SYNTHESIS OF FRAGMENTS 43-49 AND 121-125 OF INTERFERON-α_2 AND THEIR EFFECTS ON MEMORY PROCESSES

O. S. Papsuevich, G. I. Chipens,
V. A. Krauz, A. L. Drozdov,
M. Yu. Galushko, and T. A. Petrova

UDC 615.339:578.254].012.7:612.821.2].07

The multiple biological effects of interferon (IFN) result from the large number of active centers on the surface of the molecule, and from the formation of many low molecular weight fragments due to limited proteolysis. The structure and positions of these fragments, which represent the active centers, have not been widely studied; associations with one or another part of the molecule have merely been suggested [1, 3, 4, 8, 9].

It has been shown [2, 7] that the tetrapeptide formed by amino acids 122-125 of IFN-δ_2, which is positioned on the evolutionarily conserved domain, produces significant stimulation of macrophage phagocytosis, stimulates the cytolytic activity of K-cells, activates learning and memory processes, and produces a number of other neuro- and psychotropic effects.

In the present work we continue the study of the structure-function organization of the KFN molecule. We have synthesized fragments 43-49 and 121-125 of the human IFN-α_2 molecule, and studied their effects on learning and memory processes in animals.

The heptapeptide IFN-α_2 (43-49) (compound I) was synthesized by standard aqueous phase peptide chemistry (Fig. 1).

Synthesis started with N^{α}-tert-butoxycarbonyl (Boc) amino acid derivatives (Reanal, Hungary). These were deprotected with 3 N HCl in dioxane (for glutamyl peptides III and IX) and trifluoroacetic acid (TFA) (for the asparaginyl peptide V). Peptide fragments were condensed by the NN'-dicyclohexylcarbodiimide (DCHC) method in the presence of 1-hydroxybenztriazole (for the synthesis of the pentapeptide VII) and by the azide method (for synthesis of the heptapeptide XI). After removal of protective groups (Boc, Z) with 2.4 N HBr in acetic acid, the heptapeptide I was first desalted with Amberlite IRC-50 (H^+ form), followed by purification by gel filtration on Sephadex G-15 in 0.2 N acetic acid, and further purification by reverse phase HPLC on octylsilyl-modified silica gel.

The starting material for the synthesis of the pentapeptide IFN-α_2 (121-125) (Lys-Tyr-Phe-Gln-Arg, compound II) was the tetrapeptide Tyr-Phe-Gln-Arg, whose synthesis has been described previously [2]. An intermediate product was first synthesized by reacting this with Boc-Lys (Z)-ONp; II was prepared by removal of the Boc, Z protecting group.

EXPERIMENTAL (CHEMICAL)

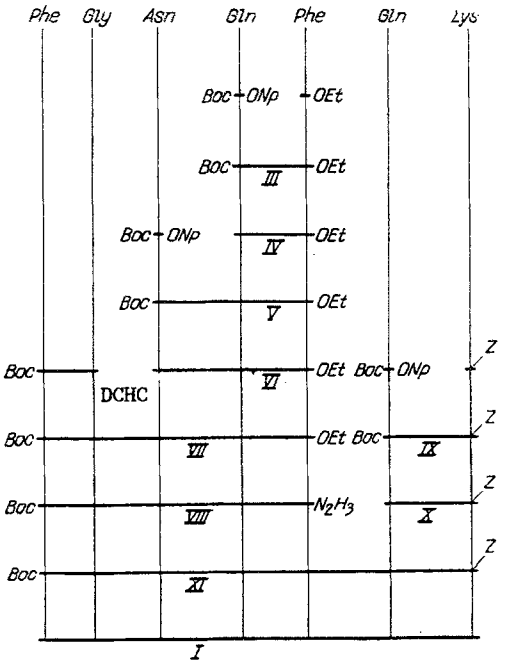
Melting temperatures were determined in capillaries (without correction). The purity of compounds was confirmed by thin layer chromatography on silic gel 60 F_{254} pates (Merck, FRG) developed with tert-butanol:n-butanol-acetic acid:water 2:2:1:1 (system A), with n-butanol:acetic acid:pyridine:water 15:3:10:6 (system B), and with chloroform:ethanol:ethyl acetate: acetone 50:25:5:5 (system C). Chromoatograms were stained with ninhydrin and chlorbenzidine. Electrophoretic mobilities were measured on Filtrak FN17 paper (GDR) in 5 N acetic acid pH 1.9 with a potential gradient of 30 V/cm for 2 h.

