

**Augmented Expression of the Tight Junction Protein Occludin
in Brain Endothelial Cell Line TR-BBB by Recombinant Rat Angiopoietin-1**

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The formation of tight junctions (TJs) is facilitated by the increased expression and phosphorylation of occludin, an integral membrane protein localizing at TJs in endothelial cells, but the physiological regulator of occludin expression is not known. Angiopoietin-1 (Ang-1) is a recently identified ligand of the endothelium-specific tyrosine kinase receptor Tie-2. Ang-1 knockout mice have hemorrhage from blood capillaries, while Ang-1 transgenic mice have leakage-resistant blood vessels. These reports suggest that Ang-1 may control blood-brain barrier (BBB) permeability *in vivo*. However, the regulation mechanism of BBB permeability by Ang-1 is unclear.

In this study, we isolated a cDNA encoding a 498-amino acid protein from rat placenta using reverse transcription-polymerase chain reaction (RT-PCR). The amplified DNA was cloned and sequenced. The rat Ang-1 was expressed in sf plus insect cells using the Bac-to-Bac baculovirus expression system. Moreover, we examined the effect of Ang-1 on TJs function through its effect on the expression of occludin.

At the level of amino acid sequence, the rat Ang-1 exhibited 97% and 96% identity to its mouse and human homolog, respectively. A major band of 65 kDa was detected mainly in the culture supernatant by western blot analysis. The observed molecular mass of the major band was larger than the calculated that of recombinant rat Ang-1 (50 kDa) since Ang-1 contained several potential glycosylation sites. Treatment of rat Ang-1 in conditionally immortalized rat brain capillary endothelial cell line TR-BBB13 for 48h induced approximately three-fold higher expression and phosphorylation of occludin in comparison with control. The upward band shift of occludin reveals the phosphorylation of occludin. Occludin phosphorylation has been implicated in the regulation of TJs function.

We show here that this is the first report of cloning and expressing the rat Ang-1 in the Bac-to-Bac baculovirus expression system. The rat Ang-1 induces the expression and phosphorylation of occludin in TR-BBB13 *in vitro*. Therefore, we suggest that Ang-1 regulates BBB permeability by formation of TJs.