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Expression and Characterization of Kinase-active v-*erbB* Protein Using a Baculovirus Vector System

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The v-*erbB* gene is an oncogene of the avian erythroblastosis virus encoding a protein that is a truncated version of the epidermal growth factor receptor. The v-*erbB* protein was expressed alone or as polyhedrin-*erbB* fusion proteins using the *Bombyx mori* nuclear polyhedrosis virus vector. The expression level of the fusion protein whose polyhedrin portion consisted of only 8 amino-terminal amino acids was more than ten times higher than that of the non-fusion protein. Studies with tunicamycin showed that the recombinant v-*erbB* proteins were glycosylated. The recombinant protein autophosphorylated tyrosine residues, and phosphorylated a synthetic tyrosine-containing peptide and lipocortin I. These observations indicate that functional v-*erbB* protein can be expressed in silkworm-derived cells, and furthermore, that this system can be used for large-scale production.

Key words: v-*erbB* — Tyrosine kinase — Baculovirus

The v-*erbB* gene is an oncogene of the avian erythroblastosis virus[1-3] and has been cloned and sequenced.[4] The v-*erbB* gene has extensive homology with the human epidermal growth factor (EGF) receptor gene.[5] Comparison of the nucleotide sequence of the genes reveals that the v-*erbB* protein is a truncated version of the EGF receptor. It consists of a short extracellular domain, a transmembrane region, and a tyrosine kinase domain, but both the extracellular domain of the EGF receptor that is necessary for the EGF binding and the carboxy-terminal region are deleted.[6]

Another *erbB*-related gene that is distinct from the EGF receptor was isolated from the genomic library of human placenta and designated as c-*erbB*-2.[7] The c-*erbB*-2 protein also has tyrosine kinase activity but does not bind either EGF, transforming growth factor (TGF), fibroblast growth factor (FGF), or several other growth factors.[8,9] In addition, the c-*erbB*-2 gene is the same as the *neu* oncogene that is active in a series of rat neuroblastomas.[10-14]

To analyze the biochemical and physico-chemical properties of the v-*erbB* protein, a large quantity of the protein is necessary. Therefore, we employed the *Bombyx mori* nuclear polyhedrosis virus (BmNPV) vector system to obtain the v-*erbB* protein. Previously, the EGF receptor and intracellular domain of EGF receptor have been expressed using the baculovirus vector system.[15-18] In addition, the expression level of the polyhedrin-*sis* fusion protein has been higher than that of the non-fusion protein in this system.[19] On the basis of these findings, the v-*erbB* proteins were expressed alone or as polyhedrin-fusion proteins. Furthermore, to characterize the recombinant v-*erbB* proteins, the tyrosine kinase activity of these proteins was investigated.

MATERIALS AND METHODS

Chemicals, enzymes and plasmids The reagents used and their suppliers were as follows: restriction enzymes, Takara Shuzo, Toyobo, Nippon Gene, Bethesda Research Laboratories and New England Biolabs; the Klenow fragment of DNA polymerase I, T4 DNA ligase, bacterial alkaline phosphatase and thioredoxin, Toyobo; lysozyme (chicken egg white, 6×crystallized), Seikagaku Kogyo; [γ-^{32}P]ATP, [^{35}S]methionine, Aquasol-2 and Enlightning, New England Nuclear; phosphate-buffered saline (PBS), Nissui Seiyaku; mouse anti-polyhedrin monoclonal antibody was a gift from Dr. Kitahara, Daiichi Pharmaceutical, Tokyo. Partially purified recombinant lipocortin I which had been expressed in *Escherichia coli*[20] was a gift from Dr. Kita, Daiichi Pharmaceutical, Tokyo. All other reagents were of the highest grade commercially available.

Construction of the v-*erbB* gene transfer plasmid The plasmid pAE7.7[4] was digested with *Hap*II followed by filling-in to obtain the v-*erbB* gene fragment. It was subcloned at the *Hinc*II site of pUC19 to generate pSCE. The pUC13 was digested with *Sal*I and *Aat*I, and ligated with the 0.2 kb *Sal*I-*Aat*I polylinker fragment from pBM030.[21] The resulting plasmid was digested with

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*Nco*I, followed by filling-in, and ligated with a synthetic 43mer DNA fragment corresponding to the 5′ region of the v-*erbB* gene (positions 4 to 46) to generate pADE. The pADE was digested with *Aat*I and *Pst*I, followed by filling-in, and ligated with the *Aat*I-*Sma*I fragment containing the v-*erbB* gene from pSCE to generate pUC-*erbB*. The transfer vector pBM010[21] was digested with *Eco*RV and *Xba*I, and ligated with the *Eco*RV-*Xba*I v-*erbB* gene fragment from pUC-*erbB* to generate pBM-*erbB*.

