FEBS Letters 412 (1997) 86–90 FEBS 18900

Induction of chymase that forms angiotensin II in the monkey atherosclerotic aorta

Shinji Takai[a], Naotaka Shiota[a], Shigeyoshi Kobayashi[b], Eiko Matsumura[b], Mizuo Miyazaki[a,*]

a

b

Received 12 May 1997; revised version received 4 June 1997

Abstract Chymase shows a catalytic efficiency in the formation of angiotensin (Ang) II. In the present study, the characterization and primary structure of monkey chymase were determined, and the pathophysiological role of chymase was investigated on the atherosclerotic monkey aorta. Monkey chymase was purified from cheek pouch vascular tissue using heparin affinity and gel filtration columns. The enzyme rapidly converted Ang I to Ang II (K_m = 98 μM, k_{cat} = 6203/min) but did not degrade several peptide hormones such as Ang II, substance P, vasoactive intestinal peptide and bradykinin. The primary structure, which was deduced from monkey chymase cDNA, showed a high homology to that of human chymase (98%). The mRNA levels of the aorta chymase were significantly increased in the atherosclerotic aorta of monkeys fed a high-cholesterol diet. These results indicate that monkey chymase has a highly specific Ang II-forming activity and may be related to the pathogenesis of atherosclerosis.

Chymase; Monkey; Angiotensin II; Purification; Cloning; Atherosclerosis

1. Introduction

Angiotensin (Ang) II, a potent pressor substance, is known to be generated from Ang I by angiotensin converting enzyme (ACE: EC 3. 4. 15. 1) in blood and several tissues. However, the Ang II-forming pathways in human do not always follow the classical renin-angiotensin system. Our previous study demonstrated that only 30% of the Ang I-induced vasoconstriction of the isolated human gastroepiploic artery is inhibited by ACE inhibitors and the rest by chymostatin [1]. Human chymase efficiently generates Ang II [1–3], but some non-human chymases do not generate Ang II; for example, rat chymase hydrolyzes the Tyr[4]-Ile[5] bond of Ang I to yield inactive fragments [4,5], and the chymase-dependent Ang II-forming pathway does not exist in rabbit vascular tissues [1]. Therefore, human tissues have two Ang II-forming pathways, whereas rats and rabbits have only the ACE pathway. The species difference in chymase's Ang II-forming ability should be considered in selecting experimental models for studying the pathogenesis of human disease where Ang II is involved.

Recently, it has been emphasized that Ang II play a crucial role in the promotion of vascular tissue remodeling. ACE inhibitors suppressed the development of atherosclerosis in rabbit [6,7]. These results suggest that the activation of Ang II formation in vascular tissues has an important role in the pathogenesis of atherosclerosis. However, human vascular tissues contain Ang II-forming chymase in addition to ACE, and it is not clear whether vascular chymase is related to atherosclerosis.

To establish animal models for exploring the role of the chymase-dependent Ang II-forming pathway in humans, we purified and cloned monkey chymase from vascular tissues, and investigated the pathophysiological roles of vascular chymase in atherosclerosis.

2. Materials and methods

Twelve male monkeys ( ) were obtained from Japan SLC (Shizuoka, Japan). In atherosclerotic models, monkeys were assigned to normal or high-cholesterol (4% cholesterol and 6% corn oil, Oriental Yeast Co. Ltd., Osaka, Japan) diets for 6 months. The monkeys were sacrificed by bleeding under pentobarbital anesthesia, and their cheek pouch vascular tissues and thoracic aorta were excised. The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

The tissue extract was obtained from cheek pouch vascular tissues by the procedure described previously [8]. The tissue extract was applied to a heparin affinity column (5 ml; HiTrap Heparin packed column, Pharmacia, Tokyo, Japan) which was pre-equilibrated with 20 mM Tris-HCl buffer, pH 8.0, containing 0.1 M KCl. The column was eluted with a linear gradient (0–100%) of 20 mM Tris-HCl buffer, pH 8.0, containing 1.8 M KCl and 0.1% (v/v) Triton X-100. The active fractions with Ang II-forming activity were applied to a gel filtration column (2.6×40 cm; Toyopearl HW-55, Tohsoh, Tokyo, Japan) and eluted with 20 mM KCl, pH 8.0, containing 0.5 M KCl and 0.1% (v/v) Triton X-100. Ang II-forming activity was assayed according to Okunishi et al. [9]. One unit of chymase activity was defined as 1 μmol of Ang II formed/minute. The protein concentration was measured by bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL).

