correlate with that of tissue edema [19, 20]. In the present study, no statistically significant difference in tissue water content was present between the control (0.830±0.047), ischemic (0.764±0.083), and reperfused (0.769±0.051) groups, indicating that edematous alteration was not caused by ischemia-reperfusion treatments.

Tissue glycogen content

Tissue glycogen content was estimated as glucose equivalents after enzymatic hydrolysis of glycogen by amyloglucosidase. Tissue glycogen (in μmol glucosyl units/g wet tissue) in the control heart (25.8±13.6) was decreased by ischemia (16.5±12.9) and further decreased by reperfusion (8.2±7.3). Although there was a decrease between groups, the difference was not statistically significant due to scattered values between specimens.

Protein composition in SR-enriched microsomal fraction

Figure 1 shows SDS-PAGE profiles of SR-enriched microsomal fractions. The amount of many proteins varied after ischemia and after reperfusion. The 97-kDa protein apparently increased after 2 to 3 h of ischemia, though additional ischemic treatment for 6 h resulted in no further increase (data not shown). Reperfusion for 40 min after 1 h of ischemia apparently decreased the content of the 97-kDa protein.

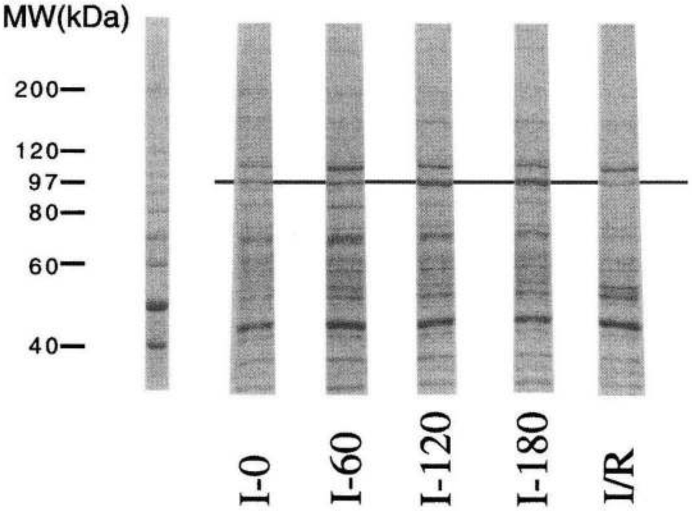

Fig. 1. Six percent SDS-PAGE profiles of proteins in SR-enriched microsomes. SR-enriched microsomes (30 μg protein) were applied to each lane. Samples were obtained after ischemia for 0 (I-0), 1 h (I-60), 2 h (I-120), and 3 h (I-180) and also after reperfusion for 40 min following 1-h ischemia (I/R). Four samples having a similar amount of protein were mixed and applied to SDS-PAGE for analysis of protein composition. The bar indicates the position of the 97-kDa protein. Proteins were stained with CBB. Leftmost lane contained molecular weight standards.

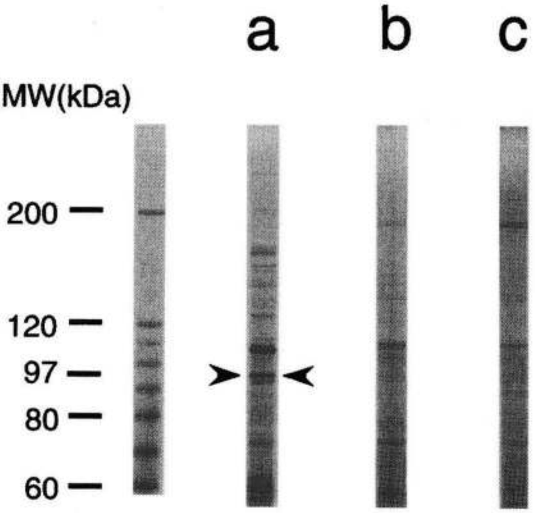

Fig. 2. SDS-PAGE profiles of proteins in SR-enriched microsomes that were separated by centrifugation in a discontinuous concentration gradient of sucrose. Samples (0.6 μg) were separated on a 6% polyacrylamide gel in the presence of 0.1% SDS. Proteins were stained with silver nitrate. Lane a, fraction between 10 and 28% sucrose; b, between 28 and 40%; c, between 40 and 60%. The leftmost lane contained molecular weight standards. Arrowheads indicate the 97-kDa protein.