All optically active amino acids were L isomers. Specific optical rotations were measured in a Perkin-Elmer (USA) model 141 M polarimeter.

Peptides were hydrolyzed with 6 N HCl at 105-110°C for 24 h; the amino acid composition of the resulting hydrolysates was determined using a Liquimat III (Sweden) analyzer. Reverse phase HPLC was carried out using a Du Pont 83 instrument (France) with a Zorbax C_8 column (2.12 × 25 cm), and a mobile phase consisting of acetonitrile and 0.2 M ammonium acetate (10:90). The results obtained corresponded with those expected.

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Dnepropetrovsk Medical Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 6, pp. 30-33, June, 1990. Original article submitted June 14, 1989.

0091-150X/90/2406-0418$12.50



Boc-Gln-Phe-OEt (III). To a solution containing 6.88 g (30 mmole) of HCl·Phe-OEt in 40 ml of dimethylformamide (DMF) was added 10 g (27 mmole) of Boc-Gln-ONp and 5.2 ml (30 mmole) of diisopropylethylamine (DIEA); the mixture was shaken for 24 h and was diluted with 0.05 N HCl. The settled oil was extracted with ethyl acetate, and the organic layer was washed with 0.05 N HCl, water, 5% $NaHCO_3$, and water, and was dried over Na_2SO_4 to remove the solvent. The residue was dissolved in hexane, and was filtered and dried. The yield was 9 g (79%), with melting point 102-104°C, $[\alpha]_D^{20}$ −4.4° (0.5; DMF); R_f 0.89 (system A), 0.91 (B), and 0.70 (C). The formula was $C_{21}H_{31}N_3O_6$.

Boc-Asn-Gln-Phe-OEt (V). **Five grams (11.9 mmole) of compound III was dissolved in 25 ml** of 3 M HCl in dioxane; after 40 min the solvent was evaporated and the residue was dissolved in ether, filtered, and dried. The hydrochloride of compound IV thus obtained was dissolved in 30 ml DMF, and 3.56 g (109.1 mmole) of Boc-Asn-ONp was added along with 2.1 ml (11.9 mmole) DIEA. After 24 h the mixture was diluted with ethyl acetate, and the dried residue was washed with ether, dried, and recrystallized from DMF in water. The yield was 4.22 g (78%), with melting point 192-194°C, $[\alpha]_D^{20}$ −17.8° (0.5; DMF); R_f 0.79 (system A), 0.84 (B), and 0.58 (C). The formula was $C_{25}H_{37}N_5O_8$.

Boc-Phe-Gly-Asn-Gln-Phe-OEt (VII). Compound V (3.48 g, 6.5 mmole) was dissolved in 20 ml TFA; the mixture was diluted with ether after 30 min, and insoluble material was separated and redissolved in ether, washed, and dried. The trifluoroacetate of compound VI thus obtained was dissolved in 20 ml of DMF, cooled to −5°C, and 2.09 g (6.5 mmole) of Boc-Phe-Gly, 0.97 g (7.2 mmole) of 1-hydroxybenztriazole, 1.2 ml (6.5 mmole) of DIEA, and 1.64 g (7.2 mmole) of DCHC in 10 ml DMF were added. The mixture was shaken for 2 h at −5°C, and was then stored at 15°C for 18 h. Insoluble material was removed by filtration, and the solution was diluted with 0.05 N HCl; the resulting precipitate was collected, washed with water, dried, recrystallized from a mixture of DMF and ethyl acetate. The yield was 3.76 g (78%), with melting point 158-160°C, $[\alpha]_D^{20}$ −9.8° (0.5; DMF); R_f 0.76 (system A), 0.79 (B), and 0.36 (C). The formula was $C_{40}H_{49}N_7O_{10}$.

Boc-Phe-Gly-Asn-Gln-Phe-N_2H_3 (VIII). Compound VII (3.50 g, 4.7 mmole) was dissolved in 40 ml of DMF;:ethanol (1:1), and 1.2 ml (24 mmole) hydrazine hydrate was added. After 48 h the mixture was diluted with ether, the recrystallized material was separated, washed with ether, and dried. The yield was 3.16 g (92%), with melting point 230°C, $[\alpha]_D^{20}$ −33.4° (0.50; DMF); Rf 0.88 (system A), 0.84 (B), and 0.00 (C). The formula was $C_{34}H_{47}N_9O_9$.