SDS-PAGE was performed on the purified enzyme of 0.5 μg and the gel was silver-stained. The optimum pH for Ang II formation was determined with Briton-Robinson's wide range buffer of pH 6.0–10.0. The susceptibility of the purified enzyme to various inhibitors was examined. These inhibitors included ethylenediaminetetraacetic acid (EDTA), phosphoramidon, pepstatin, ACE inhibitors (captopril and lisinopril), soybean trypsin inhibitor (SBTI), phenylmethylsulfonyl fluoride (PMSF), chymostatin, and aprotinin. To determine the substrate specificities and the kinetic parameters of the purified enzyme for Ang II-processing, 12 ng of the purified enzyme were incubated in 20 mM Tris-HCl buffer, pH 8.0, containing 0.5 M KCl and 0.1% (v/v) Triton X-100 at 37°C. Amino-terminal sequencing of the purified enzyme was performed on an Applied Biosystems model 477A protein sequencer (Foster City, CA).

Monkey chymase cDNA was cloned using a polymerase chain reaction (PCR). Total RNA was isolated from monkey cheek pouch vascular tissues [10]. The single-stranded cDNA was synthesized from 1 μg of the total RNA using a cDNA synthesis kit (Gibco

*Corresponding author. Fax: (81) (726) 846518.

S0014-5793(97)00752-7

```
                                                ttgctcaggcagcctctctgggaag  25

MetLeuLeuLeuProLeuProLeuLeuLeuPhePheLeuCysSerArgAlaGluAlaGly[-2]
atgctgcttcttcctctccccctgctgctctttttttgtgctccagagctgaagctggg  85

GluIleIleGlyGlyThrGluCysLysProHisSerArgProTyrMetAlaTyrLeuGlu[19]
gagatcatcgggggcacagaatgcaagccacactcccgcccctacatggcctacctggaa 145

IleValThrSerAsnGlyProSerLysSerCysGlyGlyPheLeuIleArgArgAsnPhe[40]
attgtcacctccaacggtccctcaaaatcttgtggtggtttcctgataagacggaacttt 205

ValLeuThrAlaValHisCysAlaGlyArgSerIleThrValThrLeuGlyAlaHisAsn[59]
gtgctgacagccgttcattgtgcaggaaggtctataacagtcacccttggagcccataac 265

IleThrGluLysGluAspThrTrpGlnLysLeuGluValIleLysGlnPheArgHisPro[79]
ataacagagaaagaagacacatggcagaagcttgaggttataaagcaattccgtcatcca 325

LysTyrAsnThrSerThrLeuHisHisAspIleMetLeuLeuLysLeuLysGluLysAla[99]
aaatataacacttctactcttcaccacgatatcatgttactaaagttgaaggagaaagcc 385

SerLeuThrLeuAlaValGlyThrLeuProPheProSerGlnPheAsnPheValProPro[119]
agcctgaccctggctgtggggacactccccttcccatcacaattcaactttgtcccacct 445

GlyArgMetCysArgValAlaGlyTrpGlyArgThrGlyValLeuLysProGlySerAsp[139]
gggagaatgtgccgggtggctggctggggaagaacaggtgtgttgaagccgggctcagac 505

ThrLeuGlnGluValLysLeuArgLeuMetAspProGlnAlaCysSerHisPheArgTyr[159]
actctgcaagaggtgaagctgagactcatggatccccaggcctgcagccacttcagatac 565

PheAspHisAsnLeuGlnLeuCysValGlyAsnProArgLysThrLysSerAlaPheLys[179]
tttgaccacaatcttcagctgtgtgtgggcaatcccaggaagacaaaatctgcatttaag 625

GlyAspSerGlyGlyProLeuLeuCysAlaGlyValAlaGlnGlyIleValSerTyrGly[199]
ggagactctgggggccctcttctgtgtgctggggtggcccagggcattgtatcctatgga 685

ArgLeuAspAlaLysProProAlaValPheThrArgIleSerHisTyrArgProTrpIle[219]
cggttggatgcaaagccccctgctgtcttcacccgaatctcccattaccggccctggatc 745

AsnLysIleLeuGlnAlaAsn***                                    [226]
aacaagatcctgcaggcaaattaaccctggatcctgagccagcctgaa 793
```

Fig. 1. Nucleotide sequence and deduced amino-acid sequence of monkey chymase. The deduced encoded amino acids are indicated below the corresponding nucleotides. The amino-terminal sequence determined with the purified monkey chymase is underlined. The nucleotide sequence data is available from DDJB/EMBL/GenBank with accession No. AB000823.

