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Biological activities of human interferon and 2′–5′ oligoadenylates in plants

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Abstract

Exogenous human interferon 2 (IFN) and 2′–5′ oligoadenylates (2–5A) have been shown to cause at least a dual physiological effect in tobacco and wheat: (i) increased cytokinin activity and (ii) induced synthesis of numerous proteins, among which members of two groups of stress proteins have been identified, namely pathogenesis-related (PR) and heat shock (HS) proteins. These effects were observed only by low concentrations of these substances: IFN at 0.1–1 u/ml and 2–5A at 1–10 nM.

Introduction

Interferons are proteins encoded in the genome of vertebrates and induced by viruses, double-stranded RNAs, and some other agents [36]. In the vertebrate tissues, the biological activity of IFN manifests itself as antiviral and antiproliferative action (suppression of cell growth, modification of cell differentiation, etc.) [5]. These effects result from the interaction of IFN with special receptors on the surface of the target cells. One of the mechanisms of IFN action is based on the induction of 2′–5′ adenylate synthetase, which in the presence of double-stranded RNA catalyses ATP oligomerization to 2′–5′-oligoadenylates (2–5A) possessing an antiviral activity of their own [36]. A distinguishing feature of IFN is its species specificity: a given IFN is only active in the cells of the same species as the one from which it has been isolated [34]. Quite unexpected in this context has been the report [32] that human IFN2 suppresses the reproduction of tobacco mosaic virus (TMV) in tobacco plants. Subsequently it has been shown that human IFNs can also inhibit the accumulation of some other plant viruses [16, 30, 41]. As to 2–5A, they were also inhibitory to TMV reproduction in plants [7]. However, several other groups have failed to detect any antiviral activity of human IFN in plants [3, 13, 24].

Cytokinins have been reported to be capable of inducing antiviral resistance and synthesis of stress proteins in plants, in particular the ones called pathogenesis-related (PR) proteins [1, 15, 38, 39]. The latter are elicited by infection with viruses, viroids, bacteria and fungi, by certain

chemicals (e.g. polyacrylic or salicylic acid), and under some other stress conditions [39]. Thus more than ten PR proteins can be induced in tobacco cultivars reacting hypersensitively to TMV. PR proteins P and Q have chitinase activity whereas PR-2, -N, and -O are 1, 3-glucanases; these hydrolytic enzymes may be involved in the plant defence reactions and in the breakdown of bacterial and fungal cell walls or insect exoskeletons [17, 22]. Induction of PR proteins is often accompanied by the evolvement of 'acquired antiviral resistance' in the plants [39].

Induction of heat shock (HS) proteins with a concomitant reduction in the synthesis of normal cell proteins upon a heat shock is a common stress reaction of various organisms (for review, see [27, 28, 35]). Production of HS proteins (HSPs) has also been observed in some other stress situations [28]. The most clearly observable HSPs form the following families differing in molecular mass: HSP 90, 70, 60, 20 and 8.5 K [27]. One of the probable functions of HSPs is protecting the cells from heat shock [18, 27, 28, 35].

Here we demonstrate that IFN and 2–5A evoke multiple physiological reactions in plant cells, accompanied by enhanced cytokinin activity and *de novo* synthesis of a set of proteins including PR proteins and HSPs. Some aspects of this work have been reported as preliminary communications [14, 37].

Materials and methods

Reagents

Interferon was a preparation of recombinant human IFN 2 produced by *Escherichia coli* cells (Minmedmikrobioprom, USSR).

The mixture of 2′–5′ oligoadenylates (general formula A $(pA)_n$ where n is 1 through 5) was obtained by treating the product of adenosine 2′, 3′ cyclic phosphate polymerization in the presence of diphenyl chlorophosphate with enzymes that hydrolyze the 3′–5′ internucleotide bonds in RNA to leave the phosphate on the 3′-terminal residue [26].

Plant material

The experiments were conducted with *Nicotiana tabacum* L. cv. Samsun NN and Samsun. Plants were grown in soil under glasshouse conditions. Leaves were taken from 10-week-old plants. The effects of IFN and 2–5A on protein synthesis in plant tissues were studied on wheat cv. Saratovskaya 29. The plants were grown in a climatic chamber (temperature 23 °C, relative humidity 70%, 16 h day, illumination 5000 lux). First leaves of 14-day-old plants were used for experiments.

Cytokinin assay

To study the influence of IFN and 2–5A on cytokinin content, tobacco leaves were placed in water (control) or IFN and 2–5A solutions and incubated for 72 h at 25 °C. Leaves were homogenized in liquid nitrogen. Cytokinins were extracted with 80% ethanol containing 200 g/ml diethyl dithiocarbamate, and purified chromatographically on Dowex 50W × 8 (4 M NH_4OH was used for elution). Dried eluates were dissolved in ethanol, and applied to Silufol UV-254 plates for chromatographic separation of zeatin and zeatin riboside. Cytokinins were quantitated with the competitive version of the enzyme-linked immunosorbent assay (ELISA) as previously described [19].

Antibodies were raised against a conjugate of zeatin riboside with bovine serum albumin. These antibodies showed high affinity to *trans*-zeatin ribozide and zeatin, and low affinity to dihydrozeatin and isopentenyladenine.

