Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 911–913

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Hydrophobic Modifications at 1-Phosphate of Inositol 1,4,5-Trisphosphate Analogues Enhance Receptor Binding

Waka Nakanishi,[a] Kazuya Kikuchi,[a,b] Takanari Inoue,[a] Kenzo Hirose,[c] Masamitsu Iino[c] and Tetsuo Nagano[a,*]

[a]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan*
[b]*Presto, JST Corporation, Kawaguchi, Saitama, Japan*
[c]*Department of Pharmacology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

Received 19 November 2001; accepted 11 January 2002

Abstract—Inositol 1,4,5-trisphosphate (IP_3) analogues were synthesized in order to investigate the importance of the environment of 1-phosphate of IP_3 for strong binding to the IP_3 receptor. Our results show that hydrophobic modifications of the 1-phosphate moiety enhance the binding affinity, with considerable latitude of substituent structure.

Inositol 1,4,5-trisphosphate (IP_3) is an important second messenger that evokes the release of Ca^{2+} from the intracellular stores through the IP_3 receptor (IP_3R). The Ca^{2+} signals thus generated regulate many aspects of cellular responses, such as secretion, fertilization, muscle contraction, neuronal signaling, cell growth and death.[1–3] Many IP_3 analogues have been synthesized to investigate their structure–activity relationship with respect to IP_3R. The recognition of ligands by IP_3R is stereospecific, the vicinal 4,5-bisphosphate and 6-hydroxyl motif being the crucial structural features, and the 1-phosphate further contributing to receptor binding specificity.[4,5] Most of the IP_3 analogues synthesized showed reduced affinity to IP_3,[6] though we have reported an IP_3 analogue that has high affinity for IP_3R.[7] Our analogue carries a hydrophobic and charged moiety located near the 1-phosphate of IP_3. To investigate the importance of the hydrophobicity, the bulkiness and the charge at this location, four analogues modified at the 1-position were newly synthesized and their binding affinity was measured using surface plasmon resonance (SPR). Our results suggest that highly hydrophobic substitution at the 1-phosphate position of IP_3 enhances the affinity to IP_3R, with a wide latitude of substituent structure. Additionally, cationic charge further contributes to strong interaction with IP_3R.

*Corresponding author. Tel.: +81-3-5841-4850; fax: +81-3-5841-4855; e-mail: tlong@mol.f.u-tokyo.ac.jp

Method

Synthesis

1-*O*-(3-Aminopropyl) ester of IP_3, **2**, was synthesized from DL-*myo*-inositol by the method previously reported.[8–10] Other IP_3 analogues were synthesized by coupling *N*-hydroxysuccinimide (NHS)-activated substituents with **2**. 1-Naphthoic acid *N*-hydroxysuccinimide ester (SE) was coupled to produce **3**, 1-pyrenebutyric acid SE to give **4**, leuco malachite green SE to give **5**, and 5-carboxytetramethyl-rhodamine to give **6** (Fig. 1). Enantiomers of **3** (**3′**), **4** (**4′**) and **5** (**5′**) were synthesized by a similar method. The synthesized compounds were all characterized by ^1H NMR and by MS(FAB).

