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# Synthesis of Some 1-Methyladenine Analogs and Their Biological Activities on Starfish Oocyte Maturation

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**Starfish oocytes are naturally arrested at the prophase stage of the first meiotic division and resume meiosis in response to the maturation-inducing hormone 1-methyladenine. Five analogs of 1-methyladenine including three novel ones were synthesized and tested for biological activities as 1-methyladenine agonists or antagonists in triggering reinitiation of meiosis of starfish *Asterina pectinifera* oocytes, as well as for competition in binding to putative 1-methyladenine receptors with respect to 1-methyladenine. 1-Ethyladenine was an effective agonist, but 1-propyladenine served as a weak antagonist to 1-methyladenine, indicating strict specificity for a relatively small N-1 substituent. Analogs in which carboxymethyl or methyl group substitutes for a hydrogen of 6-amino group still retained oocyte maturation-inducing activity, but to a much lesser degree. The results of the competitive binding assay with cortices of oocytes demonstrated that these agonists or antagonist inhibited the binding of [$^3$H]1-methyladenine to receptors. 8-methylamino-1-methyladenine competed only weakly with [$^3$H]1-methyladenine for the binding to cortices, although it behaved as a potent antagonist.**

**Key words:** maturation-inducing hormone; 1-methyladenine; oocyte maturation; receptor; starfish

Fully grown oocytes of starfish are arrested at the prophase stage of the first meiotic division. Meiosis is resumed in response to 1-methyladenine (1-MeAde), the maturation-inducing hormone (MIH) of starfish,[1] which is produced and released by the ovarian follicle cells under the influence of a peptide hormone (gonad-stimulating substance) from the radial nerve.[1]

Upon exposure of starfish oocytes to 1-MeAde, MPF becomes activated in the cytoplasm.[2] Although maturation-promoting factor (MPF), first discovered in frog oocytes,[3] has been identified as a complex of *cdc2* kinase (*cdk1*) with cyclin B,[4,5] the hormonal signal transduction pathway has largely not yet been described. Putative receptors for 1-MeAde have not yet been characterized biochemically, although the specific binding of 1-MeAde to the isolated cortices of starfish oocytes was reported by Yoshikuni *et al.*[6,7] and Tadenuma *et al.*[8] 1-MeAde-induced maturation of oocytes was inhibited by microinjection of pertussis toxin, suggesting the involvement of pertussis toxin-sensitive G-protein in the signal transduction pathway.[9] A starfish G-protein serving as a pertussis toxin substrate was purified from the plasma membranes of oocytes.[10] The cDNA of the $\alpha$ subunit was cloned and the deduced amino acid sequence was reported.[11] The $\beta\gamma$ subunits of starfish G-protein were shown to induce oocyte maturation when injected into cytoplasm[12] and to coexist with cytokeratin filaments in starfish oocytes.[13]

Isolation and characterization of 1-MeAde receptors would be beneficial to understanding the signal transduction from the receptors to MPF. Many analogs of 1-MeAde have been synthesized and tested for oocyte maturation-inducing activity.[14–17] However, the relationship between their biological activity and competitive binding to the 1-MeAde receptors has not yet been reported except for a few analogs of 1-MeAde.[6] Information about the structure-binding relationship for 1-MeAde analogs would be required for designing affinity adsorbents or photoaffinity labeling reagents for 1-MeAde receptors.

In this paper, we report the synthesis of five analogs of 1-MeAde including three novel ones and their biological activities as 1-MeAde agonists or antagonists. A method for synthesizing [*adenine*-2-$^3$H]1-MeAde, a novel radiolabeled 1-MeAde, and its use in competitive binding assay between [$^3$H]1-MeAde and unlabeled analogs for 1-MeAde receptors are also described here.