In vivo *protein labeling and extraction*

To study the effects of IFN and 2–5A on protein synthesis, tobacco leaf discs (1 cm in diameter) or wheat leaf cuttings (2 cm) were incubated in distilled water (control) or IFN and 2–5A solutions for 18 h at 25 °C (tobacco) or 23 °C (wheat). Then leaf samples were incubated for 2.5 h at

25 °C (tobacco), at 23 °C (wheat) or 40 °C (tobacco and wheat) with IFN or 2–5A. For the last 2 h the incubation was carried out in the presence of [^{35}S]methionine (50 μCi/ml, sp. act. 18 MBq/mol). Then the samples were washed with a cold solution of unlabelled methionine, frozen in liquid nitrogen, and kept at −70 °C until protein isolation.

For protein isolation, the plant tissue (1 g) was ground in liquid nitrogen, transferred in the cold into 2 ml of 50 mM Tris-HCl pH 8.8 containing 2% SDS, 2% 2-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride, then mixed with 5 ml acetone and kept overnight at −20 °C. The mixture was centrifuged and the pellet was washed with cold 96% ethanol, ethanol/ether (3:1), ethyl ether, and dried overnight at −20 °C. The residue was then dissolved in the isolation buffer with heating to 100 °C for 3 min, reprecipitated with acetone at −20 °C, and the protein pellet after centrifugation was redissolved in the same buffer containing 10% glycerol. The samples were assayed for [^{35}S]-methionine incorporation and protein content according to Esen [11].

Two-dimensional gel analysis

This was performed as described by O'Farrell [29]. Samples for analysis contained 50 to 100 μg protein; isoelectric focusing was carried out for 5200 V.h in rod gels with a pH gradient of 4.5 to 8.2. In the second dimension, proteins were resolved in gradient (7.5–15%) polyacrylamide with SDS. The gels were fluorographed according to Laskey and Mills [21].

Extraction of PR proteins

To obtain a standard PR protein preparation, leaves of *N. tabacum* cv. Samsun NN were incubated with TMV (5 μg/ml), 0.1 u/ml IFN or 0.01 μM 2–5A and the plants were kept at 25 °C for 5 to 7 days. Then 1 g of tobacco leaf tissue was ground in 84 mM sodium citrate pH 2.8 containing 14 mM 2-mercaptoethanol and 6 mM sodium ascorbate. The homogenate was centrifuged at 8000 × *g* for 15 min, and the supernatant was used as the crude protein solution. PR proteins were salted out with ammonium sulphate (25% satu-

Table 1. Influence of IFN and 2–5a on cytokinin content in tobacco leaves

Agent	Concn.	Cytokinin content[1] (pmol/g fresh wt.)		
		Exp. I	Exp. II	
		zeatin + zeatin riboside	zeatin	zeatin riboside
H_2O		8.4 ± 0.15	9.5 ± 0.1	0.06
IFN	100 u/ml	6.5 ± 0.03	–	–
	10 u/ml	10.8 ± 0.05	–	–
	1 u/ml	29.6 ± 0.17	39.2 ± 0.03	0.6
	0.1 u/ml	24.5 ± 0.12	18.6 ± 0.10	0.5
	0.01 u/ml	–	8.6 ± 0.02	0.66
+ antibodies[2]	1 u/ml	9.5 ± 0.05	–	–
2–5A	100 nM	–	28.2 ± 0.35	2.5 ± 0.05
	10 nM	34.1 ± 0.20	32.1 ± 0.10	3.0 ± 0.01
	1 nM	18.6 ± 0.03	20.3 ± 0.18	6.6 ± 0.02

[1] Cytokinins were assayed by a competitive version of ELISA [19] using serial dilutions of cytokinins as concentration standards. The data are averaged for three parallel experiments; 5–6 leaves from different plants were used in each experiment.
[2] Antibodies to IFN were incubated with the IFN preparation (250 ng per unit of IFN) for 15 min at 37 °C before treating the tobacco leaves.

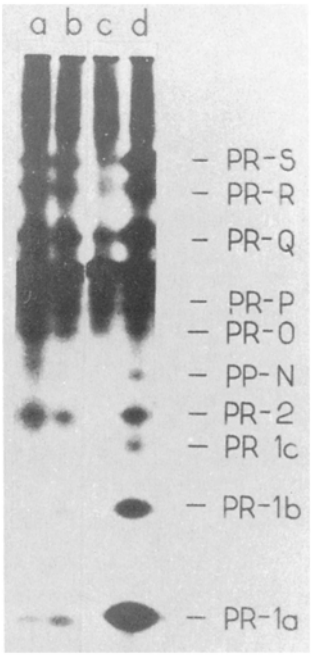


Fig. 1. Synthesis of PR proteins in Samsun NN tobacco leaves. Non-denaturing PAGE of proteins soluble at pH 2.8. The leaves were incubated with: (a) 0.1 u/ml IFN; (b) 10 nM 2–5A, (c) buffer, or (d) TMV to obtain a standard PR protein preparation. The positions are shown for PR proteins according to the nomenclature of van Loon *et al.* [40].

ration); the precipitate was dissolved in 5 mM Tris-HCl pH 8.3 and dialysed against the same buffer.