BRL, Gaithersburg, MD). PCR primers for amplification of monkey chymase were designed based on the 5′ untranslated region and the 3′ untranslated region of human chymase cDNA [11], (sense strand: 5′-TTGCTCAGGCAGCCTCTCTG-3′, position 234–253; antisense strand: 5′-TTCAGGCTGGCTCAGGATCC-3′, position 2994–2975). Two μl of the reverse transcriptase reaction mixture were added to 98 μl of the PCR buffer containing 25 pmol each of the upstream and downstream primers, 100 μM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase (Boehringer Mannheim GmbH, Mannheim, Germany). The amplification profile was run for 40 cycles: 1 min at 94°C, 1 min at 63°C, and 1 min at 72°C. A single 793 bp product was subcloned into a pBS II KS vector, and subjected to nucleotide sequencing. For the determination of the accurate sequence, we used the five PCR products amplified independently.

2.5. Quantitative reverse transcription (RT)-PCR analysis

Total RNA was isolated from the thoracic aorta as described previously [10]. Single-stranded cDNA was synthesized from 4 μg of the total RNA. A competitive RT-PCR reaction was performed using a modified procedure of Shiota et al. [12]. The PCR primers were selected according to the monkey chymase cDNA sequence (sense primer: 5′-GGAAGATGCTGCTTCTTCCTC-3′, position 21–41; antisense primer: 5′-TTTGTCTTCCTGGGATTGCC-3′, position 612–593). The competitor DNA was obtained by deleting the 201-bp fragment using the restriction enzymes StuI and EcoRV. The PCR products for the target monkey chymase and the competitor were 592 and 391 bp, respectively.

2.6. Histological analysis

The area of the atherosclerotic lesion of the thoracic aorta was measured as described previously [13]. The thoracic aortas were stained with oil red O for visualization of the presence of lipid depos-

Table 1
Purification of chymase from monkey cheek pouch vascular tissues

Purification step	Total protein (mg)	Chymase activity (mU)	Specific chymase activity (mU/mg protein)	Recovery (%)
1. Membrane extract	1825	6000	3.29	100
2. Filtration on PM-10	1386	5124	36.97	85.4
3. Heparin affinity column	2.120	3794	1789.6	63.2
4. Gel filtration column	0.240	1042	4340.8	17.4

```
          1       10        20        30        40        50        60
Monkey    IIGGTECKPHSRPYMAYLEIVTSNGPSKSCGGFLIRRNFVLTAAHCAGRSITVTLGAHNI
Human     ----------------------------F-------------------------------
Dog       ------S---------H---L-LRNHLA----------------------F-M--------
RMCP I    ----V-SR--------H---T-ER-YKAT-----VT-Q--M-----K--ET-----V-DV
RMCP II   ----V-SI--------H-D---EK-LRVI------S-Q--------K--E---I----DV

                 70        80        90       100       110       120
Monkey    TEKEDTWQKLEVIKQFRHPKYNTSTLHHDIMLLKLKEKASLTLAVGTLPFPSQFNFVPPG
Human     --E-------------------------------------------------------
Dog       QKE-----------------DDL--R-----------N---------LSP--------
RMCP I    SKT-S-Q--IK-E--IV--N--FYSNL--------QK--KV-P--DVI-L-QPSD-LK--
RMCP II   RKR-STQ--IK-E--II-ES--SVPNL--------EK-VE--P--NVV-L--PSD-IH--

                130       140       150       160       170       180
Monkey    RMCRVAGWGRTGVLKPGSDTLQEVKLRLMDPQACSHFRYFDHNLQLCVGNPRKTKSAFKG
Human     ------------------------------------D--------------------
Dog       ---------KRQ-NGS-----------------R-YMA----------------------
RMCP I    K---A----Q---T--T-N--R---Q-I--KE--KNYFHYNY-FQV---S---IR--Y--
RMCP II   A--WA----K---RD-T-Y--R--E--I--EK--VDY--YEYKF-V---S-TTLRA--M-

                190       200       210       220
Monkey    DSGGPLLCAGVAQGIVSYGRLDAKPPAVFTRISHYRPWINKILQAN
Human     -------------------S-------------------Q-----
Dog       -------------------QN-------------------V-KQ-KA
RMCP I    ------V-----H-------G------------P-V-----VIKGKD
RMCP II   ------------H------HP------I---V-T-V----AVINTSS
```

Fig. 2. Comparison of deduced amino-acid sequence of between monkey chymase with those of other chymases. The deduced amino-acid sequence of monkey chymase compared with those of human chymase [16,17], dog chymase [25], RMCP I [26] and RMCP II [20].

its. The atheromatic area was calculated as the ratio of the oil-red stained area to all of the intima area.