Construction of the polyhedrin-*erbB* fusion gene transfer plasmid The polyhedrin fusion gene transfer vectors[19] pPH120, pPH240, and pPH596 were digested with *Eco*RI and *Sca*I, followed by filling-in, and ligated with the *Eco*RV-*Sca*I fragment containing the v-*erbB* gene from pBM-*erbB* to generate pPH120-*erbB*, pPH240-*erbB*, and pPH596-*erbB*. The DNA fragment containing the v-*erbB* gene which was excised from pPH596-*erbB* using *Sca*I and *Apa*I, followed by filling-in, was ligated with the fragment containing the polyhedrin gene which was excised from p9B312[19] using *Sca*I and *Xma*I followed by filling-in, to obtain pPH312-*erbB*.

Cells, silkworm larvae and recombinant viruses BM-N cells were cultivated as described by Volkman and Goldsmith.[22] Silkworm larvae were provided by Kyodo Shiryo (Japan) and reared as described by Marumoto *et al.*[23] The recombinant viruses were prepared by the marker rescue method as described by Maeda *et al.*[24] and Horiuchi *et al.*[21]

Chemical synthesis of oligodeoxynucleotides Oligodeoxynucleotides were synthesized by the phosphoramidite solid-phase method using a DNA synthesizer (model 380 DNA synthesizer; Applied Biosystems). Deblocking and purification were performed as described by Crea *et al.*[25]

Peptide synthesis The R-R-SRC-Peptide (Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly) was synthesized by a liquid-phase method as described by Honzl and Rudinger,[26] and Yajima and Fujii.[27] The desired peptide was purified by partition chromatography on Sephadex G-15.

Expression of the v-*erbB* proteins in BM-N cells BM-N cells infected with recombinant viruses were harvested by centrifugation at 72 h post infection. The cell pellets were washed with phosphate-buffered saline (PBS), and lysed in 10 m*M* Tris-HCl, pH 7.5. The lysates were mixed with an equivalent volume of sample buffer (130 m*M* Tris-HCl, 4% sodium dodecyl sulfate (SDS), 10% mercaptoethanol, 40% glycerol, 0.004% bromophenol blue, pH 6.8), heated at 100°C for 3 min, and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Phosphorylation reaction Lysates (185 μg protein) were suspended in reaction buffer A (50 μM [γ-^{32}P]ATP (1.5 Ci/mmol), 6.25 m*M* Hepes, 10 m*M* $MgCl_2$, 20 μM $ZnCl_2$, 4 m*M* sodium *p*-nitrophenylphosphate, 1.25 m*M* 2-mercaptoethanol, 0.1% Nonidet P-40, pH 7.5) in a final volume of 20 μl at 30°C. The reaction was terminated by the addition of 20 μl of sample buffer, heated at 100°C for 3 min, and analyzed by SDS-PAGE. The gel was stained with Coomassie Brilliant Blue R-250, dried, and subjected to autoradiography using Kodak X-Omat AR film.

Analysis of phosphorylated amino acids The radiolabeled v-*erbB* protein was extracted from gels in 50 m*M* NH_4HCO_3 containing 0.1% SDS, as described by Beemon and Hunter.[28] Carrier bovine gamma globulin was added (75 μg), and the protein was precipitated by 20% trichloroacetic acid at 4°C. The precipitate was washed with ethanol and ethanol:ether (1:1), and partially hydrolyzed with 6 *M* HCl at 100°C for 2 h in tubes sealed under vacuum. The HCl was removed by evaporation and the hydrolysates were dissolved in a marker mixture containing phosphotyrosine, phosphoserine, phosphothreonine (1 mg/ml each), and phosphoric acid (5 μg/ml), and analyzed on cellulose (0.1 mm) thin layer plates (Merck) as described by Nishimura *et al.*[29]

Phosphorylation of synthetic peptide Lysates (185 μg protein) were suspended in reaction buffer A containing 2 m*M* R-R-SRC-peptide and bovine serum albumin (2 mg/ml) in a final volume of 40 μl at 30°C. The reaction was terminated by the addition of 50 μl of 10% trichloroacetic acid, and then 10 μl of bovine serum albumin (10 mg/ml) was added. The reaction mixture was left on ice for 1 h, and centrifuged at 10,000*g* for 10 min. A 50 μl aliquot of the supernatant was spotted on a square of phosphocellulose paper (1.5×1.5 cm) and washed in 0.5% phosphoric acid four times and acetone once as described by Casnellie *et al.*[30] The papers were dried and placed in vials with 5 ml Aquasol-2 for counting.

