SYNTHESIS OF DESGLYCINAMIDE-VASOPRESSIN ANALOGUES

By

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Syntheses are reported for three analogues of vasopressin in which the *C*-terminal glycinamide moiety has been deleted from the parent hormones. All three analogues have been synthesized by the solid-phase method, and purified by gel filtration in two steps.

Vasopressin and other neurohypophyseal peptides influence memory and learning processes. Structure-activity studies in the pole-jumping avoidance test revealed that arginine-vasopressin (AVP) is the most potent peptide, followed by lysine-vasopressin (LVP) [1, 2]. Removal of the *C*-terminal glycinamide (dGAVP and dGLVP) decreases the potency by approximately 50 per cent [3]. Such peptides have practically no classical endocrine activities (*e.g.* blood pressure, antidiuresis and ACTH release) [4]. These findings indicate a dissociation between the endocrine and behavioural effects of neurohypophyseal hormones. DGLVP has been isolated from porcine pituitary glands [5]. These results have started a search for vasopressin analogues that influence learning and memory, but do not cause water retention as do LVP, AVP and some of their analogues.

In this paper we describe the synthesis of three (9-desglycinamide)-vasopressin analogues. The synthesized analogues are shown in Fig. 1.

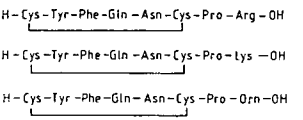

Fig. 1

The peptides were prepared by the general stepwise solid-phase method on chloromethylated polystyrene resin [6], as applied in the synthesis of oxytocin [7, 8]. At the end of the synthesis, the fully protected peptides were cleaved by alcoholysis from the solid support [9, 10], instead of the rather drastic cleavage with HBr/TFA or HF. This resulted in protected peptide esters, which were hydrolyzed with NaOH in DMF/pyridine to give protected octapeptides with a *C*-terminal free carboxyl groups. After the hydrolysis racemization was not detected.

All three protected octapeptides were deblocked with Na in liquid ammonia [11], using some modifications as described by MANNING ET AL. [12]. The linear compounds were oxidatively cyclized with aqueous $K_3[Fe(CN)_6]$ [13]. The cyclized analogues were desalted and purified by gel filtration on a Sephadex G-15 column in two steps [14]. The schematic synthesis of dGAVP is shown in Fig. 2.

The final peptides were pure on different TLC systems, and the amino acid compositions were confirmed by amino acid analysis.

The physical data on the three analogues are summarized in Table I.

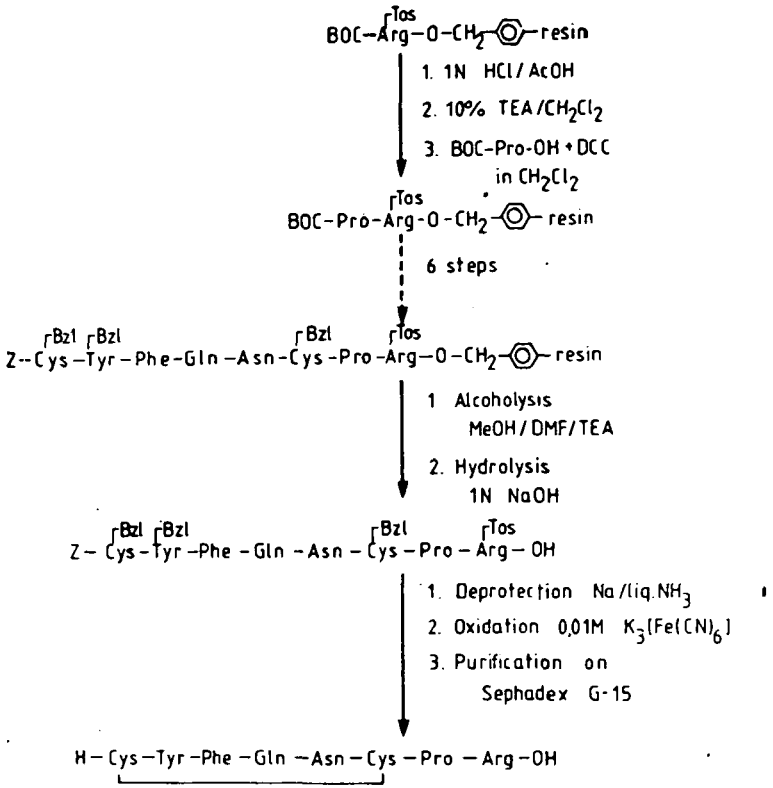

Fig. 2

Experimental

Melting points were determined on a PHMK (VEB Analytik Dresden) apparatus and are uncorrected. Optical rotations were measured with a Carl Zeiss polarimeter (Polamat A). Amino acid analyses were performed using a Czechoslovak AAA 881 analyzer. Peptide samples were hydrolyzed during 24 h in 6N HCl at 110 °C in vacuum-sealed tubes.