## Materials and Methods

*Materials.* Adenine and 1-MeAde were obtained from Sigma. The analogs of 1-MeAde used in this study (Fig. 1) were synthesized as described below. [2-$^3$H]Adenosine (20 Ci/mmol) was purchased from Amersham. All other chemicals were reagent-grade commercial

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*Abbreviations*: ASW, artificial seawater; 8-BrAdo, 8-bromoadenosine; CaFASW, $Ca^{2+}$-free artificial seawater; 1-CM-Ado, 1-carboxymethyladenosine; $N^6$-CM-Ado, $N^6$-carboxymethyladenosine; $N^6$-CM-1-MeAde, $N^6$-carboxymethyl-1-methyladenine; $N^6$-CM-1-MeAdo, $N^6$-carboxymethyl-1-methyladenosine; 1, $N^6$-$Me_2$Ade, 1, $N^6$-dimethyladenine; 1, $N^6$-$Me_2$Ado, 1, $N^6$-dimethyladenosine; 1-EtAde, 1-ethyladenine; GVBD, germinal vesicle breakdown; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate; MIH, maturation-inducing hormone; MPF, maturation-promoting factor; 1-MeAde, 1-methyladenine; $N^6$-MeAdo, $N^6$-methyladenosine; 8-MeNH-1-MeAde, 8-methyl amino-1-methyladenine; 8-MeNH-Ado, 8-methylaminoadenosine; 8-MeNH-1-MeAdo, 8-methylamino-1-methyladenosine; 1-PrAde, 1-propyladenine; TLC, thin-layer chromatography.

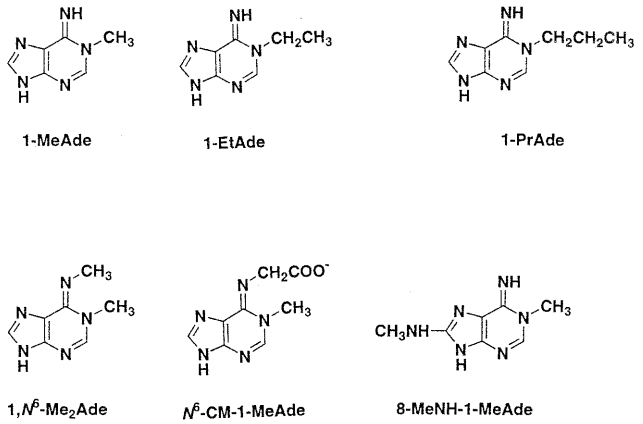


**Fig. 1.** Structures of the 1-MeAde Analogs Used in This Study.

products.

*Analytical procedures.* Protein was measured by the method of Lowry *et al.*[18] Visible and ultraviolet absorption spectra were measured at pH 1 (0.1 N HCl), 7 (0.1 M potassium phosphate buffer) and 13 (0.1 N NaOH) on a Union model SM-401 or Milton Roy recording spectrometers. Thin-layer chromatography (TLC) was done on Merck silica gel 60 $F_{254}$ or cellulose F coated glass plates. The solvent systems used were: (A) water-saturated 2-butanol; (B) solvent A containing 1% acetic acid; (C) solvent A containing 1% $NH_4OH$; (D) 2-propanol-water (1:1, v/v); (E) 3% (w/v) $NH_4Cl$. $^1$H-NMR spectra were obtained on a Varian VXR-500 NMR spectrometer operating in the Fourier transform mode. Tetramethylsilane in $CD_3OD$ sealed in a capillary tube immersed in the solution was used as a reference.

*Synthesis of 1-ethyladenine (1-EtAde) and 1-propyladenine (1-PrAde).* 1-EtAde and 1-PrAde were synthesized by the general directions of Jones and Robins[19] and purified by HPLC on a reverse-phase column (Cosmosil $5C_{18}$) using 1% acetic acid as the mobile phase. Both compounds thus obtained were homogeneous by the criteria of TLC ($R_f$ on silica gel in solvent B, 0.14 for both 1-EtAde and 1-PrAde) and HPLC.

1-EtAde. $^1$H NMR ($D_2O$): $\delta$ 0.79 (t, 3H, $J$=7.4 Hz), 3.67 (q, 2H, $J$=7.3 Hz), 7.64 (s, 1H), 7.82 (s, 1H). UV: $\lambda_{max}$ in nm ($\varepsilon \times 10^{-3}$ in $M^{-1}$ $cm^{-1}$) 260.5 (10.7) at pH 1, 266 (10.2) at pH 7, 272.5 (12.9) at pH 13.