RESULTS

Preparation of recombinant viruses coding for the v-*erbB* proteins We have expressed v-*erbB* proteins either alone or as polyhedrin-fusion proteins using the BmNPV vector system.[24, 31] The v-*erbB* gene or the polyhedrin-*erbB* fusion genes, whose v-*erbB* genes were connected to 5′-terminal portions of the polyhedrin gene, were placed downstream from the polyhedrin promoter. To investigate the relation between the lengths of the polyhedrin portions of the fusion proteins and the expression levels of the proteins, we constructed a series of polyhedrin-*erbB* fusion genes containing different lengths of the 5′-terminal region of the polyhedrin gene (Fig. 1). These genes encode the polyhedrin-*erbB* fusion proteins that contain 112, 70, 32, and 8 amino-terminal amino acids of the polyhedrin protein (designated as pPH120-*erbB*, pPH240- *erbB*, pPH 596-*erbB*, pPH 312-*erbB*, respec-

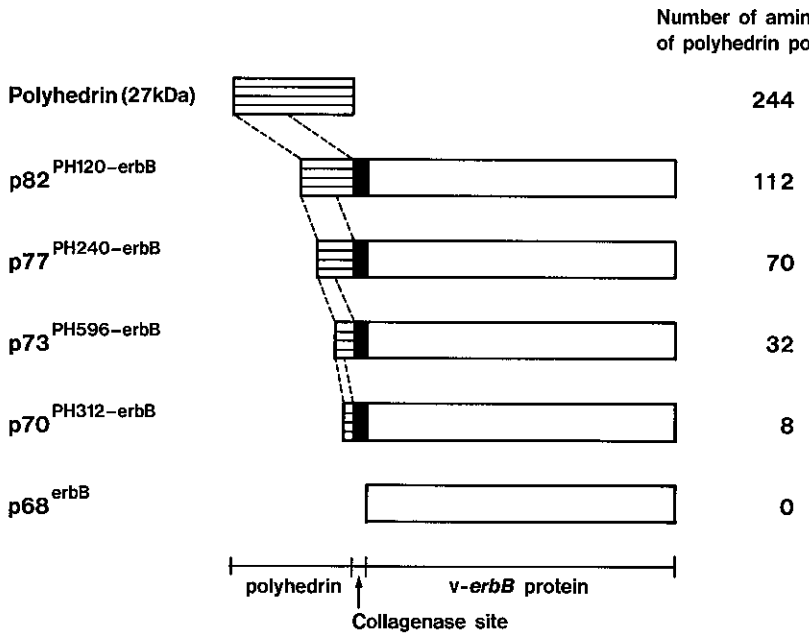

Fig. 1. Molecular structure of v-*erbB* and polyhedrin-*erbB* proteins. Polyhedrin portions and collagenase sites are shown by horizontally hatched regions and the closed boxes. The number of amino-terminal residues of each polyhedrin portion is shown in the right column.

tively) and the non-fusion v-*erbB* protein (designated as pBM-*erbB*). These polyhedrin-*erbB* fusion genes contain the sequence coding for the collagenase cleavage site at the junctions. The recombinant viruses were prepared by the marker rescue method, and designated as Bm120-*erbB*, Bm240-*erbB*, Bm596-*erbB*, Bm312-*erbB*, and Bm*erbB*, respectively.

Expression of the v-*erbB* proteins The polyhedrin-*erbB* fusion proteins (designated as $p82^{PH120\text{-}erbB}$, $p77^{PH240\text{-}erbB}$, $p73^{PH596\text{-}erbB}$, and $p70^{PH312\text{-}erbB}$, respectively) and the non-fusion v-*erbB* protein (designated as $p68^{erbB}$) were expressed in *Bombyx mori* (silkworm)-derived BM-N cells (Fig. 1). The expression of these proteins was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). The lysate of BM-N cells infected with BmNPV (BmNPV lysate) contained a 31 kDa polyhedrin (lane 1). Bm120-*erbB* lysate contained 80–90 kDa proteins representing $p82^{PH120\text{-}erbB}$, which were absent in the BmNPV lysate (lane 2). Similarly, the lysates of the cells infected with other recombinant viruses carrying the polyhedrin-*erbB* fusion genes contained proteins representing their products. However, no obvious band of a protein representing the $p68^{erbB}$ non-fusion protein was detected in Bm*erbB* lysate (lane 6). These results indicate that the expression levels of polyhedrin-*erbB* proteins, even the one in which the polyhedrin portion included only 8 amino-terminal amino acid residues, were much higher than that of non-fusion v-*erbB* protein.