Determination of PR-1A by ELISA

The serum raised against purified PR-1a was kindly provided by Dr Y. Ohashi. The indirect ELISA using immunoglobulin $F(ab)_2$ fragments was carried out as described by Barbara and Clark [4] and Antoniw *et al.* [2]. Leaf samples

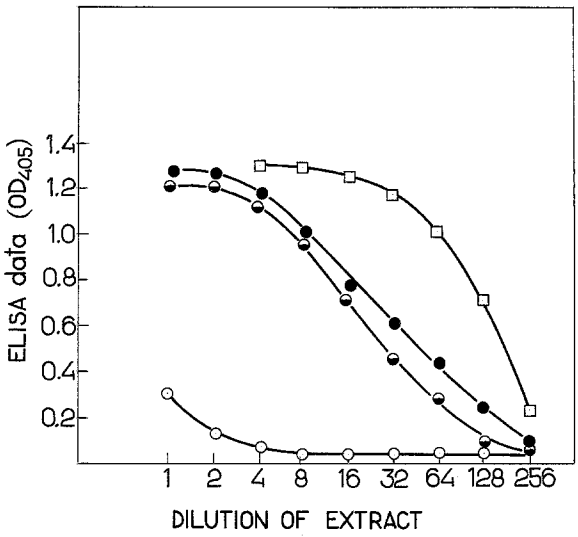


Fig. 2. ELISA, using PR-1a-specific antiserum, of 2-fold serial dilutions of extracts from untreated (open circles) and treated with IFN (half open circles) and 2–5A (closed circles) plants. A standard preparation of PR proteins was also included (squares).

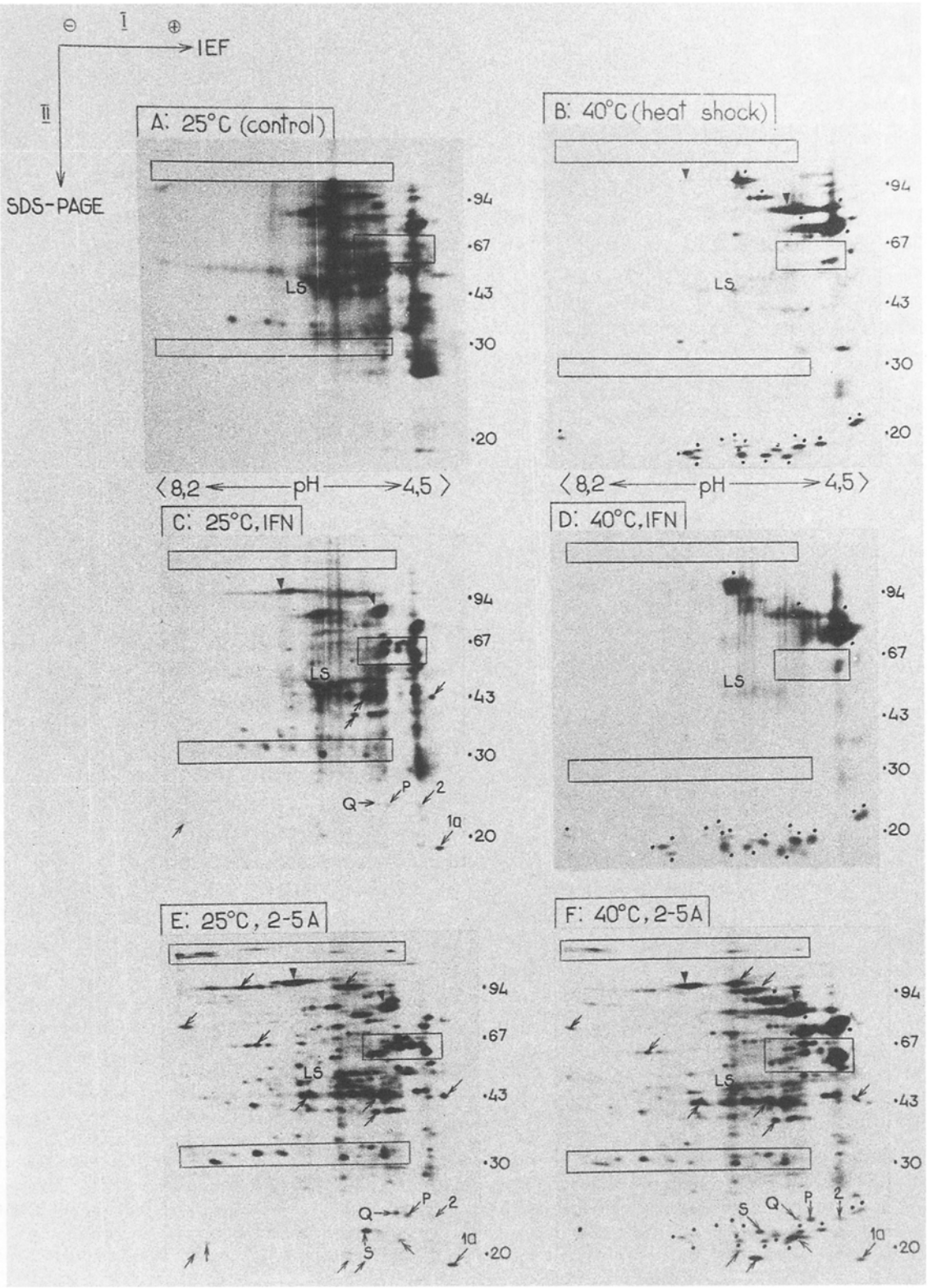
(1 g) were extracted with 4 ml of phosphate-buffered saline containing 0.5 ml/l Tween 20, 20 g/l polyvinylpyrrolidone (mol. wt. 44 000), and 2g/l ovalbumin. Each sample was assayed in duplicate as eight consecutive two-fold dilutions in the same buffer. A standard preparation of PR proteins was included in each microtitre plate to determine the relative content of PR-1a in the samples.