1-PrAde. $^1$H NMR ($D_2O$): $\delta$ 0.27 (t, 3H, $J$=7.4 Hz), 1.1–1.2 (m, 2H), 3.54 (t, 2H, $J$=7.5 Hz), 7.52 (s, 1H), 7.68 (s, H). UV: $\lambda_{max}$ in nm ($\varepsilon \times 10^{-3}$ in $M^{-1}$ $cm^{-1}$) 261 (10.4) at pH 1, 266.5 (11.8) at pH 7, 272 (12.2) at pH 13.

*Synthesis of 1, $N^6$-dimethyladenine (1, $N^6$-$Me_2$Ade).* To a solution of 100 mg of $N^6$-methyladenosine ($N^6$-MeAdo) in 2 ml of *N,N*-dimethyl acetamide was added 0.2 ml of methyl iodide. The mixture was left at room temperature for 4 days. As judged by TLC, the majority of $N^6$-MeAdo had been converted to 1,$N^6$-dimethyladenosine (1,$N^6$-$Me_2$Ado) by this time. The reaction mixture was diluted with 8 ml of water and put on a column of CM-cellulose ($H^+$ form). After washing the column successively with water and 30% ethanol, the desired product was eluted with 30% ethanol containing 1 N $NH_4OH$. The eluate containing 1,$N^6$-$Me_2$Ado was evaporated to dryness. 1,$N^6$-$Me_2$Ado obtained was dissolved in 2 ml of 0.5 N HCl and heated for 1 hr on a boiling water bath. After being cooled, the solution was neutralized with 1 N NaOH. The desired product, 1,$N^6$-$Me_2$Ade, was then purified by HPLC on a reverse-phase column (Cosmosil $5C_{18}$) using 1% acetic acid as the mobile phase. The compound thus obtained was homogeneous by the criteria of TLC ($R_f$ on silica gel in solvent B, 0.11) and HPLC. $^1$H NMR ($D_2O$): $\delta$ 2.93 (s, 3H), 3.16 (s, 3H), 7.64 (s, 1H), 7.74 (s, 1H). UV: $\lambda_{max}$ in nm ($\varepsilon \times 10^{-3}$ in $M^{-1}$ $cm^{-1}$) 262.5 (10.4) at pH 1, 269 (9.6) at pH 7, 275 (10.8) at pH 13.

*Synthesis of $N^6$-carboxymethyl-1-methyladenine ($N^6$-CM-1-MeAde).* 1-Carboxymethyladenosine (1-CM-Ado) was synthesized from adenosine and iodoacetic acid by the method of Lindberg and Mosbach[20] for the synthesis of 1-CM-AMP. 1-CM-Ado was almost quantitatively converted to $N^6$-carboxymethyl-1-methyladenosine ($N^6$-CM-Ado) by heating at 70–72°C for 2 hr at pH 11 (Dimroth rearrangement). The desired product was purified by DEAE-cellulose (acetate form) chromatography. To a solution of 100 mg of $N^6$-CM-Ado in 2 ml of *N,N*-dimethylacetamide was added 0.2 ml of methyl iodide. The mixture was left at room temperature for 6–12 days. The majority of $N^6$-CM-Ado had been converted to $N^6$-CM-1-MeAdo by this time. The reaction mixture was diluted with 8 ml of water and put on a column of phosphocellulose ($H^+$ form). After washing the column successively with water and 30% ethanol, the desired product $N^6$-carboxymethyl-1-methyladenosine ($N^6$-CM-1-MeAdo), was eluted with 30% ethanol containing 0.1 N $NH_4OH$. $N^6$-CM-1-MeAde was obtained by acid hydrolysis of $N^6$-CM-1-MeAdo and purified by HPLC, as described above for the synthesis of 1,$N^6$-$Me_2$Ade. The compound thus obtained was homogeneous by the criteria of TLC ($R_f$ on silica gel in solvent B, 0.10) and HPLC. $^1$H NMR ($D_2O$): $\delta$ 3.22 (s, 3H), 3.95 (s, 2H), 7.54 (s, 1H), 7.76 (s, 1H). UV: $\lambda_{max}$ in nm ($\varepsilon \times 10^{-3}$ in $M^{-1}$ $cm^{-1}$) 268 (9.9) at pH 1, 272 (9.6) at pH 7, 276 (10.4) at pH 13.

