Transport Performance of Nucleosides
through Nucleic Acid Bases Conjugated Hyaluronan

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The transport performance of nucleosides through the membranes of hyaluronic acid and deacetylated hyaluronan conjugated with nucleic acid base derivatives has been studied under varied temperature. Partition coefficient values of the permeants and permeabilities of the membranes showed the selectivity of nucleosides due to the effect of specific interaction between the permeants and nucleic acid base moiety in the membrane.

Since hyaluronic acid (HA) and hyaluronan (IUB-IUPAC nomenclature of hyaluronic acid derivatives)[1] are a potentially useful biomaterial, there are a number of reports dealing with the use of HA and its derivatives as a membrane and the studies on the properties of diffusion through the matrices of the membrane.[2] However, few reports are concerned about the modification or functionalization of HA derivatives with specific applications.[3] On this point of view, our group has focused on a new type of HA derivatives, the conjugation with nucleic acid analogs, which is expected to be a series of biocompatible materials owing to its specific interactions with DNA. Through this strategy, in the field of synthesis, our group has succeeded in the preparation of sulfonated[4] and deacetylated hyaluronan (DA-HA)[5] conjugated with nucleic acid bases, which were proved to show specific interaction and water soluble properties in basic study. Therefore, it is of our interest to study one of the potential application as a functional membrane. The transport mechanisms of this type of membrane would be not only due to the hydrophilicity and the microporous system of membrane matrices, in which the permeation was ascribed to the predominant mechanism of diffusion.[6] It would be also involved with the properties of nucleic acid base moiety in side chains. Thus, it is important to notify the hydrogen bonding between the solute and the functional group in the system to imply the partition mechanism. In this paper, thymine conjugated hyaluronan was applied to the materials for the membrane, and the nucleosides were used for the experiments as the model permeants to study the transport property of the membrane.

Deacetylated hyaluronan (DA-HA) conjugated with thymine base (DA-HA-Thy, figure 1) was synthesized as reported previously.[5] Membrane fabrication was as follows ; 5 ml of 2.5 wt% (w/v) HA-Na in formic acid (20wt% aqueous solution) containing 20 mg of DA-HA-Thy was prepared and degassed. To this solution, glutaric dialdehyde was added for 5 wt%, together with catalytic amount of 6 mol/L HCl. The mixture was casted on the petri dish at 40 °C for 8 h followed by drying at room temperature under reduced pressure for 2 h. In the case of HA membrane, the fabrication steps were the same but without adding DA-HA-Thy. Prior to use, membranes were
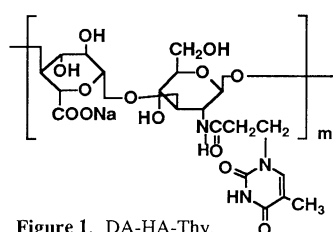

Figure 1. DA-HA-Thy.

presoaked in pH 7 buffer (1/10 M KH_2PO_4 - 1/20 M $Na_2B_4O_7$ ·10H2O) to remove unreacted crosslinker. HPLC assays were developed for quantitative analysis to all model permeants.

Membrane : buffer partition coefficients (K) of the model permeants were obtained by a solution depletion method. The partition coefficient values were calculated as the ratio of the concentration of model permeants in the membrane to the concentration in buffer.

The permeation of nucleic acid conjugated HA membrane was determined using two compartment glass diffusion cells. The obtained membranes were equilibrated for one hour in the buffer at room temperature before assembly into the cell. The initial nucleoside concentration of the donor compartment was adjusted to the UV absorbance value of 3.0 at λ_{max}. The permeation of the nucleoside interests through the membrane was studied by determining its concentration in the receiver compartment as a function of time at 5, 25 °C. Permeability coefficients(P) were calculated from the slope of the linear of the cumulative amount diffused versus time graphs, according to the method described by J. A. Hunt et al.[2]

Apparent diffusion coefficient values (D_{app}) of the model permeants were calculated using the equation $D_{app} = P / K$. Membrane hydration was calculated from the difference of final hydrated weight and dry membrane weight compared to dry membrane weight. The volume of hydrated membranes was estimated from swollen membrane in the buffer.

The obtained membrane showed the UV absorbance peak at 266 nm for 0.477, suggesting that thymine derivative contained in DA-HA-Thy membrane was 3.36 x 10^{-7}mol. Hydration study of HA and DA-HA-Thy membrane showed that DA-HA-Thy membrane was hydrated 17.9% less than that of HA membrane. This suggests that thymine moiety led the membrane to be less hydrophilic as expected by the relative hydrophobicity of functional groups.

