The effect of increasing DEAE-dextran concentration. (b and c) The effect of increasing ionic strength.

<table>
<thead>
<tr>
<th>PGM control/mixture concn. (mg/ml)</th>
<th>DEAE-dextran control/mixture concn. (mg/ml)</th>
<th>Ionic strength</th>
<th>PGM control $\gamma_{20}$ ($\text{S}^{-1}$)</th>
<th>PGM/DEAE-dextran mixture $\gamma_{20}$ ($\text{S}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 2.0</td>
<td>0.6</td>
<td>0.1</td>
<td>18.3 ± 0.4</td>
<td>21.7 ± 0.4</td>
</tr>
<tr>
<td>2.0</td>
<td>1.3</td>
<td>0.1</td>
<td>17.3 ± 0.4</td>
<td>20.8 ± 0.3</td>
</tr>
<tr>
<td>2.0</td>
<td>1.6</td>
<td>0.1</td>
<td>18.3 ± 0.4</td>
<td>23.4 ± 1.0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.9</td>
<td>0.1</td>
<td>17.9 ± 0.3</td>
<td>25.0 ± 0.9</td>
</tr>
<tr>
<td>2.0</td>
<td>2.6</td>
<td>0.1</td>
<td>18.3 ± 0.5</td>
<td>21.2 ± 0.9</td>
</tr>
<tr>
<td>2.0</td>
<td>3.4</td>
<td>0.1</td>
<td>18.4 ± 0.5</td>
<td>21.1 ± 0.6</td>
</tr>
<tr>
<td>(b) 1.3</td>
<td>1.0</td>
<td>0.1</td>
<td>21.9 ± 1.1</td>
<td>25.9 ± 1.0</td>
</tr>
<tr>
<td>1.3</td>
<td>1.0</td>
<td>0.2</td>
<td>—</td>
<td>21.7 ± 1.1</td>
</tr>
<tr>
<td>(c) 1.3</td>
<td>1.0</td>
<td>0.3</td>
<td>21.7 ± 0.9</td>
<td>25.8 ± 0.6</td>
</tr>
</tbody>
</table>

Under a given set of conditions (including the relative concentrations of the macromolecular components), the polymer was said to interact with PGM if the components in the mixture sedimented with a larger sedimentation coefficient than the controls after concentration effects had been taken into account (Table 1a). PGM concentrations were kept fixed (2.0 mg/ml) and the DEAE-dextran concentrations were varied (from 0.6 to 3.4 mg/ml): up to a DEAE-dextran concentration of 1.9 mg/ml, $x_20$ values for the faster moving PGM component showed a steady increase (Table 1a) compared with the PGM controls, strongly indicative of an interaction. The increase in sedimentation coefficient of the PGM in the mixture cell (compared with the controls) was in the same sense as increase in turbidity of the suspension and loss of apparent areas under the Schlieren boundaries.

At higher values of DEAE-dextran concentration the $x_20$ fell back to near the 'control' value again; this would appear to suggest that the interaction is very concentration sensitive, although the effects of the increased viscosity on increasing the concentration of the slower moving component (DEAE-dextran) cannot be excluded. Increase of the ionic strength in two separate experiments (Table 1b and 1c) also showed loss of interaction, indicating that the interactions between PGM and the polymer are electrostatic in nature.

In conclusion, the increases in (apparent) sedimentation coefficient of the PGM (and also DEAE-dextran) observed could be a direct result of the loss of concentration of PGM and DEAE-dextran in forming large turbid aggregates. Attempts at a more exact quantitative description of the interaction is difficult because of the presence of Johnston-Ogston [7]-related effects. These aspects, together with a consideration of the complications arising from the presence of other potentially interacting substances in the gastrointestinal tract (e.g. bile salts) and a comparison of interactions with other cationic and bifunctional polymers (and also non-interaction with polyanionics) will be considered in a future publication.


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### Brain microsomes bind ryanodine and contain ryanodine-sensitive calcium channels

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Ca$^{2+}$ channels from striated muscle sarcoplasmic reticulum (SR) have been functionally reconstituted in planar lipid bilayers [1] and purified as high-affinity ryanodine-binding proteins [2]. A deduced amino acid sequence for a skeletal muscle ryanodine-binding protein is now available from cloned cDNA [3]. All the reconstituted channels (including the purified protein) are activated by Ca$^{2+}$, ATP and caffeine, which increase the likelihood of the pore being open without altering the actual rate of ion transport. Some muscle channels are also activated by inositol triphosphate (Ins$P_3$) [4]. Given that the SR is merely a specialized endoplasmic reticulum, are similar channel proteins, possibly gated by chemical messengers, also present in non-contractile cells?1

Rat forebrain microsomes were prepared by homogenization and differential centrifugation and binding isotherms for $[^3H]$(ryanodine (0.5–50 nm, 54.7 Ci/mmol) were constructed following a protocol based on that in Table 2 of [5]. Unlabelled ryanodine (50 μM) was added to parallel samples to measure non-specific binding (the signal/noise ratio was at least 5:1 at the measured $x_20$). A typical binding isotherm, and associated Hill plot, are presented in Fig. 1(a). Channels
were reconstituted by osmotically driven fusion of microsomal membrane vesicles with planar lipid bilayers (phosphatidylethanolamine dispersed in decane) separating two chambers containing 50–500 mM-choline chloride and voltage-clamped at 0 mV by a high-impedance current amplifier with a feedback resistor of 10 MΩ. After Cl−-currents appeared the solutions were changed by perfusion, keeping the bilayer intact but exposing the channels to new components. (Ca2+ as the main permeant ion was used in most experiments) Ryanodine-sensitive Ca2+-permeable channels were co-incorporated (Fig. 1b). The reconstituted channels were activated (like striated muscle channels) by ATP (and the non-hydrolysable ATP-derivative AMPPNP) and also by caffeine and ATP (and the non-hydrolysable ATP-derivative midyl tartarate; AMPT). Preliminary work suggests that such activation is again effected by a modification of channel gating rather than conductance.

These data confirm a more general distribution for intracellular Ca2+ channels and suggest that investigation of their chemical activation, particularly by second messengers, will be worthwhile. In addition, the availability of the DNA sequence for a related muscle channel should allow the construction and deployment of DNA probes.

I am grateful to Alan Williams for advice, and to the Wellcome Trust for a Research Training Fellowship in Mental Health.

Use of antibodies to detect chemically cross-linked products from sarcoplasmic reticulum

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Introduction

Sarcoplasmic reticulum (SR) contains four major protein components, (Ca2+−Mg2+)−ATPase (M, 110000), a 160000-M, and a 53000-M, glycoprotein, and calsequestrin (M, 60000) [1]. Although the roles of the ATPase and calsequestrin in SR function are understood, the roles of the other two components are less clear, although it has been suggested that the 53000-M, glycoprotein is involved in modulating ATPase activity [2, 3]. In addition, all of these proteins are localized to particular regions of the SR, but the mechanism(s) involved in maintaining this localization is not known. In this study, we have used cross-linking agents in conjunction with monoclonal antibodies raised against particular components to identify protein–protein associations and thus highlight candidate proteins which may have modulatory functions or be involved in maintaining the spatial arrangement of proteins in SR.

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