*Synthesis of 8-methylamino-1-methyladenine (8-MeNH-1-MeAde).* To 4 ml of 30% aqueous methylamine solution were added 100 mg of 8-bromoadenosine (8-BrAdo). After 22 hr at room temperature, the mixture was evaporated to dryness. The majority of the 8-BrAdo had been converted to 8-methylaminoadenosine (8-MeNH-Ado) as judged by TLC. The residue was dissolved in 2 ml of *N,N*-dimethylacetamide, and 0.3 ml of methyl iodide was added to the solution. After 5 hr at room temperature, the mixture was diluted with 18 ml of water, adjusted to pH 2.8 with HCl, and put on a column of phosphocellulose ($H^+$ form). After the column was washed successively with water and 30% ethanol, 8-methylamino-1-methyladenosine (8-MeNH-1-MeAdo) was eluted with 30% ethanol containing 0.1 N

$NH_4OH$. 8-MeNH-1-MeAde was obtained by acid hydrolysis of 8-MeNH-1-MeAdo and purified by HPLC, as described above for the synthesis of $1,N^6$-$Me_2$Ade. The compound thus obtained was homogeneous by the criteria of TLC ($R_f$ on silica gel in solvent B, 0.13) and HPLC. $^1H$ NMR ($D_2O$): $\delta$ 2.29 (s, 3H), 3.15 (s, 3H), 7.57 (s, 1H). UV: $\lambda_{max}$ in nm ($\varepsilon \times 10^{-3}$ in $M^{-1}$ $cm^{-1}$) 280 (7.3) at pH 1, 293.5 (8.9) at pH 7, 291.5 (7.6) at pH 13.

*Synthesis of [2-$^3$H]1-MeAde.* A mixture of [2-$^3$H] adenosine (222 nmol, 3.2 mCi) and 10 $\mu$l of methyl iodide in 100 $\mu$l of *N,N*-dimethylacetamide was left at room temperature for 66 h. Unreacted methyl iodide and the solvent were removed by extraction with 1.5 ml of diethyl ether three times. The residue was dried under reduced pressure and dissolved in 100 $\mu$l of 0.5 N HCl. The solution was heated at 96°C for 10 min in a sealed tube. The mixture was then neutralized by passing it through a column of DEAE-cellulose (bed vol, 2 ml) and effluent (6 ml) was evaporated to dryness. The residue was dissolved in 40 $\mu$l of 70% ethanol, and [2-$^3$H]1-MeAde was isolated by TLC on cellulose F plates in solvent E. The UV-absorbing area corresponding to 1-MeAde ($R_f$, about 0.7) was scraped off and eluted with 0.5% NaCl solution. The purity and radiochemical purity of [2-$^3$H]1-MeAde thus obtained were established by spectral measurement and by paper chromatography in solvent E, respectively. The specific activity was 6.8 Ci/mmol, and the isolation yield was approximately 58% theoretical. Synthetic 1-MeAde ($R_f$ in TLC on silica gel in solvent B, 0.10) obtained in the cold run was confirmed to be active in inducing maturation of starfish oocytes.

*Animals and preparation of oocytes.* Starfish, *Asterina pectinifera*, collected during the breeding season near Hashirimizu, Asamushi, and Ushimado, Japan were kept in laboratory aquaria supplied with circulating artificial seawater (products from Senju Pharmaceutical Co., Ltd., Kobe, and Rohto Pharmaceutical Co. Ltd., Osaka) (14°C).