Boc-Gln-Lys(Z) (IX). To a solution containing 5 g (14 mmole) of Boc-Gln-ONp in 80 ml DMF, 3.92 g (14 mmole) of Lys(Z) in 50 ml of water, containing 3.9 ml (27.5 mmole) Et_3N was added; the mixture was shaken for 48 h, and was diluted with 0.1 N HCl. The oil was extracted

TABLE 1. The Effects of IFN-α_2 Fragments on the Formation of Conditioned Passive Avoidance Reactions in Rats

Compound	Dose, μg/kg	Rats with amnesia		Rats without amnesia	
		number of rats	% restoration of conditioned passive avoidance reflex 30 min after administration of peptide*	number of rats	% loss of conditioned passive avoidance reflex 30 min after administration of peptide *
Control		56	14,3	20	19,2
Arginine varopressin	1	12	25,0**	15	0
	20	11	50,0***	15	14,0
I	1	16	25,0**	14	28,6**
	20	16	43,8***	13	38,5**
II	4	21	38,5**	13	21,7
	10	18	38,8**	12	25,0
	100	13	0	16	50,0***

*Initial measure = 0.
**P < 0.05 in comparison with initial value.
***P < 0.05 in comparison with control.

with ethyl acetate, and the organic phase was washed with water, and dried over Na_2SO_4. The solvent was evaporated, and the residue was dissolved in ether, filtered, dried, and recrystallized from hot ethyl acetate. The yield was 3.40 g (48%), with melting point 135-136°C, $[\alpha]_D^{20}$ +1.2° (0.5; DMF); R_f 0.70 (system A), 0.79 (B), and 0.54 (C). The formula was $C_{34}H_{36}N_4O_8$

Boc-Phe-Gly-Asn-Gln-Phe-Gln-Lys(Z) (XI). Compound VIII (2.04 g, 2.8 mmole) was dissolved in 30 ml DMF, cooled to −25°C, and 16 ml 3 N HCl in dioxane was added, followed by 0.3 ml (2.8 mmole) of tert-butyl nitrate. After 20 min the mixture was neutralized with Et_3N, and substance X [prepared from 1.40 g (2.8 mmole) of compound IX treated with 3 N HCl in dioxane] and 0.4 ml (2.8 mmole) of Et_3N in 15 ml DMF were added. The mix was shaken for 3 h at −20°C, for 18 h at 5°C, and for 4 h at 20°C; the mix was then diluted with 0.05 N HCl, and insoluble material was collected by filtration, washed with water, dried, and recrystallized from a mixture of DMF and ethyl acetate. The yield was 1.68 g (60%), with melting point 194°C, $[\alpha]_D^{20}$ −19.0° (0.5; DMF); R_f 0.72 (system A), 0.84 (B), and 0.26 (C). The formula was $C_{53}H_{71}N_{11}O_{15}\cdot 1.5H_2O$.

Phe-Gly-Asn-Gln-Phe-Gln-Lys (I). Compound XI (1.02 g, 0.9 mole) was dissolved in 10 ml acetic acid, and 20 ml 3.7 N HBr in acetic acid was added; after 1 h the solution was diluted with ether, and insoluble material was collected and repeatedly washed with ether, filtered, dried, and dissolved in 200 ml water. The solution (pH 4.5) was passed through an Amberlite IRC-50 (H^+ form) column. The resin was washed with 0.25% acetic acid, and the column was eluted with 500 ml of 50% acetic acid. The solution was evaporated to 25 ml and lyophilized. The product (0.55 g) was dissolved in 5 ml of 0.2 N acetic acid and loaded onto a Sephadex G-15 column (100 × 5.9 cm); this column was eluted with 0.2 N acetic acid, peaks being detected at 275 nm. Lyophilization of the appropriate fractions yielded 0.35 g. After HPLC purification the yield was 0.29 g (34%) of peptide I, $[\alpha]_D^{20}$ −12.4° (0.5; 1 N acetic acid); R_f 0.04 (system A), 0.32 (B), and 0.00 (C); E_{His} 0.75; E_{Gly} 0.82; E_{Trp} 1.12. Amino acid analysis yielded: Asp 1.06 (1), Glu 1.70 (2), Gly 1.00 (1), Phe 1.70 (2), Lys 0.97 (1). The formula was $C_{40}H_{57}N_{11}O_{11}\cdot$acetic acid$\cdot 2H_2O$.