3. Results and discussion

The membrane extract of monkey cheek pouch vascular tissues on a heparin affinity column yielded a single peak at 1.6 M KCl. After the active fractions were concentrated and applied on a gel filtration column, a 1319-fold purification was achieved with a recovery rate of 17.4% and a specific activity of 4341 mU/mg (Table 1). The purified enzyme has a single band at 30 kDa on SDS-PAGE. The optimum pH of the Ang II-forming activity of the purified enzyme was between 7.5 and 8.5. The enzyme activity was inhibited by 10 μM SBTI, 1 mM PMSF and 100 μM chymostatin, but not by 1 mM EDTA, 1 mM phosphoramidon, 1 mM pepstatin, 10 mM captopril, 10 mM lisinopril and 1 mM aprotinin; therefore, the possibility that this activity was cathepsin G [14] or tonin [15] was ruled out. The sequence of the first 24 amino acids of the purified enzyme exhibited a high homology to that of human chymase (100%), dog chymase (78%), rat chymases (RMCP I, 71%; RMCP II, 71%), and hamster chymase (78%). We identified this Ang II-forming protease as monkey chymase.

Chymase is a chymotrypsin-like endopeptidase that hydrolyzes the carboxy-terminal side of aromatic amino acids such as Phe, Tyr, and Trp. Chymases isolated from human [3,16,17], dog [18,19] and rat [20–22] show similar substrate-binding profiles when 4-nitroanilide substrates are used [23]; however, they behave differently when hormonal peptides are used. RMCP I [4,5] and dog chymase [24] hydrolyze the Phe^{7}-Phe^{8} bond of substance P and the Tyr^{22}-Leu^{23} bond of vasoactive intestinal peptide (VIP), and RMCP I [4,5] cleaves the Tyr^{4}-Ile^{5} bond of Ang II, while human chymase hydrolyzes none of these bonds [3]. The monkey chymase isolated in this study rapidly converted Ang I to Ang II (K_m = 98 μM, V_{max} = 2.48 nmol/min, $k$cat = 6203/min) but did not degrade several peptide hormones including substance P, VIP, and Ang II (Table 2). These results indicate that primate chymases may have a higher substrate specificity than non-primate chymases.

The sequence of 247 amino acids deduced from the cDNA open reading frame includes peptides identical to the amino-terminal sequence of the purified monkey chymase (Fig. 1). The first 19 residues of monkey prepro chymase represent the hydrophobic signal peptide, and the next prosequence of 2 amino acids is removed to give a mature enzyme of 226 amino acids. Based on the number of basic (Arg+Lys = 29)

Table 2
Comparison of substrate specifities of monkey chymase with human chymase and RMCP I

Peptide substrates	Monkey chymase	Human chymase	RMCP I
Ang I	C	C	C
Ang II	N	N	C
Bradykinin	N	N	N
LH-RH	T	N	–
Met-Enkephalin	N	T	–
α-MSH	T	N	–
Somatostatin	N	–	C
Substance P	N	T	C
VIP	N	N	C

C, complete, T, trace, N, none.