Immunoblot analysis of the polyhedrin-*erbB* fusion proteins To identify the polyhedrin-*erbB* fusion proteins, the cell lysates infected with recombinant viruses were analyzed by SDS-PAGE and immunoblotting (Fig. 3). The 31 kDa polyhedrin and $p77^{PH240\text{-}erbB}$ proteins were detected by the anti-polyhedrin monoclonal antibody, but no immunoreactive material was observed in the Bm312-*erbB* lysate (Fig. 3, B). Because the anti-polyhedrin antibody recognizes the middle part of polyhedrin, $p70^{PH312\text{-}erbB}$ that contains only 8 amino-terminal amino acids of polyhedrin could not be recognized by the antibody.

Glycosylation of the v-*erbB* protein The recombinant v-*erbB* proteins showed broad bands on the polyacrylamide gel (Fig. 2), indicating heterogeneous molecular weights of the v-*erbB* proteins that were expressed in BM-N cells. It has been reported that the v-*erbB* protein expressed in cells transformed by the avian erythroblastosis virus is glycosylated,[32] and it is possible that the baculoviral v-*erbB* proteins also undergo glycosylation. Therefore, $p77^{PH240\text{-}erbB}$ was metabolically radiolabeled with [^{35}S]methionine in the presence of tunicamycin, a specific inhibitor of protein glycosylation, and analyzed by SDS-PAGE and autoradiography (Fig. 4). The expression level of higher-molecular-weight PH240-*erbB* proteins (78–95 kDa) decreased with increase in the concentration of tunicamycin, but that of 77kDa PH240-*erbB* protein was not influenced by tunicamycin (Table I). In addition, the molecular weight of polyhedrin was not influenced by tunicamycin (data not shown). These results indicate that the v-*erbB* protein region of the PH240-*erbB* protein underwent glycosylation.

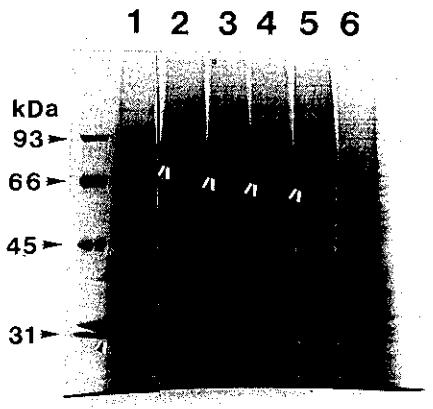

Fig. 2. Expression of v-*erbB* and polyhedrin-*erbB* proteins in BM-N cells. The lysates of BM-N cells infected with BmNPV or recombinant viruses were analyzed by SDS-PAGE (10%) under reducing conditions. Proteins were stained with Coomassie Brilliant Blue R-250. Polyhedrin (lane 1) and predicted v-*erbB* proteins (lanes 2–5) are indicated by arrows. Lane 1, BmNPV lysate; lane 2, Bm120-*erbB* lysate; lane 3, Bm240-*erbB* lysate; lane 4, Bm596-*erbB* lysate; lane 5, Bm312-*erbB* lysate; lane 6, Bm*erbB* lysate. The positions of molecular weight markers are shown on the left ($Da \times 10^{-3}$).