Results

Cytokinins in tobacco leaves treated with IFN and 2–5A

Table 1 shows that low IFN concentrations (0.1 to 1.0 u/ml) considerably increase the cytokinin

Fig. 3. Induction of proteins by IFN and 2–5A in Samsun NN tobacco. Two-dimensional IEF/ SDS-PAGE analysis of proteins synthesized in tobacco leaf discs at normal (25 °C: A, C, E) and heat-shock (40 °C: B, D, F) temperature. Total protein preparations were extracted from tissues not treated (A, B) and treated with (C, D) 0.1 u/ml IFN or (E, F) 10 nM 2–5A. The HSPs are marked by dots. The spots corresponding to HSPs induced not only by heat shock but also by IFN or 2–5A at normal temperature are marked with arrowheads. Arrows show proteins induced by each substance (but not by heat shock). Boxed areas indicate the positions of obvious alterations in the protein pattern; LS denotes the large subunit of ribulose bisphosphate carboxylase. Molecular weights (in thousands) of the protein standards are shown on the right. The positions of PR proteins are tentatively indicated with arrows and symbols 1a, 1b, 1c, 2, P, Q and S in accordance with the nomenclature of van Loon *et al.* [40].


⊖ I ⊕
IEF
II
SDS-PAGE
A: 25°C (control)
B: 40°C (heat shock)
C: 25°C, IFN
D: 40°C, IFN
E: 25°C, 2-5A
F: 40°C, 2-5A
LS
·94
·67
·43
·30
·20
〈8,2 ← pH → 4,5〉
Q
P
2
1a
S

content in tobacco leaves. On the other hand, no changes in cytokinin content can be observed at higher (10 u/ml) and lower (0.01 u/ml) IFN concentrations. Appropriate controls demonstrate that IFN activity is completely neutralized with antibodies to IFN (Table 1, Exp. I).

Endogenous cytokinins in tobacco leaves are mainly represented by the functionally active free cytokinin zeatin; its inactive conjugate – zeatin riboside, which is considered a transport form of cytokinins – constitutes less than 1% of the total in control leaves (Table 1). Upon exposure of leaves to IFN the content of zeatin increases two- to fourfold (Table 1, Exp. II). The content of zeatin riboside also increases but its portion of the total cytokinins remains very low.

It can also be seen in Table 1 that the total preparation of 2–5A enhances the cytokinin content in tobacco leaves as well as does IFN. Its effect is clearly observed in a broad range of low concentrations (1 nM to 0.1 μM). It should be noted that dimeric and trimeric 2–5A are inactive in cytokinin induction in tobacco (data not shown); hence the above effect must be caused by 2–5A molecules longer than di- or trimers.

Effect of human IFN and 2–5A on protein synthesis in tobacco leaves

The spectra of proteins revealed by native PAGE in Samsun NN plants treated with IFN and 2–5A are shown in Fig. 1a,b. For comparison, similar analysis has been performed on healthy plants treated with buffer (Fig. 1c) and on TMV-infected plants (Fig. 1d).

As can be seen, synthesis of PR1a and PR_2 proteins is induced in tobacco leaves both by TMV (Fig. 1d), IFN (Fig. 1a) or 2–5A (Fig. 1b). In conformity with this, $F(ab)_2$ ELISA demonstrates a dramatic increase in the amount of antigen reacting with PR-1a-specific antibodies: 40- to 80- fold with 0.1 u/ml IFN and 80- to 100-fold with 0.01 μM 2–5A (Fig. 2).