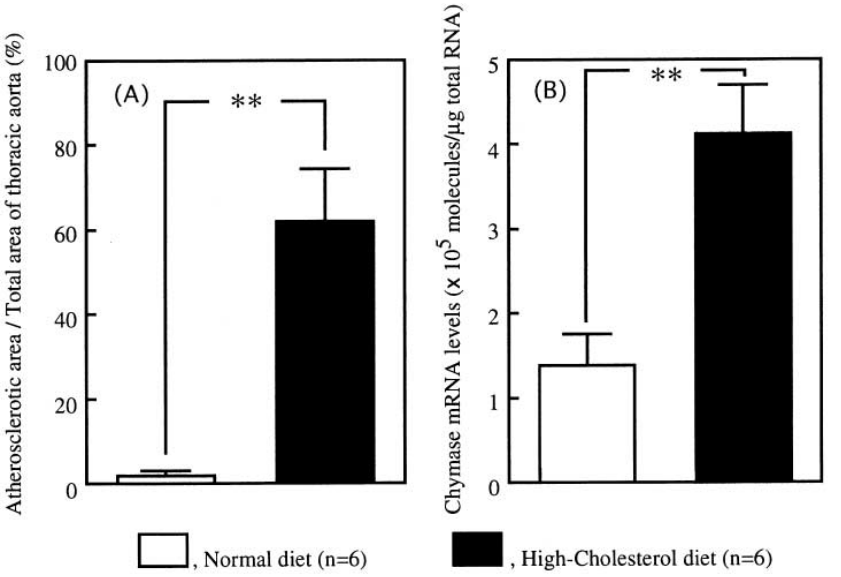

Fig. 3. (A) The atherosclerotic area of the thoracic aorta of monkeys fed a normal or a high-cholesterol diet. (B) The aortic chymase mRNA levels of monkeys fed a normal or a high-cholesterol diet. Vertical bars represent S.E.M. $^{**}P < 0.01$ vs. respective monkeys fed a normal diet.

and acidic (Asp+Glu = 14) amino acids, the monkey chymase catalytic domain has a predicted net charge of +15 (Fig. 1), and that of human chymase [16,17], dog chymase [25] and RMCP I [26] are +13, +16 and +18, respectively. Despite the differences in net charges, chymases are reported to bind to heparin glycosaminoglycan [27–29]; therefore, it is reasonable to assume that monkey chymase will bind in the same manner. Like human chymase, monkey chymase has two consensus N-linked glycosylation sites (Asn-X-Ser/Thr) at Asn^{59} and Asn^{82} (Fig. 1). Dog chymase also has two sites, but at Asn^{100} and Asn^{134} [25], and no such sites are found in RMCP I [26] and RMCP II [20]. The predicted N-linked glycosylation sites in monkey chymase, as deduced from the crystallography of RMCP II [22], are some distance away from the active-site binding cleft, which coincides with our observation that the substrate specificity of the native monkey chymase for hormonal peptides did not change after deglycosylation (unpublished data). The deduced primary structure of monkey chymase is more homologous to that of human chymase (98%) than dog chymase (84%) and rat chymases (RMCP I, 61%; RMCP II, 59%) (Fig. 2).

In the present study, we further investigated the pathophysiological role of vascular chymase in the atherosclerotic aorta. After loading with a high-cholesterol diet for 6 months, remarkable atherosclerotic degeneration was observed in the thoracic aorta and the chymase mRNA levels of monkeys fed the atherogenic diet were triple the levels of monkeys fed the normal diet (Fig. 3). Recently, we reported that an Ang II receptor antagonist significantly prevented the formation of atherosclerotic lesions in monkeys fed a high-cholesterol diet [30]. Chymase is considered to be derived from mast cells [31,32]. Kaartinen et al. demonstrated that the number of activated mast cells was increased in human atherosclerotic lesions [33]. These findings suggest that the chymase-dependent Ang II-forming pathway may be related to the pathogenesis of atherosclerosis.

In conclusion, the primary structure of monkey chymase is highly homologous to human chymase and has a higher specificity of Ang II-formation than do non-primate chymases. In the present study, we first demonstrated that vascular chymase mRNA is remarkably increased in the monkey aorta concurrently with the development of atherosclerosis.

Acknowledgements: This work was supported in part by Grant-in-Aid for Scientific Research (B) 08457031 from the Ministry of Education, Science, Sports and Culture, Japan, and by a research grant (1997) from Jinsenkai, the alumni of Osaka Medical College.