Immature oocytes without follicle cells were released from dissected ovaries by the transfer from $Ca^{2+}$-free artificial seawater (CaFASW)[6] to modified van't Hoff's artificial seawater (ASW).[21] Dejellied oocytes were prepared as described by Yoshikuni *et al.*[6]

*Biological activities of analogs as 1-MeAde agonists or antagonists.* Biological activities of 1-MeAde analogs were assayed in plastic plates with 1.5 ml wells. Immature, follicle-free oocytes were incubated at room temperature with various concentrations of 1-MeAde analogs in ASW. After 1 hr, the percentage of oocytes without germinal vesicle was measured by counting at least 100 oocytes. Oocyte maturation-inducing activity (% germinal vesicle breakdown (GVBD)) was evaluated by subtracting the percentage of spontaneous maturation (usually less than 10%). $EC_{50}$ for agonists is defined as the concentration for inducing 50% GVBD.

For the analogs that did not induce oocyte maturation, the antagonistic effect toward 1-MeAde was measured. In this case, oocytes were incubated with a mixture of various concentrations of analogs and 1-MeAde for 1 hr in ASW. $IC_{50}$ for antagonists is defined as the concentration for 50% inhibition of GVBD in the presence of 0.2 $\mu$M 1-MeAde. A 50%-inhibition index ($I_{50}$) value was defined by the following equation:

$$I_{50} = IC_{50}/[\text{1-MeAde}] = IC_{50}(\mu M)/0.2(\mu M)$$

Fertilizability of eggs which underwent GVBD in the presence of analogs was also examined by microscopic observation of elevation of the fertilization membrane and cleavage after insemination.

*Preparation of cortices.* Cortices of starfish oocytes were prepared from dejellied oocytes, essentially as described by Yoshikuni *et al.*[6] Dejellied oocytes were suspended in 5 volumes of hypotonic burst buffer (50 mM KCl/1 mM $MgCl_2$/2 mM EGTA/1 mM phenylmethanesulfonyl fluoride/10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (HEPES) buffer adjusted to pH 7.4 with KOH) on ice. After 30 min with gentle shaking, swollen oocytes were gently homogenized with a Dounce-type glass homogenizer (3 strokes by hand). The homogenates were left for 60–80 min on ice and the supernatant was removed. Ghost cells were suspended in 5 ml of cold wash buffer (burst buffer minus phenylmethanesulfonyl fluoride) and left to precipitate for 60-90 min. Ghost cells were washed again with the same buffer and finally with the cold uptake buffer (ASW (pH 7.4) buffered with 10 mM HEPES instead of borate) by repeating these procedures. A suspension of ghost cells in the uptake buffer at a concentration of about 125,000 cells/ml was used as cortices in the [$^3$H]1-MeAde binding assay.

*[$^3$H]1-MeAde binding assay and competitive binding assay.* [$^3$H]1-MeAde binding assay was done essentially as described by Yoshikuni *et al.*[6] with some modifications. Cortices (about 8,000–14,000) were incubated at 25°C for 20 min with various concentrations of [$^3$H]1-MeAde in 0.1 ml of the uptake buffer (pH 7.4) in the presence (nonspecific binding) or absence (total binding) of 100 $\mu$M unlabeled 1-MeAde. The suspension was then cooled for 5 min in an ice-water bath, diluted with 1 ml of ice-cold uptake buffer and filtered rapidly through a glass fiber filter (GC-50, 25 mm, Toyo).[8] The filter was washed 4 times with 2 ml of the same buffer. After air-drying, the radioactivity retained in the filter was measured by liquid scintillation counting using a naphthalene-dioxane counting fluid (100 g of naphthalene and 5 g of 2,5-diphenyloxazole (PPO) in 1 l of 1,4-dioxane) as scintillant.[22] The specific binding of [$^3$H]1-MeAde was evaluated by subtraction of nonspecific binding from total binding. A competitive binding assay was done in a similar manner by incubation of cortices with a mixture of 0.1 $\mu$M [$^3$H]1-MeAde and 10 or 100 $\mu$M unlabeled analogs.