Thin-layer chromatography (TLC) was carried out on Merck precoated silica gel plates (Kieselgel 60). The following solvent systems were used: **A**, 1-butanol--acetic acid-water (4:1:1, v/v); **B**, 1-butanol-pyridine-acetic acid-water (30:20:6: 24, v/v); **C**, chloroform-methanol (7:3, v/v). Ninhydrin, TMD and iodine vapour were used for detection. Solvents and reagents used for solid-phase synthesis were of analytical grade and were distilled immediately prior to use.

Table 1

Physical data on the synthesized analogues

Peptide	Yield %	Mol. weight	Formula	Melting point. °C	$[\alpha]_D^{25}$ (c=1, DMF)	A	R_f B	C	Tyr	Phe	Glu	Asp	Cys	Pro	X
prot. dGAVP-OMe (1)	40	1602.9	$C_{81}H_{95}O_{16}N_{13}S_3$	195—197	−36.5°	0.66	0.76	—							
prot. dGAVP-OH (2)	74	1588.9	$C_{80}H_{93}O_{16}N_{13}S_3$	162—165	−35.4°	0.60	0.64	—	0.78	0.92	1.02	1.08	1.75*	1.01	1.00
dGAVP (3)	37	1029.1	$C_{44}H_{64}O_{12}N_{13}S_2$	—	—	0.20	0.49	—	0.80	0.95	1.05	1.10	0.75	1.10	1.00
prot. dGLVP-OMe (4)	30	1574.9	$C_{81}H_{95}O_{16}N_{11}S_3$	210—213	−39.1°	0.71	0.72	0.90							
prot. dGLVP-OH (5)	90	1560.9	$C_{80}H_{93}O_{16}N_{11}S_3$	155—157	−40.8°	0.65	0.62	—	0.68	0.90	0.98	1.00	1.65*	1.07	1.02
dGLVP (6)	32	1001,1	$C_{44}H_{64}O_{12}N_{11}S_2$	—	—	0.15	0.45	—	0.76	1.20	1.06	1.00	0.75	1.09	1.06
prot. dGOVP-OMe (7)	67	1560.9	$C_{80}H_{93}O_{16}N_{11}S_3$	197—200	−35.7°	0.71	0.76	0.90							
prot. dGOVP-OH (8)	72	1546.9	$C_{79}H_{91}O_{16}N_{11}S_3$	160—165	−27.2°	0.47	0.63	—	0.72	1.00	0.91	1.08	1.48*	1.20	0.74
dGOVP (9)	35	987.1	$C_{43}H_{62}O_{12}N_{11}S_2$	—	—	0.12	0.42	—	0.80	1.18	0.89	1.00	0.60	1.10	1.08

* S-Bzl-Cys
X Arg, Lys or Orn

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-OMe (1)

The solid-phase synthesis procedure conformed to that described previously [6]. 1% Chloromethylated resin (Bio Beads Sx-1) was esterified with Boc-Arg(Tos)-OCs to an incorporation of 0.18 mmol/g resin [15]. 1.8 g Boc-Arg(Tos)-resin (0.32 mmol of Arg) was elongated. Seven cycles of deprotection, neutralization and coupling were carried out with the following protected amino acid derivatives; Boc-Pro, Boc-Cys(Bzl), Boc-Asn, Boc-Gln, Boc-Phe, Boc-Tyr(Bzl) and Z-Cys(Bzl) with coupling in the final step. All coupling reactions were carried out by DCC in CH_2Cl_2 [16], except in the cases of Asn and Gln which reacted as the nitrophenyl ester derivatives [17] in DMF. These couplings being facilitated by the addition of HOBt [18].

At the end of the synthesis the protected peptide resin (2.1 g) were washed out of the reaction vessel with CH_2Cl_2, MeOH, Et_2O and dried *in vacuo*.

1.97 g of the protected octapeptide-resin was suspended in 25 ml of DMF, 25 ml of MeOH and 11 ml of TEA [9, 10]. The mixture was stirred at 45 °C for 24 h. The resin was filtered and was washed with warm DMF (2×25 ml). The resin was retreated by the same procedure for another 24 h, filtered and washed again with DMF (2+25 ml) and MeOH (25 ml). The filtrates were combined and evaporated *in vacuo*. The oil residue was dissolved in AcOH (4 ml) and the protected octapeptide methyl ester was precipitated by the addition of EtOH. The crude product was reprecipitated from DMF-EtOH-Et_2O to give (1) (0.197 g).