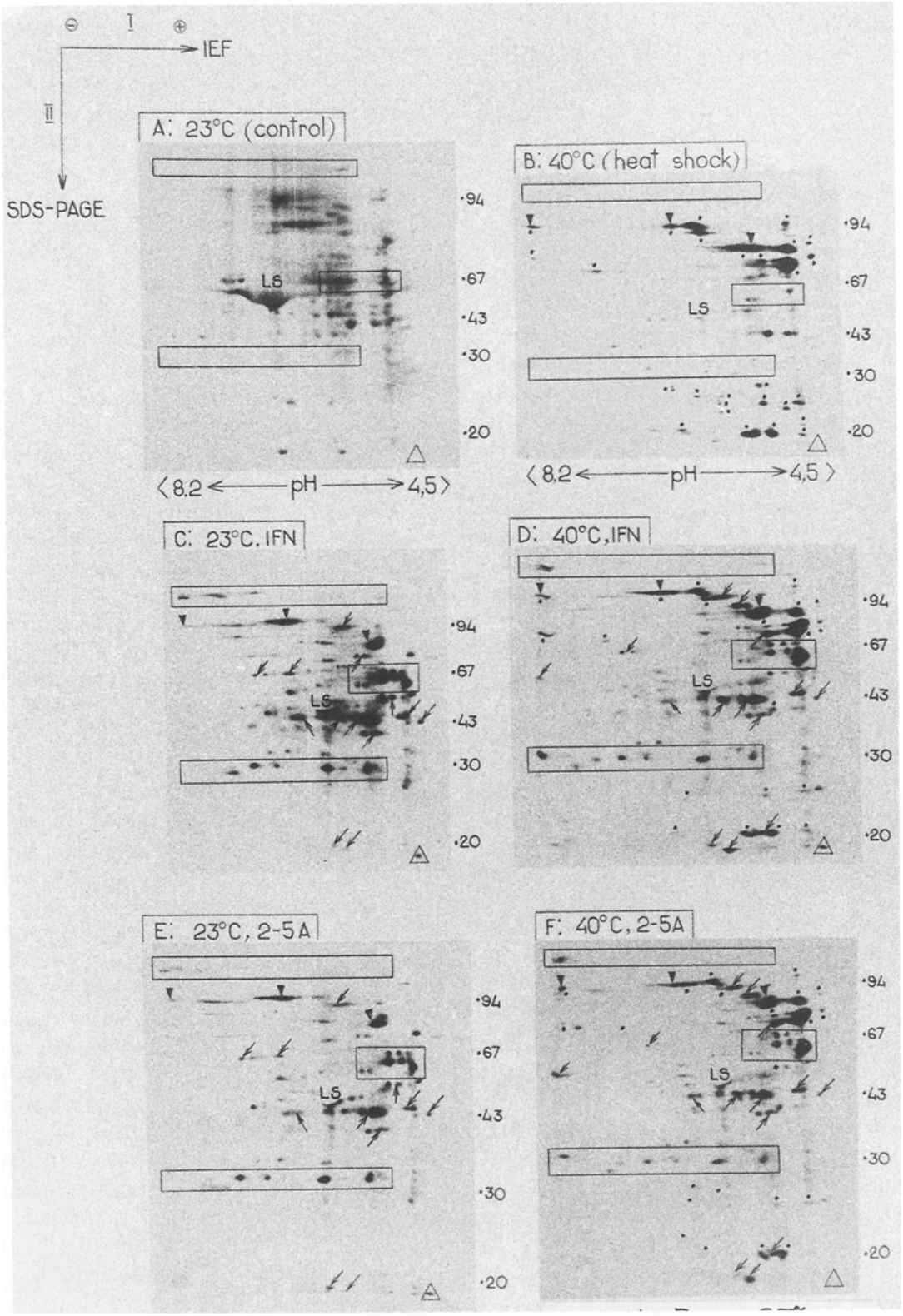
A more detailed analysis of the proteins induced in tobacco leaves by exogenous IFN and 2–5A was carried out by two-dimensional resolution (IEF/SDS-PAGE, Fig. 3).

Exposure of tobacco leaf discs to a low concentration of IFN (0.1 u/ml) for 24 h at normal temperature (25 °C) considerably alters the protein pattern (Fig. 3C) as compared with the control (Fig. 3A), inducing or stimulating the synthesis of several proteins (some of them boxed in Fig. 3).

Among the polypeptides induced by IFN at normal temperature, two species marked by arrows in Fig. 3C (98 K and 87 K) can be expected to be HSPs, by comparison with those induced by heat shock itself (Fig. 3B). Some of the IFN-induced proteins can be considered as PR proteins (PR-1a, PR-2, PR-Q, PR-P) according to their position on two-dimensional gel electrophoresis [40]. Besides, a number of additional IFN-induced proteins are revealed by two-dimensional resolution (Fig. 3C).

1. A number of new proteins of about 30 kDa were demonstrated in IFN-treated leaves (boxed in Fig. 3) and were not discovered in control leaves.
2. Changes of the polypeptide pattern of the 63 K protein family were detected (boxed in Fig. 3).
3. Increase of the synthesis of the polypeptide 43 K group mapping close to the large subunit of ribulose bisphophate carboxylase (LS) were

Fig. 4. Induction of proteins by IFN and 2–5A in wheat. Two-dimensional IEF/SDS-PAGE analysis of proteins synthesized at normal (23 °C: A, C, E) and heat-shock (40 °C: B, D, F) temperature. Total protein preparations were extracted from wheat not treated (A, B) and treated with (C, D) 0.1 u/ml IFN or (E, F) 10 nM 2–5A. The HSPs are marked by dots. Arrowheads mark the spots corresponding to HSPs induced not only by heat shock but also by IFN or 2–5A at normal temperature. Arrows show proteins induced by each substance (but not by heat shock). Proteins induced only by IFN are marked with white arrows, those induced by 2–5A are marked by white triangles. Boxed areas indicate the positions of obvious alterations in the protein pattern. LS denotes the large subunit of ribulose bisphosphate carboxylase. The triangle marks the putative PR-1a-like protein. Molecular weights (in thousands) of protein standards are indicated on the right.


⊖ I ⊕
IEF
II
SDS-PAGE
A: 23°C (control)
B: 40°C (heat shock)
C: 23°C, IFN
D: 40°C, IFN
E: 23°C, 2-5A
F: 40°C, 2-5A
LS
·94
·67
·43
·30
·20
〈8,2 ← pH → 4,5〉

observed and new polypeptides in this area can be recognized.

Figure 3E depicts the results of the two-dimensional analysis of the proteins synthesized in tobacco leaves in the presence of 0.01 μM 2–5A. 2–5A induced changes in the protein pattern are even more pronounced than with IFN (cf. Figs 3A, 3C and 3E). In this case all interferon-induced changes in the pattern of protein synthesis mentioned above are observed. Besides 2–5A-induced synthesis of the group of high-molecular-mass proteins (ca. 136 kDa) is revealed. Their synthesis is discovered by one-dimensional electrophoresis under IFN treatment as well [14].