References

[1] Okunishi, H., Oka, Y., Shiota, N., Kawamoto, T., Song, K. and Miyazaki, M. (1993) Jpn. J. Pharmacol. 62, 207–210.
[2] Reilly, C.F., Tewsksbury, D.A., Schechter, N.M. and Travis, J. (1982) J. Biol. Chem. 257, 8619–8622.
[3] Urata, H., Kinoshita, A., Misono, K.S., Bumpus, F.M. and Husain, A. (1990) J. Biol. Chem. 265, 22348–22356.
[4] Wintroub, B.U., Schechter, N.B., Lazarus, G.S., Kaempfer, C.E. and Schwartz, L.B. (1984) J. Invest. Dermatol. 83, 336–339.
[5] Le Trong, H., Neurath, H. and Woodbury, R.G. (1987) Proc. Natl. Acad. Sci. USA 84, 364–367.
[6] Chobanian, A.V. (1990) Clin. Cardiol. 13, (suppl. 7) 43–48.
[7] Fennessy, P.A., Campbell, J.H. and Campbell, G.R. (1994) Clin. Sci. Colch. 87, 685–691.
[8] Takai, S., Shiota, N., Yamamoto, D., Okunishi, H. and Miyazaki, M. (1996) Life Sci. 58, 591–597.
[9] Okunishi, H., Miyazaki, M., Okamura, T. and Toda, N. (1987) Biochem. Biophys. Res. Commun. 149, 1186–1192.
[10] Shiota, N., Okunishi, H., Fukamizu, A., Sakonjo, H., Kikumori, M., Nishimura, T., Nakagawa, T., Murakami, K. and Miyazaki, M. (1993) FEBS Lett. 323, 239–242.
[11] Urata, H., Boehm, K.D., Philip, A., Kinoshita, A., Gabrovsek, J., Bumpus, F.M. and Husain, A. (1993) J. Clin. Invest. 91, 1269–1281.
[12] Shiota, N., Saegusa, Y., Nishimura, K. and Miyazaki, M. (1997) Clin. Exp. Pharmacol. Physiol. 24, 243–248.
[13] Miyazaki, M. (1994) Am. J. Hypertens. 7, 131S–140S.
[14] Tonnesen, M.G., Klempner, M.S., Austen, K.F. and Wintroub, B.U. (1982) J. Clin. Invest. 69, 25–30.
[15] Boucher, R., Saidi, M. and Genest, J. (1972) in: Hypertension '72 (Genest, J. and Koiw, E., Eds.), pp. 512–523

[16] Caughey, G.H., Zerweck, E.H. and Vanderslice, P. (1991) J. Biol. Chem. 266, 12956–12963.
[17] Urata, H., Kinoshita, A., Perez, D.M., Misono, K.S., Bumpus, F.M., Graham, R.M. and Husain, A. (1991) J. Biol. Chem. 266, 17173–17179.
[18] Caughey, G.H., Viro, N.F., Ramachandran, J., Lazarus, S.C., Borson, D.B. and Nadel, J.A. (1987) Arch. Biochem. Biophys. 258, 555–563.
[19] Caughey, G.H., Viro, N.F., Lazarus, S.C. and Nadel, J.A. (1988) Biochim. Biophys. Acta. 952, 142–149.
[20] Benfey, P.N., Yin, F.H. and Leder, P. (1987) J. Biol. Chem. 262, 5377–5384.
[21] Yurt, R. and Austen, K.F. (1977) J. Exp. Med. 146, 1405–1419.
[22] Remington, S.J., Woodbury, R.G., Reynolds, R.A., Matthews, B.W. and Neurath, H. (1988) Biochemistry 27, 8097–8105.
[23] Powers, J.C., Tanaka, T., Harper, J.W., Minematsu, Y., Barker, L., Lincoln, D., Crumley, K.V., Fraki, J.E., Schechter, N.M., Lazarus, G.G., Nakajima, K., Nakashino, K., Neurath, H. and Woodbury, R.G. (1985) Biochemistry 24, 2048–2058.
[24] Caughey, G.H., Leidig, F., Viro, N.F. and Nadel, J.A. (1988) J. Pharmacol. Exp. Ther. 244, 2048–2058.
[25] Caughey, G.H., Raymond, W.W. and Vanderslice, P. (1990) Biochemistry 29, 5266–5277.
[26] Le Trong, H., Newlands, G.F.J., Miller, H.R.P., Charbonneau, H., Neurath, H. and Woodbury, R.G. (1989) Biochemistry 28, 391–395.
[27] Forsberg, L.S., Lazarus, S.C., Seno, N., DeVinney, R., Caughey, G.H. and Gold, W.M. (1988) Biochim. Biophys. Acta 967, 416–428.
[28] Pejler, G. and Maccarama, M. (1994) J. Biol. Chem. 269, 14451–14456.
[29] Sayama, S., Iozzo, R.V., Lazarus, G.S. and Schechter, N.M. (1987) J. Biol. Chem. 262, 6808–6815.
[30] Miyazaki, M., Sakonjo, H., Takai, S. and Shiota, N. (1996) J. Hypertens. 14, (suppl. 1) S138.
[31] Schechter, N.M., Choi, J.K., Slavin, D.A., Deresienski, D.T., Sayama, S., Dong, G., Lavker, R.M., Proud, D. and Lazarus, G.S. (1986) J. Immunol. 137, 962–970.
[32] Schwartz, L.B., Irani, A-M.A., Roller, K., Castells, M.C. and Schechter, N.M. (1987) J. Immunol. 138, 2611–2615.
[33] Kaartinen, M., Penttila, A. and Kovernen, P.T. (1994) Circulation 90, 1669–1678.