Spectral data of newly synthesized compounds. 3 ^1H NMR (CD_3OD) 1.93 (m, 2H), 3.44–3.53 (m, 3H), 3.88–4.42 (m, 7H), 7.40–7.55 (m, 3H), 7.83 (dd, $J=2.0$, 7.3 Hz, 1H), 7.85 (d, $J=8.1$ Hz, 1H), 8.10 (d, $J=8.0$ Hz, 1H), MS(FAB) 632 $(M+1)^+$; **4** ^1H NMR (CD_3OD) 1.77 (m, 2H), 2.05 (m, 2H), 2.28 (t, $J=7.3$ Hz, 2H), 3.27 (m, 2H), 3.50–4.45 (m, 10H), 7.88–8.32 (m, 9H); MS(FAB) 748 $(M+1)^+$; **5** ^1H NMR (D_2O) 1.75 (m, 2H), 3.08 (s, 12H), 3.23–4.21 (11H), 7.03 (d, $J=7.8$ Hz, 2H), 7.15 (d, $J=7.8$ Hz, 4H), 7.36 (d, $J=7.8$ Hz, 4H), 7.52 (d, $J=7.8$ Hz, 2H), MS(FAB) 834 $(M+1)^+$; **6** ^1H NMR (D_2O) 1.92 (m, 2H), 3.03 (s, 12H), 3.46–4.15 (m, 12H), 6.39 (s, 2H), 6.77 (dd, $J=10$ Hz, 2H), 7.12 (d, $J=10$ Hz, 2H), 7.60 (d, $J=7.8$ Hz, 1H), 8.00 (dd, $J=7.8$ Hz, 1H), 8.10 (s, 1H), MS(FAB) 890 $(M)^+$.

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00044-6

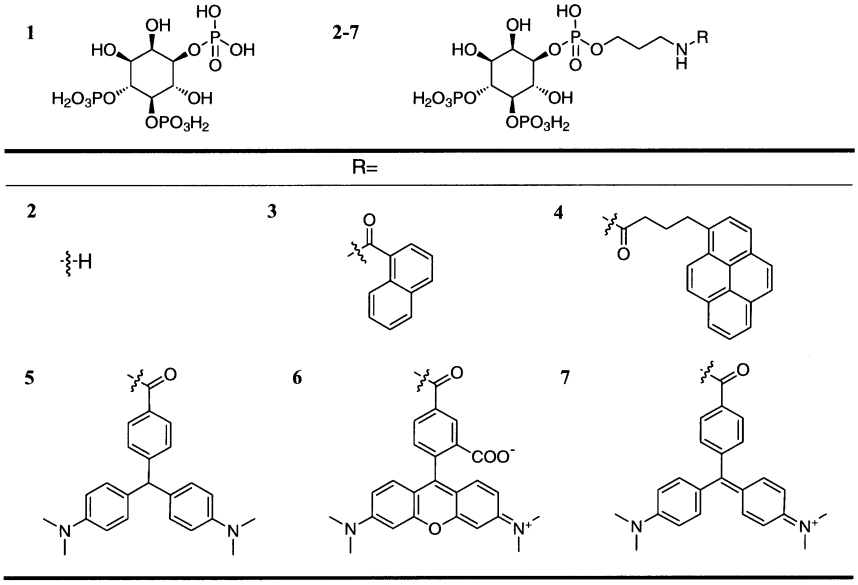

Figure 1. Structures of IP_3 (**1**) and novel (**3**–**6**) or reported (**2**, **7**) IP_3 analogues.

IP_3R binding assay

The IP_3-binding domain (IBD) of human IP_3 receptor type 1 was prepared in the same way as previously reported[7] except for purification using Talon™ Metal Affinity Resin (Clontech). The purity of the protein was confirmed by SDS–PAGE. Measurements of SPR were carried out using **2**-immobilized chips. The dissociation constant (K_d) of each compound was calculated from the SPR signals obtained from a competition assay (Fig. 2).

Calculation of stable conformations

The structures of the substituents of **5**, **6** and **7** including the amide group were energy-minimized using MM2 molecular mechanics calculations to find energetically stable conformations. After the energy minimization, the substituents were overlaid to assess their structural similarity (Fig. 3).

We have previously reported a 1-phosphate-modified IP_3 analogue (**7**) that has high affinity for IBD (170-fold higher affinity than IP_3).[7] Compound **7** has a substitutent with high hydrophobicity and cationic charge (Fig. 1).

To investigate the importance of steric factors and charges, **5** was synthesized. The substituent of **5** is a reduced form of that of **7**, and **5** was calculated to have a similar conformation to that of **7** [Fig. 3(b)]. The major difference between these analogues is that **7** has one cationic charge at the substitution site, but **5** does not. The binding affinity of **5** was estimated to be 90-fold lower than that of **7** [Table 1, Fig. 2(b)], indicating that cationic charge is important for the affinity to IP_3R.