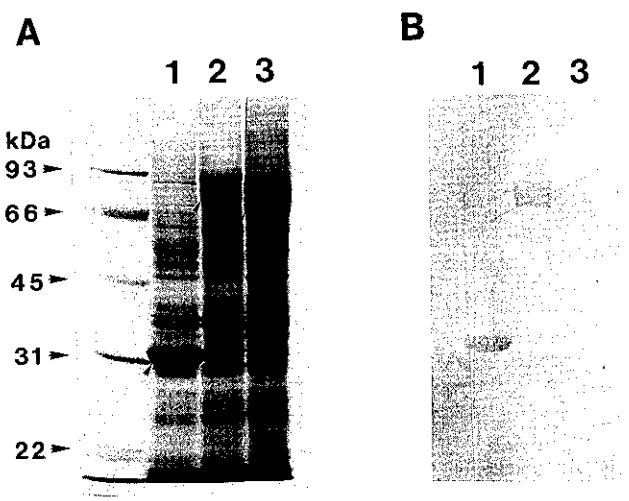

Fig. 3. Immunoblot analysis of polyhedrin-*erbB* fusion proteins. The lysates of BM-N cells infected with BmNPV (lane 1), Bm240-*erbB* (lane 2), or Bm312-*erbB* (lane 3) were analyzed by SDS-PAGE (10%) under reducing conditions (A) and immunoblotting (B). (A) Proteins were stained with Coomassie Brilliant Blue R-250. Polyhedrin (lane 1), $p77^{PH240\text{-}erbB}$ (lane 2), and $p70^{PH312\text{-}erbB}$ (lane 3) are indicated by arrows. The positions of molecular weight markers are shown on the left ($Da \times 10^{-3}$). (B) Proteins were transferred to a nitrocellulose membrane from the gel using a semi-dry blotting system. Mouse anti-polyhedrin monoclonal antibody and peroxidase-conjugated goat anti-mouse IgG antibody were used in the procedure.

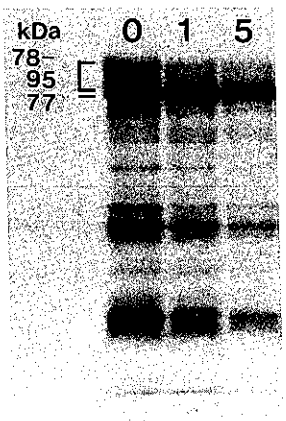

Fig. 4. Effect of tunicamycin on the synthesis of the v-*erbB* protein. BM-N cells infected with Bm240-*erbB* at 36 h post-infection were radiolabeled with [^{35}S]methionine (50 μCi/ml, 1151 Ci/mmol) for 48 h. Tunicamycin (final 1 or 5 μg/ml) was added to the medium for 8 h before labeling with [^{35}S]-methionine. The cells were harvested, washed twice with PBS, and analyzed by SDS-PAGE and autoradiography. The lane numbers indicate concentrations of tunicamycin (μg/ml).

Table I. Effect of Tunicamycin on the Synthesis of the v-*erbB* Protein

Tunicamycin (μg/ml)	[^{35}S]Methionine incorporation[a] ($cpm \times 10^{-3}$)		Ratio[b] b/a
	a 77 kDa protein	b 78–95 kDa proteins	
0	10	61	6.1
1	11	44	4.0
5	11	39	3.5

a) BM-N cells infected with Bm240-*erbB* were radiolabeled with [^{35}S]methionine in the presence or absence of tunicamycin, and analyzed by SDS-PAGE as described in Fig. 4. The radiolabeled 77 kDa protein and 78–95 kDa proteins were excised from the gel shown in Fig. 4 and their radioactivity was measured.
b) Ratio of incorporated counts for column b (78–95 kDa proteins) relative to column a (77 kDa protein).

Phosphorylation of the v-*erbB* protein The lysates of BM-N cells infected with Bm*erbB*, Bm312-*erbB*, Bm596-*erbB*, Bm240-*erbB*, and BmNPV were incubated with [γ-^{32}P]ATP, and the reaction mixtures were analyzed by SDS-PAGE and autoradiography. Proteins representing the v-*erbB* proteins were radiolabeled, but no radiolabeled material was observed in the BmNPV lysate (Fig. 5). Because the control lysate did not include protein kinase activity, the recombinant v-*erbB* proteins are

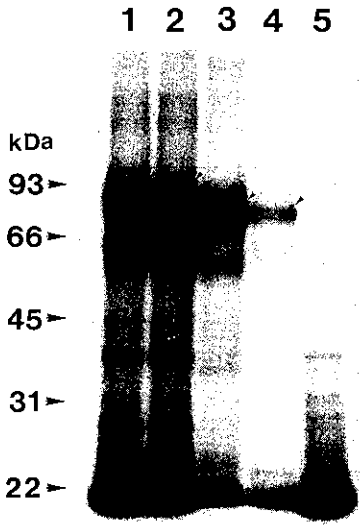

Fig. 5. Autoradiogram showing the proteins phosphorylated in the lysates of BM-N cells infected with BmNPV or recombinant viruses. Lysates (185 μg) were incubated with 50 μM [γ-^{32}P]ATP at 30°C for 30 s, and analyzed by SDS-PAGE and autoradiography as described in "Materials and Methods." Predicted v-*erbB* proteins (lanes 1–4) are indicated by arrows. Lane 1, Bm240-*erbB* lysate; lane 2, Bm596-*erbB* lysate; lane 3, Bm312-*erbB* lysate; lane 4, Bm*erbB* lysate; lane 5, BmNPV lysate. The positions of molecular weight markers are shown on the left (Da$\times 10^{-3}$).