Figure 2 compares SDS-PAGE profiles of microsomal fractions centrifuged in a discontinuous sucrose concentration gradient. The 97-kDa protein was located between the 10 and 28% sucrose layers, though the 10% sucrose layer did not contain the protein (data not shown). A Ca^{2+}, Mg^{2+}-ATPase of approximately 100 kDa was also located mainly in the 10/28% fraction.

Isolation and amino acid sequence analysis of 97-kDa protein

Proteins in the SR-enriched microsomes were electrophoretically fractionated (Fig. 3). The 97-kDa protein was eluted in fractions 8 through 12. Because the N-terminal residue was protected in the 97-kDa protein, the conventional Edman analysis was not effective. We therefore analyzed the internal peptide sequences of two fragments and found them to be T-C-A-Y-T-N-H-T-V-L-P-E and F-G-C-R-D-P-V-R. After comparing these sequences to those in a data bank, we deduced the 97-kDa polypeptide to be GP [21].

Western blot analysis

Figure 4 shows that only the 97-kDa protein was stained with anti-rabbit skeletal muscle GP-a in the Western blot analysis. The stained area of the 97-kDa band increased after 1 h of ischemia and decreased following reperfusion.

GP in other organs

Since ischemia may modulate the level of GP in other organs, we also

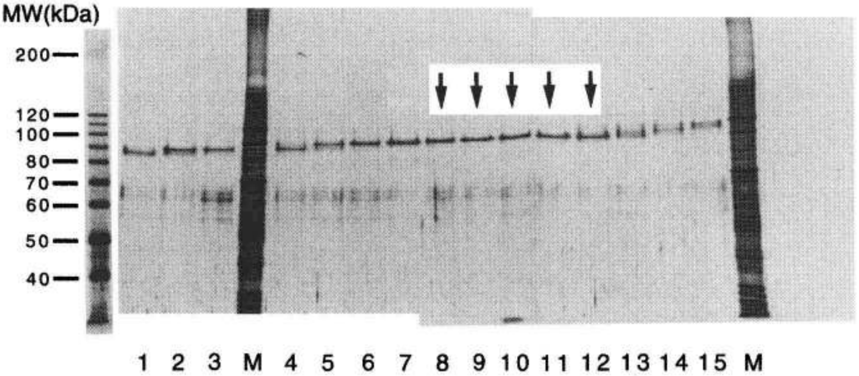

Fig. 3. SDS-PAGE profiles of electrophoretically separated proteins. Fractions were collected every minute. Proteins were stained with silver nitrate. Fractions 8 through 12 (arrows) were combined for further analysis. The leftmost lane contained molecular weight standards; and M, the original microsomes.

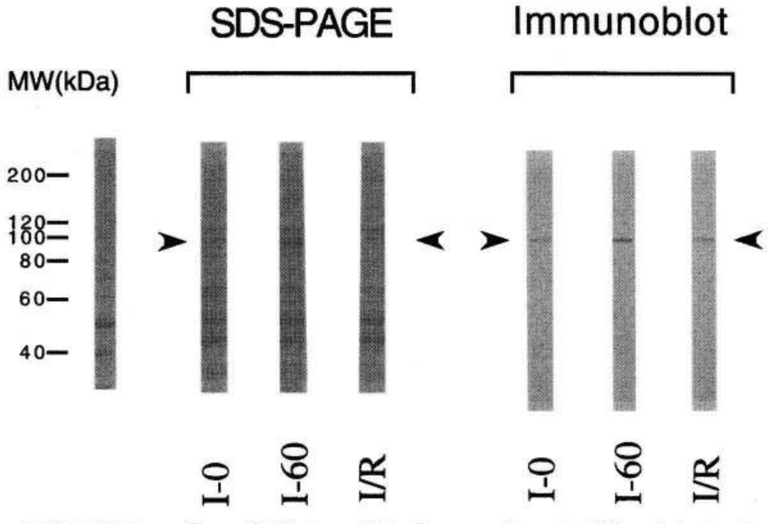

Fig. 4. SDS-PAGE profiles of Western blot figures of crude SR-enriched microsomes obtained after ischemia for 0 (I-0), 1 h (I-60), and after reperfusion for 40 min following 1-h ischemia (I/R). SR-enriched microsomes (30 μg protein) were applied to SDS-PAGE. Arrowheads indicate glycogen phosphorylase (97 kDa).