## Results

*Oocyte maturation-inducing activity of analogs*

Among the five 1-MeAde analogs tested, 1-EtAde, $N^6$-CM-1-MeAde, and 1,$N^6$-$Me_2$Ade showed GVBD-inducing activity (Table I). The oocytes incubated for 1 hr with these analogs not only underwent GVBD but also extruded two polar bodies. Furthermore, as shown in Table I, the eggs treated by them were fertilizable upon insemination. Elevation of the fertilization membrane was observed, and the fertilized eggs underwent cleavage and normal early development. Therefore, it can be concluded that these three analogs function as MIH as 1-MeAde, the natural hormone, does. From the $EC_{50}$ values shown in Table I, the effectiveness of analogs as 1-MeAde agonists were in the following order: 1-MeAde > 1-EtAde > $N^6$-CM-1-MeAde > 1,$N^6$-$Me_2$Ade, $N^6$-CM-1-MeAde and 1,$N^6$-$Me_2$Ade are the first reported agonists with a substituent in the $N^6$ position. Polarity of a substituent at $N^6$ seems dispensable for either the hormonal activity or the binding to receptors. Among the N-1-substituted analogs, 1-EtAde served as very effective MIH. 1-PrAde was essentially inactive even at a concentration up to 3 mM, although for some individuals (one per six), it functioned as a slightly active MIH at a concentration more than 3 mM. Thus, it is evident that the substitution of a bulkier alkyl group than ethyl for the methyl group on N-1 destroys the hormonal activity toward the *A. pectinifera* oocytes. This is in clear contrast to the results of Dorée *et al.*[15] using *Asterias rubens* or *Marthasterias glacialis* oocytes. 8-MeNH-1-MeAde did not induce GVBD at all even at a concentration of 2 mM.

*Inhibition of oocyte maturation by inactive analogs*

When the oocytes treated for 1 hr with 1-PrAde or 8-MeNH-1-MeAde were inseminated, neither elevation of fertilization membrane nor cleavage was observed (Table I). Thus, it was concluded that these analogs are totally inactive as MIH. When these two analogs were added to oocytes together with 0.2 μM 1-MeAde, they inhibited 1-MeAde-induced oocyte maturation in a concentration-dependent manner. This inhibition was reversed simply by washing the analog-treated oocytes with ASW, followed by re-addition of 1-MeAde, indicating that these inactive analogs served as antagonists to 1-MeAde. Their $IC_{50}$ values were measured at a 1-MeAde concentration of 0.2 μM (Table I). From $I_{50}$ values, it is evident that 8-MeNH-1-MeAde is a much stronger antagonist than 1-PrAde.

**Table I.** Biological Activities of Analogs as 1-MeAde Agonists or Antagonists.[a]

| Analog | $EC_{50}$ (μM) | $IC_{50}$ (μM) | $I_{50}$ | Induction of fertilizability |
|---|---|---|---|---|
| 1-MeAde | 0.08±0.01 | | | + |
| 1-EtAde | 0.96±0.06 | | | + |
| $N^6$-CM-1-MeAde | 78±6 | | | + |
| 1,$N^6$-$Me_2$Ade | 173±15 | | | + |
| 1-PrAde | Inactive[b] | 62±25 | 310 | − |
| 8-MeNH-1-MeAde | Inactive | 3.3±0.1 | 17 | − |

[a] Parameters are defined as described in the text. Values are the mean±S.D. of 6–8 experiments with oocytes from at least 3 individuals.

[b] For some individuals, 1-PrAde was slightly active as MIH at a concentration more than 3 mM.

*Competition between [$^3$H]1-MeAde and analogs for the binding to 1-MeAde receptors*

In order to estimate the affinity of receptors for 1-MeAde, binding assays were done using [$^3$H]1-MeAde and isolated cortices of starfish oocytes. When varied concentrations of [$^3$H]1-MeAde were incubated with oocyte cortices in the absence of analogs, specific binding of [$^3$H]1-MeAde to cortices depicted a saturation curve against free [$^3$H]1-MeAde concentration. One of the typical results is shown in Fig. 2. As illustrated in the *inset* of Fig. 2, a linear relationship was obtained in the Scatchard plot. An apparent dissociation constant ($K_d$) and a maximum number of binding sites ($B_{max}$) calculated from 2 independent experiments were 0.08±0.01 μM and 0.016±0.002 fmol/cell (0.9±0.1 pmol/mg of protein), respectively. This $K_d$ is in good agreement with the $EC_{50}$ for 1-MeAde (Table I).

