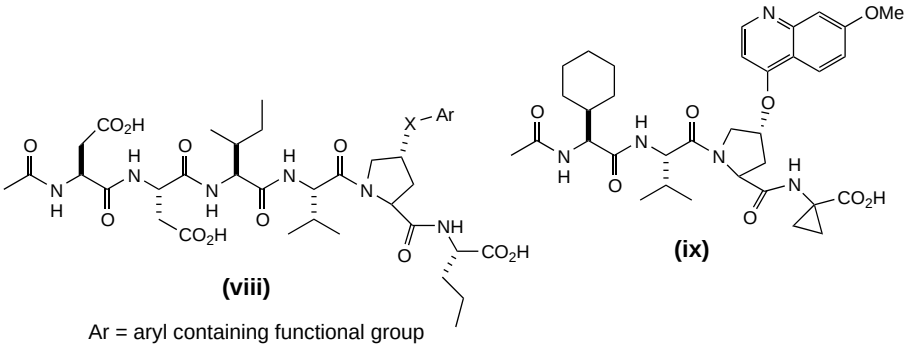

(viii)

(ix)

Ar = aryl containing functional group
X = CH_2 or O

In a recent publication, the same group focussed on optimizing this position using combinatorial chemistry[7]. A tetrapeptide was attached to a solid support and used as a scaffold for the synthesis of peptidomimetic inhibitors. The researchers then took advantage of the Mitsunobu reaction to introduce hydroxy-aryl groups at P2. Further modifications to the aryl group were achieved using the Suzuki cross-coupling reaction. SARs derived from this library suggest that, in addition to a hydrophobic interaction, a dipole–quadrupole interaction between the enzyme and the P2 substituent contributes to binding. Compound **(ix)**, having a quinoline-based P2 group (IC_{50} = 0.8 μM) was the most active analog disclosed.

6 Schecter, I. and Berger, A. (1967) On the size of the active site in proteases. I. Papain. *Biophys. Res. Commun.* 27, 157–162

7 Poupart, M-A. *et al.* (2001) Solid-phase synthesis of peptidomimetic inhibitors for the hepatitis C virus NS3 protease *J. Org. Chem.* 66, 4743–4751

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Drug delivery

A new drug delivery system for protein drugs using silicone

In general, macromolecular drugs such as proteins have an extremely short half-life in the body. Formulations that are designed to protect the drug from physiological effects until its release can be advantageous in these situations. Sustained release formulations that are designed to maintain an appropriate concentration in the body over a long period of time can also be advantageous. In this regard, a constant, zero-order release rate is usually ideal. Zero-order release formulations of lipophilic drugs can usually be achieved using formulations that contain hydrophobic polymers, but there has been limited success developing zero-order release formulations of protein drugs.

Recently, Kajihara and coworkers[1] reported the use of a new drug delivery system for protein drugs using silicone. Although this matrix formulation possessed some advantages over typical hydrophobic polymers, it still exhibited first-order release; that is, the concentration of drug released decreases over time. More recently, this group reported on the use of an improved formulation that is referred to as a covered-rod type formulation, which exhibits zero-order release characteristics for a model protein drug[2]. This latest report compares the release characteristics of the matrix- and covered-rod-type formulations. For a model drug, interferon (IFN) was chosen. Most protein drugs do not diffuse easily through silicone, especially when present in low concentrations. However, it was found that high loadings of simple, inexpensive, water-soluble proteins, such as human serum albumin (HSA) facilitate the release of protein drugs, such as IFN, from silicone. IFN–HSA powders containing a small amount of IFN were loaded into the silicone formulations and the resulting release characteristics of IFN were studied. For some *in vitro* studies, Texas-Red (sulforhodamine 101 acid chloride)-labeled HSA was used to facilitate visualization by confocal laser microscope (CLSM) analysis.

Matrix formulations were prepared by packing a mixture of silicone–IFN–HSA and additives into a polytetrafluoroethylene tube and removed after curing (hardening). Covered-rod-type formulations were made by preparing two silicone mixtures, one containing IFN–HSA and additives and another containing none of the drug mixture. The two silicone mixtures were loaded into separate syringes and extruded through a concentrically arranged die so that the silicone containing the drug mixture formed the inner, rod-shaped part, and the drug-free mixture formed the outer part. The resulting extrusion was cured at room temperature for three days. The configuration is called a covered-rod-type formulation because the inner, rod-shaped silicone that contains the drug is covered by drug-free silicone, except on the ends. Studies were then conducted to compare the matrix and covered-rod-type formulations.

The covered-rod-type formulation is prepared under mild conditions that require no heat or organic solvents. Protein drugs, including IFN, are denatured by heat and/or organic solvents. A covered-rod-type formulation was crushed in a grinder and, when the pieces were immersed in buffer, almost 100% of the IFN was recovered. This indicates that IFN is stable to the preparation conditions of the covered-rod-type formulation.

