton reaction (38) under UV irradiation, and the specific peaks were monitored by ESR spectrum. Although the ESR spin-trapped spectra were not as well resolved because of the low level of free radical generation, it was nonetheless evident that the specific peaks generated for the free radicals in the absence of P35 (Fig. 6*B Top*) were attenuated to almost basal level on the expression of the *p35* gene in transfected cells (Fig. 6*B Bottom*). Attenuation (*I*) value was determined to be about 95% in the transfected cells. Thus, these data unequivocally demonstrate that specific ESR spectra corresponding to the free radicals generated *in vitro* or *in vivo* were abolished in the presence of P35, pointing to the action of P35 as a "sink" to sequester ROS.

Table 1. ESR quenching of free radicals *in vitro* and *in vivo* by p35

Treatments		*I*		% reduction
In vitro	*In vivo*	($\times 10^{-10}$)	% *I*	in *I*
X	—	130	24.2	—
X + XO	—	537	100	
X + XO + P35	—	131	24.4	100
—	Fe + HP	144	100	—
—	$^{\beta}$pNN1 + Fe + HP	7.2	5	95

Attenuation of the ESR integrated absorption intensity (*I*) by p35 protein *in vitro* and *in vivo*. HP, hydrogen peroxide; X, xanthine; XO, xanthine oxidase; Fe, iron. β-Cells were heat-shocked for activating *p35* gene expresssion.

DISCUSSION

We selected H_2O_2 for the study of the oxidative stress-induced apoptosis in Sf9 cells because of its biological reactivity compared with many ROS and its ability to cross membranes and diffuse away from the site of generation. ROS also act as signaling molecules, among other stimuli, for the activation of the apoptotic pathway (39). H_2O_2 is often generated as a byproduct of oxidative metabolism in oxygen-dependent organisms. ROS is also central to the cytotoxic action of several drugs and pathogenic infections (1, 39). The baculovirus P35 protein is known to act in an oxidant-independent pathway at the execution step of the apoptotic process by stoichiometrically inhibiting various members of the family of ICE proteases (22). Our data clearly show that P35 could also operate via an antioxidant pathway in the inhibition of apoptosis. This was apparent from the ability of P35 to arrest H_2O_2-induced apoptosis or those induced by blocking cellular scavengers of ROS or on UV exposure. The action of P35 to block apoptosis was seen in the viral context as well as when it was present alone in the plasmid. Recombinant *Ac*NPV carrying specific *p35* deletion failed to rescue Sf9 cells from oxidative stress-induced apoptosis, once again directly pointing to the involvement of P35 in this process.

The fact that the presence of P35 protein is required well before the initiation of apoptosis by oxidative stress implies that P35 intercepts the oxidative stress-induced pathway at an upstream step besides the execution step of apoptosis (22, 24). The upstream step of action posed an important question of whether P35 protein was acting as a sink for the ROS. ROS sequestration by p35 was directly addressed by ESR spectroscopy. The ESR results clearly show that P35 was able to quench the *in vitro*- and *in vivo*-generated free radicals. *In vitro* experiments where the superoxide radicals (via xanthine/xanthine oxidase system) detected by the ESR signals were quenched in the presence of P35 protein not only confirmed the antioxidant action of P35 but also reinforced the notion that P35 functions at an upstream step of the apoptotic signaling cascade.

The ability of P35 protein to sequester ROS could be explained by the analysis of the amino acid composition of P35. It is likely that P35 protein has metal-binding site(s) that could enhance its antioxidant property, and/or its three-dimensional structure contains some amino acids that confer electrodynamically stable configuration conducive to ROS-trapping. P35 protein has five scattered histidine residues, which may form a functional metal-binding domain to enable it to bind to certain metal ions (e.g., Fe^{2+}, Mn^{2+}, etc.). Of these, the Mn^{2+}-binding activity of P35 has been observed (data not shown). P35 also contains six cysteine residues that are established chemical radioprotectors, especially under oxygenated conditions (40). These aminothiols are known to react with certain ROS with appreciable rate constants, thereby supporting the antioxidant role of P35.

Oxidative damage to cellular macromolecules such as nuclear and mitochondrial DNA and proteins caused by ROS is considered to be of key importance in the aging process (41). The chain of oxidative reactions initiated by ROS eventually knocks down the crucial biomolecules, thereby driving the cellular machinery to undergo apoptosis via activation of caspases, which ultimately brings about the execution of cell death. The ability of P35 to inhibit ICE proteases coupled with our demonstration of its antioxidant property additionally points to the "Swiss army knife-like" action of P35, similar to Bcl-2 (42)—an observation of significant therapeutic implica-

tions. Microdissection of the molecular events associated with P35-mediated inhibition of apoptosis at upstream as well as downstream steps in the cell death pathway will lead to a better understanding of its anti-apoptotic function.

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