Competitive binding assays were then done with 0.1 μM [$^3$H]1-MeAde and 10 or 100 μM unlabeled analogs. To compare the affinity of putative 1-MeAde receptors for analogs, relative specific binding of [$^3$H]1-MeAde to cortices (%) in the presence of analog to that in the absence of analog was calculated. As illustrated in Fig. 3, unlabeled 1-EtAde inhibited the binding of [$^3$H]1-MeAde to cortices most strongly among the analogs tested. $N^6$-CM-1-MeAde and 1,$N^6$-$Me_2$Ade also competed with [$^3$H]1-MeAde in a concentration-dependent manner, but to a lesser degree. 1-PrAde, a weak antagonist to 1-MeAde, also showed an inhibitory effect on the binding of [$^3$H]1-MeAde to cortices. The behavior of 8-MeNH-1-MeAde in the competitive binding assay was peculiar. Although this analog was a very strong an-

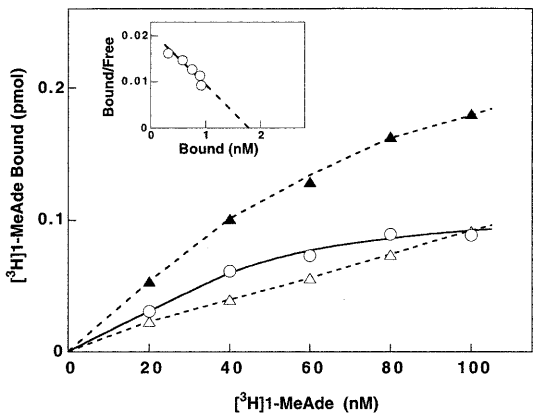


**Fig. 2.** Effect of Varying Concentration of [$^3$H]1-MeAde on the Binding to Receptors in Oocyte Cortices.

Oocyte cortices were incubated at 25°C for 20 min with the indicated concentration of [$^3$H]1-MeAde in the presence and absence of 100 μM unlabeled 1-MeAde. The specific binding was evaluated as described in the text. ▲---▲, Total binding; △---△, nonspecific binding; ○—○, specific binding. *Inset*, Scatchard plot of the data.

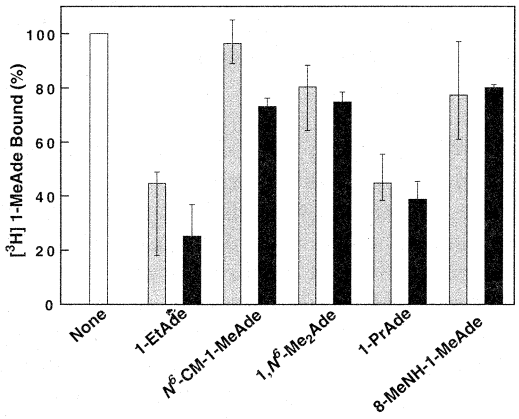


**Fig. 3.** Competition between [$^3$H]1-MeAde and Its Analogs for Binding to Receptors in Oocyte Cortices.

Oocyte cortices were incubated at 25°C for 20 min with a mixture of 0.1 $\mu$M [$^3$H]1-MeAde and 10 (*gray bars*) or 100 (*closed bars*) $\mu$M unlabeled analogs. Specific binding was evaluated as described in the text. The specific binding of [$^3$H]1-MeAde in the absence of unlabeled analog (*open bar*) was taken as 100%. The mean ± S.D. of 4 experiments are shown as histograms.

tagonist to 1-MeAde as judged from its biological effect on oocyte maturation ($I_{50}$ of 17 in Table I), it competed much more weakly with [$^3$H]1-MeAde than expected for the binding to cortices even at a concentration 1,000 times higher than 1-MeAde. Therefore, the antagonistic effect of 8-MeNH-1-MeAde seems not to be simply accounted for by its competitive binding for 1-MeAde receptors.