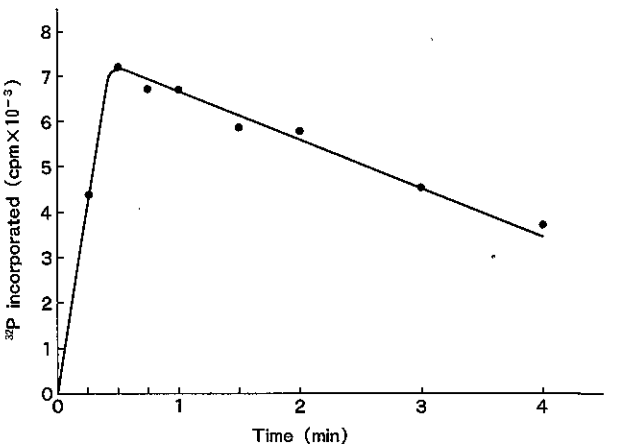

Fig. 6. Time course of autophosphorylation of p70$^{PH312\text{-}erbB}$. Bm312-*erbB* lysate was incubated with 50 μM [γ-^{32}P]ATP at 30°C, and analyzed by SDS-PAGE and autoradiography as described. Radiolabeled p70$^{PH312\text{-}erbB}$ was excised from the gels, and analyzed for radioactivity.

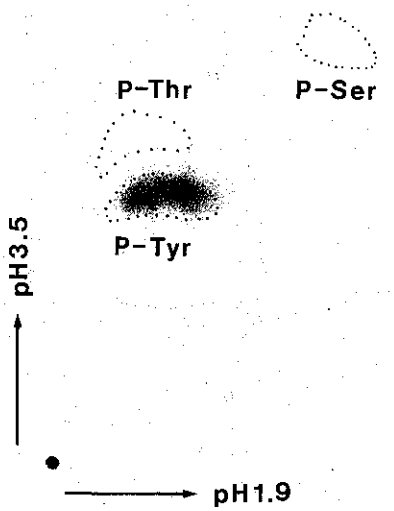

Fig. 7. Analysis of phosphorylated amino acids present in p70$^{PH312\text{-}erbB}$ eluted from the gel (shown in Fig. 5, lane 3) and hydrolyzed as described. The sample was analyzed by two-dimensional electrophoresis and autoradiography. P-Ser, phosphoserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine.

probably autophosphorylated. Since the Bm*erbB* lysate contained the phosphorylated protein representing the 68 kDa non-fusion v-*erbB* protein (Fig. 5, lane 4), the non-fusion protein was certainly expressed in the baculoviral system. However, the expression level of the non-fusion v-*erbB* protein was much lower than those of the fusion proteins.

The phosphorylation of p70$^{PH312\text{-}erbB}$ was linear for approximately 30 s (Fig. 6). After this time, the amount of phosphate incorporated into the protein began to decline.

To determine which amino acids of the v-*erbB* protein were phosphorylated, ^{32}P-labeled amino acids were analyzed from hydrolysates of the radiolabeled v-*erbB* protein. The region of the gel containing ^{32}P-labeled p70$^{PH312\text{-}erbB}$ was excised, and the protein was extracted and hydrolyzed. The hydrolysate was analyzed by two-dimensional electrophoresis and only phosphotyrosine was detected (Fig. 7). These results indicate that the v-*erbB* proteins autophosphorylated the tyrosine residue(s).

Phosphorylation of synthetic peptide by the v-*erbB* protein To confirm the tyrosine kinase activity of the recombinant v-*erbB* protein, phosphorylation of a synthetic R-R-SRC-peptide[33] that is related to the sequence of the tyrosine phosphorylation site in pp60^{src} was examined (Fig. 8). This peptide was phosphorylated by the Bm312-*erbB* lysate, but not by the BmNPV lysate. Because the peptide contains a tyrosine but neither serine nor threonine, it is clear that p70$^{PH312\text{-}erbB}$ phosphorylated