Lys-Tyr-Phe-Gln-Arg (II). The compound Tyr-Phe-Gln-Arg·acetic acid·$2H_2O$ [2] (0.71 g, 1 mmole) was dissolved in 100 ml of DMF, and 0.15 ml (1.3 mmole) of Et_3N and 0.61 g (1.2 mmole) Boc-Lys(Z)-ONp were added, the mixture was shaken for 20 h, and diluted with ethly acetate. The precipitate was collected by filtration and washed with ethyl acetate and ether. Protecting groups and purification of peptide II were carried out as described for peptide I. The yield was 0.47 g (52%). $[\alpha]_D^{20}$ +5.1° (0.5; 1 N acetic acid); R_f 0.03 (system A), 0.27 (B), and 0.00 (C); E_{His} 0.92; E_{Gly} 1.03; E_{Trp} 1.81. Amino acid analysis yielded: Glu 1.11 (1), Tyr 0.87 (1), Phe 1.02 (1), Lys 1.00(1), Arg 0.97 (1). The formula was $C_{35}H_{52}N_{10}O_8\cdot$(acetic acid)$\cdot 2H_2O$.

TABLE 2. The Effect of Arginine-vasopressin and IFN-α_2 Fragments* on Blood Pressure and Heart Rate in Rats

Compound	Initial value	5 min	10 min	20 min	30 min	60 min
	Blood pressure, mm Hg					
Arginine vasopressin	142,50±2,58	155,50±4,50**	187,75±2,78**	146,25±2,53	137,50±1,44	140,00±2,00
I	139,18±5,83	155,00±5,45	155,00±5,00	136,60±8,82	127,50±7,50	123,75±11,06
II	136,75±2,69	139,25±2,29	137,50±5,95	144,17±3,96	140,00±4,56	146,75±2,69
	Heart rate, per min					
Arginine-vasopressin	165,67±1,33	80,00±13,00**	100,00±2,00**	111,33±1,76**	120,00±1,15**	128,00±5,11**
I	221,50±16,82	167,00±23,01	217,00±6,19	214,50±5,57	213,50±4,69	253,55±11,95
II	161,50±1,50	155,75±3,75	158,00±2,15	158,00±1,58	161,01±3,13	157,00±4,10

*Dose 20 mg/kg.
**$P < 0.05$.

EXPERIMENTAL (BIOLOGICAL)

The effects of IFN-α_2 fragments on such integrative brain functions as memory were studied, along with effects on the cardiovascular system.

The effects of IFN-α_2 fragments on the formation of memory engrams was studied in 252 mature white Wistar rats (150-200 g). The recovery of memory was assessed in a conditioned passive avoidance reaction as described in [6], induced by electric shocks applied to the skin. Rats were subjected to electric shocks (20 mA for 500 msec) 2 h after formation of the passive defensive reflex, using electrodes placed on the ears. After 72 h, when rats had recovered normal levels of activity, retention of the conditioned reflex was tested. The results allowed rats to be divided into those showing amnesia (about 30%) and those retaining the passive avoidance reflex (up to 70%). The effects of the peptides were assessed 2-3 h after the selection test, in both groups. Alterations in memory recovery were determined 30-40 min, 2, 24, and 48 h after i.p. injections; control animals received the same volume of isotonic saline. Rats received IFN-α_2 fragment (43-49) at doses of 1 and 20 μg/kg, fragment (121-125) at doses of 1, 4, 10, and 100 μg/kg, and arginine-vasopressin at doses of 1 and 20 μg/kg.

The requirement to determine cardiovascular changes arose from the hormonal properties of arginine-vasopressin, which significantly limits its use as an antiamnesic agent. Experiments were performed on 16 mongrel dogs (5-10 kg), receiving the largest doses of each substance. Cardiovascular effects were studied by manometric measurement of femoral arterial blood pressure (BP), and heart rate (HR), which was determined with a PMB-01 rhythmovasometer.

RESULTS AND DISCUSSION

Table 1 shows the effects on memory recovery of IFN-α_2 fragments I and II, in comparison with those obtained with arginine-vasopressin. The passive defensive reflex recovered in 25% of rats 30 min after administration of peptide I (20 μg/kg), while 2- and 5-fold smaller doses of peptide II increases the recovery of the passive avoidance reflex in 38-39% of cases. These changes were significant as compared with initial values, but were not significantly different from those produced by arginine-vasopressin. Peptide I gave a maximum effect 2 h after dosage, with recovery of the reflex in 43.8% of animals; the effect gradually decreased at later times (24 and 48 h).

It should be noted that while arginine-vasopressin significantly reduced the number of nonamnesiac rats losing the conditioned reflex after dosage, peptides I and II had the opposite effect.