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-OH (2)

150 mg of protected octapeptide-OMe (1) was dissolved in 1 ml of pyridine and 0.5 ml of DMF. The pH was adjusted to 9—10 with 1 N NaOH and the mixture was stirred for 10 h. The pH was then adjusted to 7 with 1 N HCl and the protected octapeptide was precipitated. Removal of the solvents *in vacuo*, followed by trituration with Et_2O and drying *in vacuo* over P_2O_5, gave the protected octapeptide (2) (110 mg).

(9-desglycinamide)-arginine-vasopressin (dGAVP) (3)

The protected octapeptide (2) was deblocked by the sodium-liquid ammonia procedure [8, 13]. 100 mg of (2) was dissolved in 180 ml of boiling NH_3, freshly distilled over Na. Fresh Na stick was introduced into the solution until a light-blue colour persisted for 30 sec, 0.5 ml of acetic acid was added to discharge the colour. The solution was evaporated and N_2 was passed through the flask. The residue was dissolved in 50 ml of 20% AcOH and quickly poured into 1000 ml of ice-cold water. The pH of the solution was adjusted to 7 with NH_4OH, and 0.01 N $K_3[Fe(CN)_6]$ was added with stirring until a yellow colour persisted (12 ml). The yellow solution was stirred for further 30 min, and than for 20 min with the anion-exchange resin (Amberlyst A 26 Cl^- form) to remove ferrocyanide and excess ferricyanide ions. The resin was removed by filtration and washed with 100 ml of water. The combined filtrate and washings were lyophilized. The resulting material was desalted on a Sephadex G-15 column (110×2.5 cm), with elution with 50% AcOH at a flow rate of 8 ml/h. The eluate was fractionated, and monitored for absorbance at 277 nm. The fractions were checked by TLC, pooled and lyophilized, and the residue was further desalted by gel filtration on a Sephadex G-15 column (100×1.6 cm), with elution with 0.2 N AcOH at a flow rate of 4 ml/h. The peptide was eluted in a single peak. The lyophilization of these fractions yielded the pure dGAVP (3) as a white powder (24 mg).

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-OMe (4)

4.3 g of the protected octapeptide resin was obtained as described above, using 3.5 g Boc-Lys(Tos)-resin containing 0.55 mmol of Boc-Lys(Tos)-OH per g resin. The protected octapeptide methyl ester (4) was cleaved from 3 g of the resin in the manner detailed above for (1): yield 620 mg of (4).

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-OH (5)

200 mg of the protected octapeptide methyl ester (4) was hydrolyzed with NaOH in DMF/pyridine as for (2), to give 178 mg of (5).

(9-desglycinamide)-lysine-vasopressin (dGLVP) (6)

100 mg of the protected octapeptide (5) was reduced with sodium in liquid ammonia, reoxidized, deionized and purified in two steps on Sephadex G-15 as for (3): yield 20 mg of (6).

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Orn(Tos)-OMe (7)

2.8 g of the protected octapeptide resin was obtained as described above, using 2 g of Boc-Orn(Tos)-resin containing 0.52 mmol of Boc-Orn(Tos)-OH per g resin. The protected octapeptide methyl ester (7) was cleaved from 2.5 g of the resin in the manner detailed above for (1): yield 940 mg of (7).

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Orn(Tos)-OH (8)

400 g of the protected octapeptide methyl ester (7) was hydrolyzed with NaOH in DMF/pyridine as for (2), to give 285 mg of (8).

(9-desglycinamide)-ornithine-vasopressin (dGOVP) (9)

100 mg of the protected octapeptide (8) was reduced with sodium in liquid ammonia, reoxidized, deionized and purified in two steps on Sephadex G-15 as for (3): yield 22 mg of (9).

Abbreviations: DMF: Dimethylformamide
TEA: Triethyl amine
DCCI: Dicyclohexylcarbodiimide
TMD: N, N, N′, N′-tetramethyl-4, 4′ diamino-diphenylmethane
ACOH: Acetic acid

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СИНТЕЗ АНАЛОГОВ ДЕСГЛИЦИНАМИДО-ВАСОПРЕССИНА

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Сообщено о синтезе трех аналогов васопрессина в которых *С*-терминальный глицинамидный фрагмент был удален с соответствующего гормона. Все три аналога были синтетизированы методом реакции в твердой фазе и были очищены двухступенчатой гельфильтрацией.