## Discussion

From the structure-function relationship studied so far using many analogs of 1-MeAde,[14-17] it has been demonstrated that (a) nonpolar substituent on N-1, (b) amino group on C-6, and (c) a positive charge upon protonation of the hormone are required for triggering the reinitiation of meiosis in starfish oocytes. Any substitution in the 7- or 9-position or the substitution at C-8 with a bulky group deprives analogs of the oocyte maturation-inducing activity.

The data presented in this paper led us to the following conclusions. First, 1-EtAde was an effective MIH as reported before,[14] but a bulkier substituent than ethyl at N-1 destroyed the hormonal activity toward *A. pectinifera* oocytes although 1-PrAde behaved as an antagonist. This is in sharp contrast to the results of Dorée *et al.*[15] and Mornet *et al.*[16] They reported that analogs with a much bulkier substituent, such as hexyl or benzyl, in the N-1 position are still active as MIH for *Asterias rubens* or *Marthasterias glacialis* oocytes. It is likely that the structures of the interacting sites of 1-MeAde receptors with the N-1-substituent are somewhat different between these species of starfish. Secondly $N^6$-CM-1-MeAde and 1,$N^6$-$Me_2$Ade were active MIH with a substituent at $N^6$. Thus, it can be concluded that bulkiness and polarity of the $N^6$-substituents do not affect hormonal activity or the affinity for receptors so markedly. $N^6$-Benzyl-1-MeAde has been the only $N^6$-substituted analog of 1-MeAde examined so far for MIH activity. This analog has been reported to be slightly active toward *A. rubens* oocytes.[14,17] Thirdly, 8-MeNH-1-MeAde was strongly antagonistic to 1-MeAde with a $I_{50}$ of 17. This finding is consistent with the paper of Monsees *et al.*[17] which reported that 8-$Me_2$N-1-MeAde serves as a potent antagonist. In contrast, 8-$N_3$-1-MeAde was an effective agonist[16,17] (Tsurukai, T., Komatsu, Y. and Toraya, unpublished results). Since three nitrogen atoms of the azido group are in line, the bulkiness of the C-8-substituent rather than its length may affect the hormonal activity of the C-8-substituted analogs.

Although some analogs have been reported to be 1-MeAde antagonists, their competition with 1-MeAde for the binding to 1-MeAde receptors has been investigated so far with only a few analogs. In this study, we developed a simple procedure for synthesizing and purifying [2-$^3$H]1-MeAde, a new labeled ligand for 1-MeAde receptors. This method of synthesis of [2-$^3$H]1-MeAde seems much easier than that developed for the synthesis of [*methyl*-$^3$H]1-MeAde.[6] By using this $^3$H-labeled ligand, we directly measured competitive binding between 1-MeAde and analogs to receptors. The values of $K_d$ for 1-MeAde and $B_{max}$ from Scatchard plot analysis in the absence of analogs coincided well with the $EC_{50}$ for 1-MeAde and in reasonable agreement with the reported values.[6,8] In general, the results of the competitive binding assay were comparable with the biological effects of analogs. Although 1-PrAde served as a weak antagonist, it showed competitive binding to receptors with [$^3$H]1-MeAde. This finding indicates that the binding to receptors is a prerequisite but not sufficient for the hormonal signal transduction. 8-MeNH-1-MeAde was exceptional. It competed with [$^3$H]1-MeAde only weakly for the binding to receptors although it was a potent antagonist to 1-MeAde. Therefore, this analog may exerts its inhibitory effect on oocyte maturation by some mechanism other than competitive binding to 1-MeAde receptors in starfish oocytes.

Biological effects of 1-MeAde analogs on oocyte maturation and their competitive binding to cortices of starfish oocytes described in this paper offer supporting evidence for the presence of 1-MeAde receptors on the surface of starfish oocytes. Isolation and identification of 1-MeAde receptors will be required for further investigation of the receptors, but good affinity adsorbents for the receptors are not yet available. Mapping the interacting sites of the hormone with receptors would be primarily important for designing the adsorbents.

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