Table 1 summarizes the experimental values of permeability(P), partition(K) and apparent diffusion coefficient (D_{app}) through studied membranes. The experimentally determined partition coefficient values ranged from 0.9 to 2.0. Generally, partition coefficients provide the information on the interactions of the solutes with the polymer membranes.[7] In the case of HA membrane, the partition coefficient value of uridine (1.51) was above 20% higher than those of adenosine and thymidine at 25 or 5 ° C, probably due to the hydrophilic nature of uridine permeant. This was confirmed by the smallest partition coefficient value of uridine in hydrophobic octyl alcohol system (Ado = 0.031, Urd = 0.017, Thd = 0.055, Guo = 0.033).

For DA-HA-Thy membrane, the partition values of uridine and thymidine were similar (0.9 to 1.1), irrespective of the temperature. The results reflected the distribution of the permeants on the membrane. Note that this similarity was also observed in the case of HA membrane. However, in the case of adenosine through DA-HA-Thy membrane, the partition value showed a large increase up to 39% when the temperature was

Table 1. Permeability (P), partition (K), and diffusion coefficients (Dapp) for nucleosides through HA and DA-HA-Thy membrane [a]

Membrane	Hydration $\%$	Nucleoside Permeant	Temp $°C$	$\dfrac{P \times 10^7}{cm^2/s}$	K	$\dfrac{Dapp \times 10^7}{cm^2/s}$
HA	394.29	Ado	5	4.17	1.12	3.72
			25	21.7	1.17	18.5
		Urd	5	5.69	1.78	3.20
			25	26.8	1.51	17.7
		Thd	5	4.52	0.98	4.61
			25	23.5	1.21	19.4
DA-HA-Thy	323.36	Ado	5	11.8	1.42	8.31
			25	19.5	0.88	22.2
		Urd	5	4.36	1.07	4.07
			25	21.7	0.93	23.3
		Thd	5	3.83	0.96	3.99
			25	20.2	0.90	22.4

a) membrane thickness = 0.015 $\pm$ 0.003 cm, and area = 0.785 cm^2.

lowered to 5 ° C. The partition value of adenosine through DA-HA-Thy membrane was 27% greater than that of HA membrane. This suggests the effective partitioning of adenosine through DA-HA-Thy membrane.

The observed permeability coefficient value of HA membrane at either 25 or 5 °C showed only small differences between nucleoside permeants. However, all permeability values were drastically reduced at low temperature. In the case of DA-HA-Thy, permeability coefficient value of adenosine was similar to other nucleosides at 25 °C, while it was nearly twice as large as those of other nucleosides at 5 °C. It should be noted that in either case of nucleosides through the HA or DA-HA-Thy membrane, the permeability coefficient values observed at 5 °C turned to be one-fifth of those observed at 25 °C. It should also be pointed out that in the case of adenosine through DA-HA-Thy membrane, the value at 5 °C (11.8) was three-fifths of the value (19.5) at 25 °C. These suggest the high permeability of adenosine across DA-HA-Thy membrane at low temperature.

Table 1 also provides a comparison between transport properties of HA and DA-HA-Thy membrane. At 25 °C, apparent diffusion coefficient values of HA and DA-HA-Thy membrane were similar. At 5 °C, diffusion coefficient values of all permeants were smaller due to the decrease of permeability coefficient. However, for DA-HA-Thy membrane, diffusion coefficient value of adenosine (8.31) was approximately two times as large as those of other nucleosides (3.99-4.07).

As thymine moiety is a specific functional group in the HA matrices, the transport performances should be noted for the factor of specific interaction. It is known that hydrogen bonding of base-pairing between nucleic acic bases is more effective at the low temperature. Here, the temperature dependency of transport performance of the nucleosides should be related to the total of partition and permeation of the membrane. At 5 °C, the partition coefficient of adenosine reflected the hydrogen bonding between thymine moiety in the membrane, while the other nucleosides could not form that bonding with thymine in membrane. Permeability of adenosine through DA-HA-Thy membrane, which was higher than other nucleosides, reflected the effect of thymine moiety as a carrier. From these considerations, thymine

moiety in the membrane should be regarded as an acceptor of base-pairing by adenosine for partition mechanism and as a carrier for permeation mechanism in the transport performance.

In transport performance, guanosine permeant was significantly different from other nucleoside permeants. This may be due to the hydrophobicity and self-aggregation of guanosine, which made the performance more complicated.

When the permeation was observed over 8 h at 5 °C. The profile of cumulative mass transport of adenosine appeared to be higher than that of the other nucleosides. This result suggests the selectivity on the transport performance of the membrane.

In summary, the studies demonstrate that the diffusion coefficients in the membrane are correlated to the base-pairing specific interaction. This affinity membrane should be recognized as a new type of specific selective membrane.

References and Notes

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