examined the effect of ischemia on rabbit brain, liver, spleen, and psoas skeletal muscle (Fig. 5). Microsomal samples were prepared by a procedure similar to that for the isolation of the heart microsomes. Brain and spleen microsomes did not show any band that could be stained by muscle anti-GP-a. Liver microsomes contained a band that reacted with muscle anti-GP-a, but ischemic treatment did not change the amount of this band. In contrast, skeletal muscle showed an apparent increase in the amount of the 97-kDa protein after ischemia, although the extent of the increase was not as pronounced as in the case of the heart microsomes.

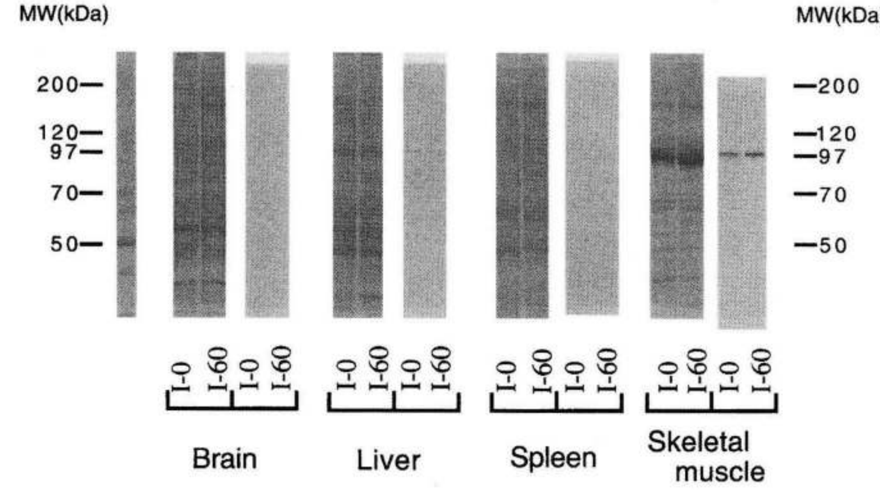

Fig. 5. SDS-PAGE profiles of microsomes obtained from rabbit brain, liver, spleen, and skeletal muscle. SR-enriched microsomes (30 μg protein) were applied to each lane. Left gel of each set was CBB stained; and the right, the Western blot. Sheep IgG against glycogen phosphorylase of rabbit skeletal muscle was used as the primary antibody. The leftmost lane contained molecular weight standards.

DISCUSSION

Many aspects of ischemia-reperfusion injury in the heart such as decreased contractility, deteriorating energy balance, and malfunctioning intracellular organelles have been extensively studied. Reperfusion injuries are often attributed to elevated oxygen tension, i.e., the 'oxygen paradox' [5] and elevated intracellular Ca^{2+}, i.e., the 'calcium paradox' [4]. The intracellular Ca^{2+} concentration is controlled by ATP-dependent Ca^{2+} pumps in the SL and SR. When the intracellular Ca^{2+} concentration is extremely elevated, Ca^{2+} precipitates in the mitochondrial matrices, leading to mitochondrial dysfunction. Homeostasis of intracellular ions, especially Ca^{2+}, is thus essential to ensure myocardial viability. We reported that Ca^{2+}, Mg^{2+}-ATPase in SR was degraded after hypothermic global ischemia with subsequent normothermic reperfusion [11, 12]. We furthermore observed that the level of a variety of proteins associated with the SR, including that of a 97-kDa protein, changed following ischemia-reperfusion. The goal of the present study was to identify this 97-kDa protein in the SR-enriched microsomal fraction and to examine the effect of ischemia-reperfusion treatment on the level of this protein. Various models have been used to study ischemia-reperfusion injury in the heart, e.g., a model of partial ischemia induced by ligation of the coronary artery *in situ* and a model of global ischemia using isolated hearts. Ischemia has been classified into two types: anoxic ischemia by perfusion of deoxygenated solution and hypoxic low-flow ischemia [22]. The present model reproduces hypoxic global