Parameters in the control of release rates

In vitro release rates of IFN from the covered-rod-type formulations were studied and compared with the release rate from matrix-type formulations. Matrix-type formulations released IFN at a rate that was initially high and gradually

decreased with time. The release rate was too low to be measured after one month. By contrast, covered-rod-type formulations released IFN at a constant, zero-order rate that could be controlled by variations in the formulation. Several parameters were studied, including the amount of IFN–HSA powder in the formulation, different particle sizes of IFN–HSA, different additives and the length of the rod. When the IFN–HSA powder content of the formulation was only 20%, the amount of IFN released over one month was minimal (only 0.65%). However, IFN was released continuously at a constant rate from other formulations containing from 30–50% powder content, and the IFN release rate increased with increasing powder content. The maximum amount of IFN released was from the formulation containing 50% powder content; the total amount of IFN released was 57%. Formulations containing 30% IFN–HSA powder content were chosen for further study.

The IFN release rate also increases with increasing IFN–HSA particle size. Particle sizes ranging from <20–150 μm were studied, and the total amount of IFN released varied from 17–39%. The additives studied included glycine, sodium glutamate and sodium chloride. Glycine caused the greatest increase in release rate of IFN. The difference in release rates was shown to correlate well to the osmotic pressure differences between the various additive formulations.

Finally, covered-rod-type formulations of two different lengths, 1 cm and 2 cm, were studied. For one month, both formulations released IFN at a constant, similar rate. After one month, the amount of IFN released from the 1 cm formulation gradually decreased. The 2 cm formulation continued to release IFN at a constant rate for two months. This demonstrated considerable control of release rate afforded by relatively simple changes in the formulation.

An *in vivo* study comparing IFN aqueous solution and similar matrix-type and covered-rod-type formulations was then conducted using nude mice. An IFN formulation was administered subcutaneously into the back of the nude mice, blood samples were collected at predetermined time points and serum-IFN concentrations were measured. In the group of mice that were administered IFN aqueous solution, the serum-IFN concentration decreased rapidly, and was undetectable in less than a day. In the group of mice that were administered the matrix-type formulation, the maximum serum-IFN concentration was observed at the initial sampling time (24 h); the concentration decreased to approximately 1/19 of the maximum value ten days after implantation, and IFN could be detected at low levels for up to 30 days. In the group of mice that were administered the covered-rod-type formulation, the maximum serum concentration was observed at 24 h, and the level at this time point was comparable to that for the matrix-type formulation. After this time, an approximately constant level was maintained; the serum IFN concentration 28 days after implantation was approximately 1/18 of the maximum value.

Geometry determines order of release

Some insight into the mechanism of drug release from the matrix-type and covered-rod-type formulations was gained from CLSM studies of formulations containing Texas-Red-labeled HSA. The silicone formulation was placed in a tube with buffer and incubated at 5°C for five days. The formulation was cut in the center and observed using CLSM. The HSA was fluorescent because of the Texas Red label, whereas the silicone was not. Therefore, the distribution of HSA in silicone could be detected by the fluorescence of HSA against the black background of silicone. These studies showed that HSA is released from all surfaces of the matrix formulation, whereas HSA is released only from the ends of the covered-rod-type formulation.

The authors believe that the differences in release rates observed from the two types of formulations are primarily a result of the difference in water infiltration patterns. They explain their interpretation of the results at considerable length and believe they can be summarized as follows: In a matrix-type formulation, channels form over the entire surface of the formulation; because the penetration front proceeds from the surface of the cylindrical formulation into the interior, the area of the front decreases with time. In a covered-rod-type formulation, because the formulation is surrounded by silicone (through which water cannot penetrate), except on the ends, the area of the penetration front remains constant. There are other details that will not be elaborated upon here, but this major difference between the formulations largely explains the differences observed in release characteristics.

These covered-rod-type formulations based on silicone have many potential advantages for protein drug delivery. They are prepared under mild conditions that require no heat or organic solvents. They exhibit zero-order release characteristics with a relatively small initial burst. The drug release rate can be controlled by several parameters, including concentration of the drug, particle size, additives and the length of the rod. The zero-order release rate has also been proven in these initial *in vivo* experiments. This formulation could prove useful for sustained release of protein drugs in the treatment of chronic diseases.

1 Kajihara, M. *et al.* (2000) Development of a new drug delivery system for protein drugs using silicone (II). *J. Control. Release* 66, 49–61

2 Kajihara, M. *et al.* (2001) Development of a new drug delivery system for protein drugs using silicone (II). *J. Control. Release* 73, 279–291

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