The cardiovascular effects of peptides I and II differed from those of arginine-vasopressin. Administration of arginine-vasopressin (20 μg/kg) increased BP in dogs from 142.50 ± 2.58 to 187.75 ± 2.78 mm Hg, i.e., by 31.8% ($p < 0.05$) 5 min after dosage. The HR decreased in parallel by 51.7% (Table 2). BP returned to the initial level 10 min after dosage with arginine-vasopressin, while HR remained reduced to the end of the 1-h observation period.

IFN-α_2 peptides I and II had no significant effect on BP or HR during the 1-h observation period after i.v. dosage.

More detailed studies on the effects of IFN-α_2 fragments on learning and memory processes, and on functions of the brain and cardiovascular system, and on brain neurochemistry will be published separately.

Thus, IFN-α_2 fragments 43-49 and 121-125 have significant effects on the recovery of memory traces, though they do not have greater antiamnesiac activity than arginine-vasopressin; the peptides significantly disturb the production of memory engrams in non-amnesiac rats. The peptides differ from arginine-vasopressin in that i.v. administration does not significantly alter BP or HR.

LITERATURE CITED

1. V. P. Vav'yalov and A. I. Denesyuk, Dokl. Akad. Nauk SSSR, 275, No. 1, 242-246 (1984).
2. O. S. Papsuevich, G. I. Chipens, V. D. Bakharev, and T. A. Petrova, Khim.-Farm. Zh., 19, No. 1, 35-39 (1985).
3. G. I. Chipens, N. B. Levinya, O. S. Pansuevich, et al., Izv. Akad. Latv. Akad. Sci. Ser. Khim., No. 3, 366-368 (1986).
4. G. Allen, Biochem. J., 207, No. 3, 397-408 (1982).
5. L. Bernardi, G. Bosisio, R. De Castiglioni, and O. Coffredo, Chem. Abstr., 72, No. 13, 67271 u (1970).
6. J. Bures and O. Buresova, J. Com. Psychol., 56, 268-272 (1963).
7. G. Chipens, R. Vegners, O. Papsuevich, et al., Chemistry of Peptides and Proteins, Berlin (1986), Vol. 3, pp. 221-234.
8. D. Gillespie, E. Pequignot, and W. A. Carter, Interferons and Their Applications, Berlin (1984), pp. 55-63.
9. G. Rosenthal, R. Vegners, O. Papsuevich, et al., Synthetic immunomodulators and Vaccines, Prague (1986), pp. 236-242.

ANTIINFLAMMATORY ACTIVITY OF DIPHENYLDI[AROYLOXY]SILANES

A. D. Dzhuraev, K. M. Karimuklov,
A. G. Makhsumov, U. B. Zakirov,
Sh. D. Radzhabova, and N. Kh. Tsinzadze

UDC 615.276:547.77.012.1

The identification of antiinflammatory drugs of low toxicity is a continuing task for pharmaceutical chemists [1, 2].

We have now examined the antiinflammatory activity of some diphenyldi(aroyloxy) silanes (I-XIV), obtained by reacting benzoic acids with diphenyldichlorosilane.

$$[(X)C_6H_4C(O)O]_2SiPh_2 \text{ (I—XIV)}$$

X=2-Cl(I), 4-Cl(II), 2,2-Cl_2(III), 2-I(IV), 3-I(V), 4-I(VI), 2-Br(VII), 4-Br(VIII), 2,4-Br_2(IX), 2-NO_2(X), 3-NO_2(XI), 2,4-NO_2(XII), 4-OH(XIII), 2,4-$(OH)_2$(XIV).

The purity of the products was checked by TLC on grade II alumina in the solvent system toluene—acetic acid. The structures of the compounds were established by their IR spectra and elemental analyses.

For example, the IR spectra of (I-XIV) showed absorption for the carbonyl group (1710-1750 cm^{-1}) and for the benzene ring (1500-1600 cm^{-1}).

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a UR-20 spectrophotometer in KBr disks. The elemental analyses were in agreement with the calculated values.

Diphenyldi(o-chlorobenzoyloxy)silane (I). A mixture of 3.12 g (0.01 mole) of o-chlorobenzoic acid and 2.53 ml of diphenyldichlorosilane in 100 ml of benzene was stirred at ~20°C for 6 h. The product was then extracted with ether, the solvent removed, and the material purified by TLC on grade II alumina in the system toluene—acetic acid (4:10) (Table 1).

Tashkent Institute of Medicine. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 6, pp. 33-34, June, 1990. Original article submitted August 1, 1989.

0091-150X/90/2406-0422$12.50