The heat shock induced the synthesis of different HSP groups in tobacco leaves (Fig. 3B) and at the same time markedly inhibited the synthesis of proteins produced at 25 °C.

The leaves treated with 2–5A and exposed to heat shock synthesized the HSP as well as proteins induced by 2–5A at 25 °C (Fig. 3F). Besides this, many proteins typical of control leaves at normal temperature continued to be produced. One can suggest that the thermal resistance of protein synthesis is enhanced in the presence of 2–5A.

The effect of IFN on protein synthesis in tobacco (but not wheat, see below) leaf tissues upon transition to high temperature (40 °C) is weaker than that of 2–5A, and in a number of experiments the corresponding protein patterns did not differ from the typical HSP ones (Fig. 3D and 3B).

Effect of IFN and 2–5A on protein synthesis in wheat leaves

In the preliminary experiments IFN effects on protein synthesis in wheat leaves were studied within the wide range of concentrations of this substance: 0.01, 0.1, 1.0 and 10 u/ml. No effect was detected at 0.01 u/ml. 10 u/ml of IFN inhibited protein synthesis in leaf cells. 0.1 and 1 u/ml had a remarkable effect on the pattern of proteins synthesized in wheat leaves. The 2–5A effect on protein synthesis was studied in the range of concentrations from 0.1 nM to 0.1 μM. A concentration of 0.1 uM of 2–5A had no effect on this process, 1–10 nM enhanced protein synthesis and altered the pattern of labelled polypeptides, and 0.1 μM gave an inhibitory action.

The results of two-dimensional IEF/SDS-PAGE analysis of proteins synthesized under IFN and 2–5A treatment in wheat leaves are presented in Fig. 4. As with tobacco, in wheat IFN at low concentrations (0.1 u/ml) and 2–5A (10 nM) induced or increased the synthesis of numerous new proteins at normal temperature (Fig. 4C, E) and simultaneously decreased the synthesis of a number of polypeptides. The sets of proteins synthesized under the influence of IFN and 2–5A are very similar. Among the IFN- (or 2–5A)-induced proteins one can also find HSP-like proteins (98 K, 87 K) and a protein similar to the tobacco PR-1a (Fig. 4C, E). Recently immunoelectroblotting with an affinity-purified antiserum to the tobacco PR-1a has been used to demonstrate that such a protein is induced in wheat upon infection with brome mosaic virus [42]. We did not undertake any further identification of the wheat PR proteins.

In wheat as in tobacco, IFN and 2–5A induce a group of 30 K polypeptides (boxed in Fig. 4), high-molecular-weight proteins (ca. 136 K boxed in Fig. 4), new proteins of 43 K family located in the LS region, and polypeptides of 63 K family (boxed in Fig. 4). The synthesis of a number of other proteins is inhibited (see the groups of polypeptides with a M_r lower than 94 K and LS in Fig. 4C, E).

Upon transfer of IFN- or 2–5A-treated wheat leaves to heat-shock conditions, they synthesize not only HSP but also practically all other proteins elicited by these compounds as well as many proteins typical of control leaves at normal temperature (Fig. 4D, F). Thus IFN and 2–5A apparently enhance the thermal resistance of protein synthesis in wheat plants. The protein patterns obtained upon treatment of wheat with IFN and 2–5A (Fig. 4C, E) prove to be similar enough albeit there are minor differences. However, in contrast to tobacco, the effect of IFN on wheat is somewhat more pronounced than that of 2–5A both at 23 °C and at 40 °C.

Discussion

In the present work it is shown that human interferon and one of the mediators of its action in vertebrates 2–5A cause significant changes in the set of synthesized proteins in the leaves of tobacco and wheat. They induce or enhance synthesis of a large number of polypeptides (more than 100). Besides, appearance of several isoforms of proteins close in their molecular weight (for example, the 43 K family) makes probable influence of IFN and 2–5A on the post-translational modification of proteins as well.

At least part of proteins induced by IFN and 2–5A in plant tissues can be classified as stress proteins: HSPs (in tobacco and wheat cells) and PR proteins (in tobacco and, possibly, wheat).

It is known that genes of PR proteins are not activated by heat shock [25]; as far as we know HSP cannot be induced in plants by pathogens and other inductors of PR proteins [27, 28]. Consequently, responses to pathogens and heat shock are two different stress reactions of plants. Moreover heat shock blocks synthesis of PR proteins [27, 31, 33] in spite of high homology of RE_sHSP genes and PR genes. Here we describe the first example of simultaneous induction of PR and HS proteins. It is important to stress that in 2–5A treated tobacco leaves HS does not inhibit PR proteins synthesis (Fig. 3F). Obviously the last becomes thermostable. In the same way HS does not alter synthesis of a lot of other proteins induced in the leaves of tobacco and wheat by IFN and 2–5A, although it is known that HS inhibits synthesis of plant proteins typical of normal temperature conditions and it is well seen on the control leaves (Fig. 3A, B). Thus IFN and 2–5A enhance stress resistance (thermotolerance) of processes connected with protein synthesis in leaves.