Another analogue which has a similar structure [Fig. 3(c)] but has both cationic and anionic charges, **6**, was synthesized. Although **6** has a cationic charge, binding affinity was 300-fold lower than that of **7** (Table 1, Fig. 2(a)]. The reduced affinity might be due to the electronic repulsion between the anionic charge of **6** and IBD. However, the possibility remains that steric hindrance of oxygen at the bottom rings or the carboxyl moiety at the upper ring of the substituent [Fig. 1 (**6**)] might have lowered the affinity.

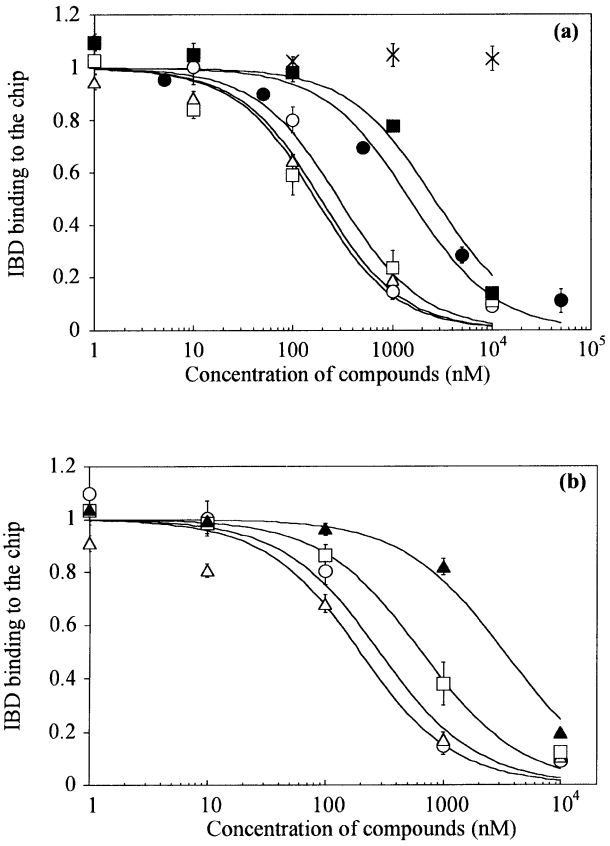

Figure 2. Binding assay of compounds. Dose–response relationship in competition assay of: (a) IP_3 (○), **2** (●), **3** (△), **4** (□), **4′** (■) and 1-naphthoic acid (×); (b) IP_3 (○), **5** (△), **5′** (▲) and **6** (□). Inhibition of IBD binding to **2**-immobilized chip by these compounds is shown. The values are mean ± SEM of at least three experiments.