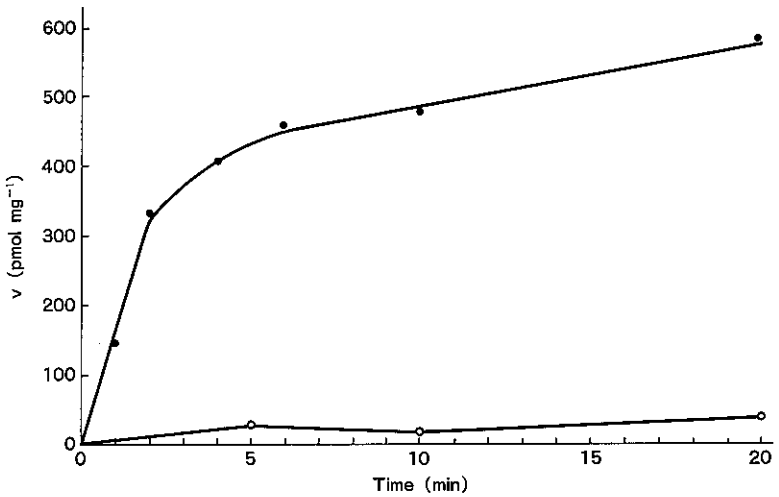

Fig. 8. Time course of R-R-SRC-peptide phosphorylation by $p70^{PH312\text{-}erbB}$. The lysate of BM-N cells infected with Bm312-*erbB* (●) or BmNPV (○) was incubated with 2 m*M* R-R-SRC-peptide in the presence of 50 μM [γ-^{32}P]ATP at 30°C as described.

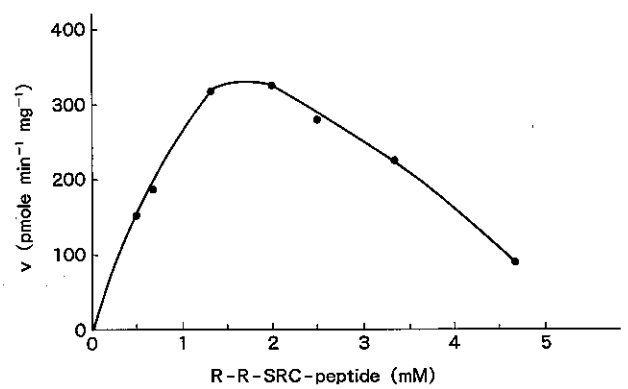

Fig. 9. Phosphorylation of R-R-SRC-peptide by $p70^{PH312\text{-}erbB}$ as a function of peptide concentration. The lysate of BM-N cells infected with Bm312-*erbB* was incubated with increasing concentrations of R-R-SRC-peptide in the presence of 50 μM [γ-^{32}P]ATP at 30°C for 2 min as described.

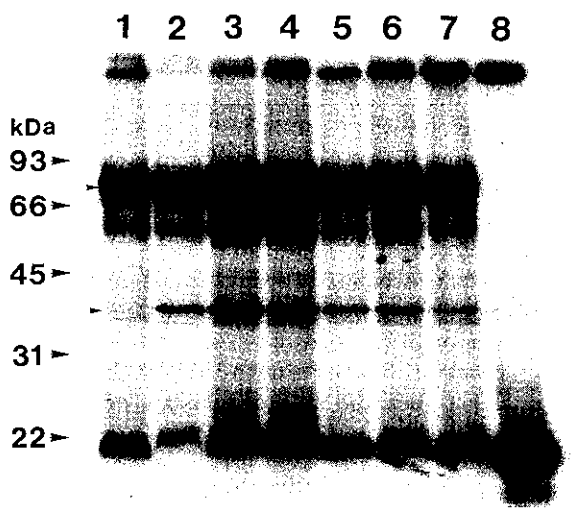

Fig. 10. Autoradiogram showing the time course of lipocortin I phosphorylation by the v-*erbB* protein. Two μg of partially purified lipocortin I was incubated with 20 μg of Bm312-*erbB* lysate (lanes 2–7) or BmNPV lysate (lane 8) in the presence of 50 μM [γ-^{32}P]ATP at 30°C as described. The reactions were stopped at 15 s (lane 2), 30 s (lane 3 and 8), 60 s (lane 4), 90 s (lane 5), 2 min (lane 6), and 4 min (lane 7). The reaction was also carried out in the absence of lipocortin I for 30 s (lane 1). The samples were analyzed by electrophoresis and autoradiography. The upper and lower arrows indicate $p70^{PH312\text{-}erbB}$ and lipocortin I, respectively. The positions of molecular weight markers are shown on the left ($Da \times 10^{-3}$).

a tyrosine residue. Phosphorylation of the peptide was linear for approximately 2 min, after which the phosphorylation rate decreased.