It still remains unclear if there are common elements in the reaction to IFN and 2–5A of the cells of vertebrates and plant leaves. The mechanisms involved in the actualization of IFN activities in plants are totally obscure. The interpretation is further complicated by the data that similar effects are caused by 2–5A. Notwithstanding, one can hardly suggest the existence of a 2–5A system mediating IFN action in plants; it seems more probable that the similar effects produced by these two agents on plants are unrelated phenomena. Human IFN and 2–5A may mimic some substances causing or attending stress in plants, which results in formation of stress (PR and HS) proteins. It cannot be excluded that IFN and 2–5A act upon plant leaves as stressors causing hardening of plants to unfavourable conditions (HS, pathogens). At the same time their action is exerted in an extremely low concentration range (0.1–1 u/ml for IFN and 1–10 nM for 2–5A), which makes more probable their influence on plant regulatory systems. Enhancement of cytokinin content in tobacco leaves under the action of IFN and 2–5A confirms this assumption. Other stresses, for example, heat shock (our unpublished data) strictly lower cytokinin levels in plants. As IFN and 2–5A concentrations causing enhancement of cytokinin content in leaves and those changing the pattern of proteins synthesized coincide one can expect a mediative role for cytokinins in IFN and 2–5A action on plants. In this connection it seems interesting that cytokinin enhances plant virus-resistance [1, 15]. Along with this both high content of endogenous cytokinins in transgenic plants [6] and the action of high concentrations of exogenous cytokinins cause stress reaction in plants switching on PR protein synthesis [1, 15, 38].

The appearance of PR proteins in plant tissues is often accompanied by the evolvement of acquired antiviral resistance [39]. Though participation of PR–1a in the induction of antiviral resistance has not been directly demonstrable in transgenic tobacco plants expressing the PR–1a gene [6, 23], probably some other PR proteins or their combinations enhance plant resistance to viruses. The induction of PR and HS proteins by IFN and 2–5A allows one to speculate that IFN and 2–5A can protect the plants against different stresses and/or pathogens. In particular, IFN and 2–5A apparently stabilize protein synthesis in plants subjected to heat shock.

References

1. Antoniw JF, Kueh J, Walkey DGA, White RF: The presence of pathogenesis-related proteins in callus of Xanthi-nc tobacco. Phytopath Z 101: 179–184 (1981).
2. Antoniw JF, White RF, Barbara DJ, Jones P, Longley A: The detection of PR (b) protein and TMV by ELISA in systemic and localised virus infections of tobacco. Plant Mol Biol 4: 55–60 (1985).
3. Antoniw JF, White RF, Carr JP: An examination of the effect of human interferons on the infection and multiplication of tobacco mosaic virus in tobacco. Phytopath Z 109: 367–371 (1984).
4. Barbara DJ, Clark MF: A simple indirect ELISA using F (ab') 2 fragments of immunoglobulin. J Gen Virol 58: 315–322 (1982).
5. Clemens MJ, McNurlan MA: Regulation of cell proliferation and differentiation by interferons. Biochem J 226: 345–360 (1985).
6. Chen Y, Varner YE: Isolation and characterisation of cDNA clones for carrot extensin and proline-rich 33-KDa protein. Proc Natl Acad Sci USA. 82: 4399–4403 (1985).
7. Cornelissen BJC, Horowitz LY, van Kan YAL, Goldberg RB, Bol JF: Structure of tobacco genes encoding pathogenesis-related proteins from the PR-1 group. Nucl Acids Res 15: 6799–6811 (1987).
8. Cutt JR, Harpster MH, Dixon DC, Carr JP, Dunsmuir P, Klessig DF: Disease response to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related PR 1b gene. Virology 173: 89–97 (1989).
9. Devash Y, Gera A, Willis DH, Reichman M, Pfleiderer W, Charubala R, Sela I, Suhadolnik RJ: 5'-dephosphorylated 2', 5'-adenylate trimer and its analogs. Inhibition of tobacco mosaic virus replication in tobacco mosaic virus-infected leaf discs, protoplasts, and intact tobacco plants. J Biol Chem 259: 3482–3486 (1984).
10. Engelbrecht L, Mothes K: Weitere Untersuchungen zur experimentellen Beeinflüssung der Hitzewirkung bei Blättern von *Nicotiana rustica*. Flora 154: 279–298 (1964).
11. Esen A: A simple method for quantitative semiquantitative and qualitative assay of protein. Anal Biochem 89: 264–273 (1978).
12. Hooft van Huijsduijnen RAM, Alblas SW, De Rijk RH, Bol JF: Induction by salycylic acid of pathogenesis-related proteins and resistance to alfalfa mosaic virus infection in various plant species. J Gen Virol 67: 2135–2143 (1986).
13. Huisman MJ, Broxterman HJG, Schellekens H, van Vloten-Doting L: Human interferon does not protect cowpea plant cell protoplasts against infection with alfalfa mosaic virus. Virology 143: 622–625 (1985).
14. Kaplan IB, Malyshenko SI, Fedina AB, Taliansky ME, Karpeisky MYa, Ogarkov VI, Atabekov JG: Effect of human interferon and 2'–5'-oligoadenylates on protein synthesis in plants. (Russian.) Dokl Akad Nauk SSSR 297: 1018–1022 (1987).
15. Kaplan IB, Malyshenko SI, Shakulova ER, Dubinina EN, Taliansky ME: Induction of pathogenesis-related proteins and acquired antiviral resistance in tobacco plants under the influence of kinetin. (Russian.) Fiziol Rastenii 35: 849–856 (1988).
16. Kaplan IB, Taliansky ME, Malyshenko SI, Ogarkov VI, Atabekov JG: Effect of human interferon on reproduction of plant-and mycoviruses. Arch Phytopath PflSchutz 24: 3–8 (1988).
17. Kauffmann S, Legrand M, Geoffroy P, Fritig B: Biological function of 'pathogenesis-related' proteins: four PR proteins of tobacco have 1, 3-β-glucanase activity. EMBO J 6: 3209–3212 (1987).
18. Kimpel IA, Key IL: Heat shock in plants. Trends Biochem Sci 10: 353–357 (1985).
19. Kudoyarova GR, Veselov SYu, Karavaiko NN, Guli-Zade VZ, Cheredova EP, Mustafina AR, Mashkov IE, Kulaeva ON: Immunoenzyme test system for assaying cytokinins. (Russian.) Fiziol Rastenii 37: 193–199 (1990).
20. Kulaeva ON: Cytokinins: Their Structure and Function (Russian.) Nauka, Moscow (1973).
21. Laskey RA, Mills AD: Quantitative film detection of H and C in polyacrylamide gels by fluorography. Eur J Biochem 56: 335–342 (1975).
22. Legrand M, Kauffmann S, Geoffroy P, Fritig B: Biological function of 'pathogenesis-related' proteins: four tobacco PR-proteins are chitinases. Proc Natl Acad Sci USA 84: 6750–6754 (1987).
23. Linthorst HJM, Meuwissen RLJ, Kaufmann S, Bol JF: Constitutive expression of pathogenesis-related proteins PR-1, GRP and PR-S in tobacco has no effect on virus infection. Plant Cell 1: 285–291 (1989).
24. Loesch-Fries LS, Halk EL, Nelson SE, Krahn KJ: Human leucocycyte interferon does not inhibit alfalfa mosaic virus in protoplasts or tobacco tissue. Virology 143: 626–629 (1985).
25. Matsuoka M, Ohashi Y: Induction of pathogenesis-related proteins in tobacco leaves. Plant Physiol 80: 505–510 (1986).
26. Michelson AM: Polynucleotides. Part 1. Biosynthesis and properties of some polyribonucleotides. J Chem Soc 4: 1371–1394 (1959).
27. Neumann D, Nover L, Parthier B, Rieger R, Schard KD, Wollgiehm, Nieden U: Heat shock and other stress response systems of plants. Biol ZentBl 108: 1–155 (1989).
28. Nover L, Hellmund D, Neumann D, Scharf K-D, Serfling E: The heat shock response of eucariotic cells. Biol ZentBl 103 AB: 358–435 (1984).
29. O'Farrell PH: High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021 (1975).
30. Ogarkov VI, Kaplan IB, Taliansky ME, Atabekov JG: Reduction of potato viruses reproduction by human interferon. (Russian.) Dokl Akad Nauk SSSR 276: 743–745 (1984).