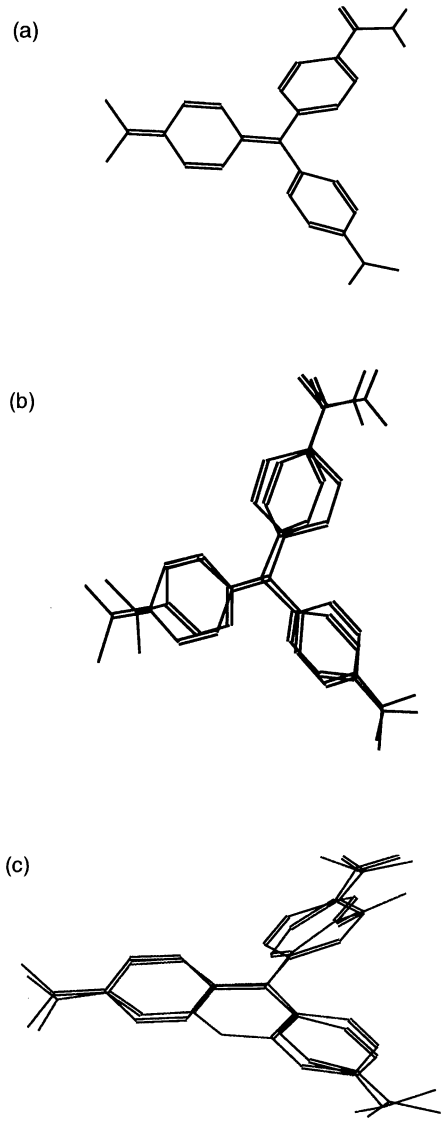

Figure 3. Substituent of **7** (a) and comparison of substituents between **7** and **5** (b) or **6** (c). These structures were all energy-minimized using MM2.

In order to analyze the importance of a hydrophobic substituent for increasing the affinity to IP_3R, **3** and **4** were synthesized. Though the modification of 1-phosphate to 1-*O*-(3-aminopropyl) ester (**2**) resulted in a 6-fold lower affinity than IP_3, modification of amine to naphthalene amide restored the affinity to the same level as that of IP_3. A more hydrophobic substituent, for example butyl pyrene, further enhanced the affinity. So far, adenophostins A and B are the most potent agonists to be reported.[11] Adenophostins have a bulky hydrophobic moiety at the putative 1-phosphate position. It was suggested that long-range interaction at this position might enhance the activity of adenophostins. Our finding that hydrophobic substitution at 1-phosphate enhanced the affinity for IP_3R supports the above suggestion, and also suggests that there might be a wide hydrophobic pocket proximal to the 1-phosphate site of IP_3R. By modifying the length of the linker, it should be possible to determine the size of the hydrophobic pocket.

Table 1. Dissociation constant of test compounds

Compound	K_d (nM)	Relative affinity compared with **2**[b]
1	260±82[a]	5.8
2	1500±350[a]	1
3	160±47[a]	9.4
3′	8400±2700[a]	0.18
4	140±58[a]	11
4′	2600±720[a]	0.58
5	170±70[a]	8.8
5′	3200±510[a]	0.47
6	620±87[a]	2.4
7	1.17	950

[a]Data are presented as mean±standard error of at least three experiments.
[b](K_d of **2**)/(K_d of the compound).

Enantiomers **3′**, **4′** and **5′** had about 20- to 50-fold lower affinity than their respective isomers [Table 1, Fig. 2(a) and (b)]. No non-specific interaction with 1-naphthoic acid was seen [Fig. 2(a)]. These results are consistent with highly specific ligand recognition by IP_3R.

In conclusion, linking a hydrophobic moiety to 1-phosphate of IP_3 enhances the binding of IP_3 analogues to IP_3R with considerable latitude of substituent structure. For strong interaction with IP_3R, a cationic charge adjacent to the 1-phosphate of IP_3 is important.

Acknowledgements

We thank Dr. M. Tanabe for her technical assistance and Drs. K. Mikoshiba and T. Furuichi for their kind gift of cDNA of human IP_3 receptor type 1. This work was supported in part by the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1. Berridge, M. J. *Nature* **1993**, *361*, 315.
2. Putney, J. W.; Bird, G. S. *Cell* **1993**, *75*, 199.
3. Clapham, D. E. *Cell* **1995**, *80*, 259.
4. Nahorski, S. R.; Potter, B. V. *Trends Pharmacol. Sci.* **1989**, *10*, 139.
5. Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933.
6. Wilcox, R. A.; Primrose, W. U.; Nahorski, S. R.; Challiss, R. A. J. *Trends Pharmacol. Sci.* **1998**, *19*, 467.
7. Inoue, T.; Kikuchi, K.; Hirose, K.; Iino, M.; Nagano, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1697.
8. Prestwich, G. D.; Marecek, J. F.; Mourey, R. J.; Theibert, A. B.; Ferris, C. D.; Danoff, S. K.; Snyder, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 1822.
9. Ozaki, S.; Kondo, Y.; Shiotani, N.; Ogasawara, T.; Watanabe, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 729.
10. Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 423.
11. Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, *269*, 369.