The phosphorylation of the R-R-SRC-peptide increased with increasing concentrations of peptide up to 1.3 m*M* (Fig. 9). Above 1.3 m*M* peptide, the enzyme appeared to become saturated, and higher concentrations of peptide (above 2 m*M*) seemed to be inhibitory. Half-maximal phosphorylation occurred at about 0.53 m*M*.

Phosphorylation of lipocortin I by the v-*erbB* protein

Partially purified recombinant lipocortin I was incubated with Bm312-*erbB* lysate or BmNPV lysate in the presence of [γ-^{32}P]ATP and the reaction mixture was analyzed by SDS-PAGE and autoradiography (Fig. 10).

Lipocortin I was a major radiolabeled protein after incubation with the Bm312-*erbB* lysate. However, lipocortin I incubated with the BmNPV lysate was not radio-

labeled. These results indicate that lipocortin I is a good substrate for the v-*erbB* protein.

The phosphorylation of lipocortin I reached a maximum by 30 s, after which the amount of incorporated phosphate began to decline. The time course for phosphorylation of lipocortin I was similar to that for the autophosphorylation of $p70^{PH312\text{-}erbB}$.

DISCUSSION

In the present study, the v-*erbB* protein was expressed alone or as polyhedrin-fusion proteins in silkworm-derived BM-N cells using the *Bombyx mori* nuclear polyhedrosis virus vector system. The expression levels of the polyhedrin-fusion v-*erbB* proteins were much higher than that of the non-fusion v-*erbB* protein, even for the fusion protein whose polyhedrin portion included only 8 amino-terminal amino acid residues. The reason for the enhanced expression levels of the fusion proteins is not yet known, but similar results have been obtained for the expression of the v-*sis* protein[19] and interferon-α (unpublished observations).

To isolate the v-*erbB* protein from the fusion proteins, a collagenase site was inserted into the junction region. We have reported that the v-*sis* protein is isolated from the polyhedrin-*sis* fusion protein by collagenolysis.[19] However, the v-*erbB* protein has not been separated from the fusion proteins yet.

The molecular weights of the recombinant polyhedrin-*erbB* fusion proteins were heterogeneous on SDS-PAGE gels. Tunicamycin reduced the expression of the higher-molecular-weight fusion protein (78–95 kDa PH240-*erbB* protein), but did not affect that of the lower-molecular-weight protein (77 kDa PH240-*erbB* protein). These results indicate that the PH240-*erbB* protein was heterogeneously glycosylated. Because polyhedrin is not a glycosylated protein, only the v-*erbB* protein portion of the fusion product would be glycosylated. The v-*erbB* proteins expressed in chicken erythroblasts and fibroblasts are glycosylated homogeneously.[32] Therefore, glycosylation of the v-*erbB* protein in silkworm cells may be different from that in mammalian cells. This conclusion is supported by the observation that the EGF receptor expressed in SF9 insect cells using the *Autographa californica* baculovirus system[15] is glycosylated, but lacks the complex-type oligosaccharides present on EGF receptors expressed in A431 cells.

The recombinant v-*erbB* proteins possess tyrosine kinase activity, because these proteins autophosphorylated only tyrosine residue, and phosphorylated the R-R-SRC-peptide. At peptide concentrations above 2 m*M*, substrate inhibition was observed. This suggests that peptide binding to the free enzyme results in steric hindrance that precludes ATP from access to the active site. Similar substrate inhibition has been observed for phosphorylation of [Val^5]angiotensin II by the v-*src* protein.[33]

Lipocortin I is a physiological substrate of the EGF receptor.[34, 35] Recombinant lipocortin I was phosphorylated by the fusion v-*erbB* protein more predominantly and rapidly than other cellular proteins. Therefore, lipocortin I could be a physiological substrate of the v-*erbB* protein as well as of the EGF receptor.

Time course studies of autophosphorylation of recombinant v-*erbB* proteins and of phosphorylation of lipocortin I showed that the amount of phosphate incorporated into these proteins began to decline after reaching the maximum. These results indicate that dephosphorylating activity is present in the lysates.

Expression of adequate quantities of functional v-*erbB* protein for biochemical and physico-chemical analysis was achieved. Provided that elimination of the polyhedrin portion from the polyhedrin-*erbB* fusion proteins and purification of the separated v-*erbB* protein can be achieved successfully, large-scale production of v-*erbB* protein appears to be feasible.

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