31. Ohshima M, Matsuoka M, Yamamoto N, Tanaka Y, Kano-Murakami Y, Ozeki Y, Kato A, Harada N, Ohashi Y: Nucleotide sequence of the PR-1 gene of *Nicotiana tabacum*. FEBS Lett 225: 243–246 (1987).
32. Orchansky P, Rubinstein M, Sela I: Human interferons protect plants from virus infection. Proc Natl Acad Sci USA 79: 2278–2280 (1982).
33. Pfitzner UM, Pfitzner AJP, Goodman HM: DNA sequence analysis of a PR-1a gene from tobacco: molecular relationship of heat shock and pathogen responses in plants. Mol Gen Genet 211: 290–295 (1988).
34. Richtsmeier WJ: Interferon – present and future prospects. CRC Crit Rev Clin Lab Sci 20: 57–93 (1984).
35. Schlesinger MY: Heat shock proteins. J Biol Chem 265: 12111–12114 (1990).
36. Staeheli P: Interferon-induced proteins and the antiviral state. Adv Virus Res 38: 147–200 (1990).
37. Taliansky ME, Malyshenko SI, Kaplan IB, Lozjnikova VN, Dudko ND, Karpeisky MYa, Mikhailov SN, Padukova NSh, Ogarkov VI, Atabekov JG, Chailakhyan MKh: Induction of cytokinin activity in Amaranthus caudatus plants by human interferon and 2′–5′-oligoadenylates. (Russian.) Dokl Akad Nauk SSSR 293: 253–256 (1987).
38. van Loon LC: The induction of pathogenesis-related proteins by pathogens and specific chemicals. Neth J Plant Path 89: 265–273 (1983).
39. van Loon LC: Pathogenesis-related proteins. Plant Mol Biol 4: 111–116 (1985).
40. van Loon LC, Gerritsen YAM, Ritter CE: Identification, purification, and characterization of pathogenesis-related proteins from virus-infected Samsun NN tobacco leaves. Plant Mol Biol 9: 593–609 (1987).
41. Vicente M, De Fazio G, Menezes ME, Golgher RR: Inhibition of plant viruses by human gamma interferon. J Phytopath 119: 25–31 (1987).
42. White RF, Rybicki EP, von Wechmar MB, Dekker JL, Antoniw JF: Detection of PR 1-type proteins in Amaranthaceae, Chenopodiaceae, Graminae and Solanaceae by immunoelectroblotting. J Gen Virol 68: 2043–2048 (1987).