Table IV. Effect of KCl and AMP on the inhibition of the citrate synthase from *Rhodospirillum rubrum* by NADH

Concentration of KCl (mM)	$(I)_{0.5}$ (μM)	Apparent n
0	4.7	-2.25 ± 0.07
35	6.1	-2.37 ± 0.24
105	9.6	-1.79 ± 0.19
210	23.0	-1.70 ± 0.04
Concentration of AMP (μM)		
0	6.1	-2.37 ± 0.24
2.5	16.8	-2.03 ± 0.13
5.0	21.5	-2.34 ± 0.20
7.5	31.0	-2.22 ± 0.10
10.0	36.0	-2.14 ± 0.17

The assay conditions were as previously described[11], except for the concentrations of AMP and KCl which were as stated in the Table, and that of NADH, which was varied from 0 to 70 μM. The concentration of KCl in the experiments related to the effect of AMP was kept constant at 35 mM. 0.3 μg of purified enzyme were used per assay. The apparent n values ($\pm$ probable EM; $n = 5$) and $(I)_{0.5}$ values for NADH (NADH concentration for half-maximal inhibition) were calculated as previously described[19].

Table V. Deinhibition of the citrate synthase from *Rhodospirillum rubrum* by AMP

Concentration of NADH (μM)	$(A)_{0.5}$ (μM)	Apparent n
6.4	1.6	1.66 ± 0.05
16.1	4.3	1.84 ± 0.26
32.0	10.4	2.17 ± 0.07
64.0	17.5	2.79 ± 0.11

The experimental conditions were similar to those given in the legend to Table IV, except for the fixed NADH concentrations stated, the concentrations of AMP, which were varied between 0 and 20 μM, and that of KCl, which was kept constant at 105 mM. The apparent n values ($\pm$ probable EM; $n = 5$) and $(A)_{0.5}$ values for AMP (AMP concentration for half-maximal stimulation of enzyme activity) were calculated from Hill plots considering only the portion of the enzyme activity due to the AMP effect, as previously described[19].

synthase from *R. spheroides* has also been reported to be inhibited by ATP, although in the latter case the inhibition was non-competitive towards both substrates[4]. The enzyme from *R. capsulata* was reported not to be inhibited by ATP[2].

The *R. rubrum* citrate synthase, like the enzymes from *R. capsulata*[2] and *R. spheroides*[4], was strongly inhibited by NADH (Table IV); the kinetics of inhibition was sigmoidal. The effect of NADH was counteracted by KCl, which increased the $(I)_{0.5}$ for NADH with some decrease in the apparent n, and by AMP, which also caused an increase in the $(I)_{0.5}$ without significant variation in the apparent n (Table IV). The curves of enzyme de-inhibition by AMP were also sigmoidal; both the $(A)_{0.5}$ for AMP and the apparent n increased with the concentration of NADH (Table V). The inhibition by NADH was non-competitive towards oxaloacetate; the kinetic pattern for acetyl-CoA became sigmoidal in the presence of the inhibitor. The apparent n value for acetyl-CoA increased from 1.08 ± 0.01 in the absence of NADH to 1.73 ± 0.04 in the presence of 6 μM NADH; the corresponding $(S)_{0.5}$ values (concentration of acetyl-CoA required for half-maximal velocity) were 0.6 and 1.1 mM, respectively. The enzyme was not inhibited by α-oxoglutarate at concentrations up to 10 mM, either in the absence or in the presence of KCl. Therefore, the citrate synthase from *R. rubrum* shows the properties to be expected from similar enzymes from Gram negative bacteria which do not ferment glucose[3,16], being distinctly different from those of Gram positive bacteria. The effects of NADH and AMP are similar to those described for the enzymes from *R. capsulata*[2] and *R. spheroides*[4]. There are some differences, however, in the effect of ATP; the activation by KCl has not been reported for the *Rhodopseudomonas* enzymes[2,4].

The inhibitory response of the *R. rubrum* enzyme to NADH and ATP suggests that it will be strongly inhibited under photosynthetic conditions, where both the concentration of NADH and the adenylate energy charge can be expected to be high[2,4]. Although the effects reported have been studied in vitro, it is possible that they are involved in the enzyme regulation in vivo, since they were evident at rather high concentrations of KCl, which might be present inside living bacterial cells.

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Effect of Heat Treatment on the ATPase Activity of Various Sarcoplasmic Reticulum Preparations

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Summary. Ca^{2+}-stimulated ATPase activity of sarcoplasmic reticulum (SR) preparations is activated after a short period of preincubation at temperatures between 40 and 45 °C, but for temperatures higher than 48 °C pronounced denaturation is observed. Heat denaturation is decreased if Mg^{2+} or K^+ are present during heat treatment.

Leaky sarcoplasmic reticulum (SR) vesicles can be prepared by treatment with various agents such as phospholipase A[2], diethylether[3], EDTA[4] and X-537A[5], a Ca^{2+} ionophore. After each of these treatments, the membrane vesicles lose the ability to accumulate Ca^{2+}, although they retain the ATPase activity in some form, and have been utilized by several investigators to study phenomena related to the enzyme[2-7].

In this work we studied the behaviour of SR preparations previously treated with diethylether, EDTA and X-537A with respect to heat denaturation of the ATPase enzyme and its stabilization by cations.

Materials and methods. Sarcoplasmic reticulum membranes were isolated from rabbit skeletal muscle as described previously[8]. Immediatly after isolation, samples of SR were treated with 8% (v/v) diethylether ac-

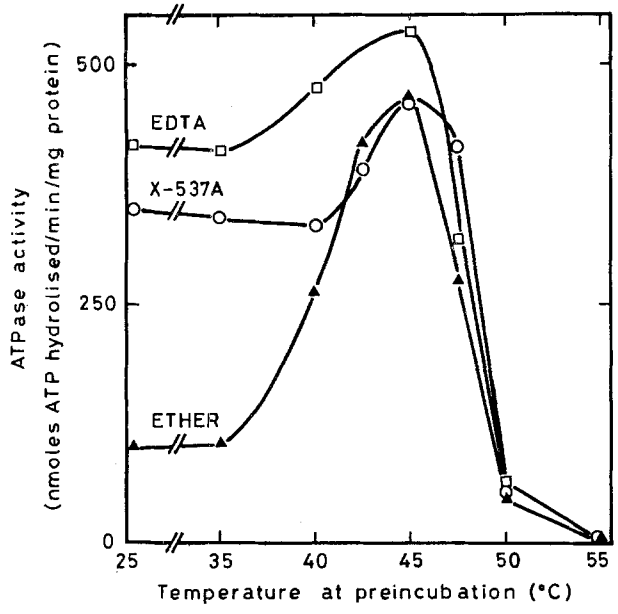

Fig. 1. Effect of temperature of preincubation on the Ca^{2+}-ATPase activity of SR preparations. ○, SR treated with X-537A; △, SR treated with diethylether; □, SR washed with EDTA. Samples of 1.5 mg of SR protein suspended in 0.6 ml media containing 5 m*M* *Tris*-Cl pH 6.9, 50 m*M* KCl and 5 m*M* $MgCl_2$, were incubated for 4 min at the temperatures indicated, and then were cooled down to room temperature (20 °C). Samples of these suspensions containing 0.5 mg of protein were withdrawn and used to measure the ATPase activity. In the case of SR treated with X-537A 100 nmoles of the ionophore were added per mg of protein.

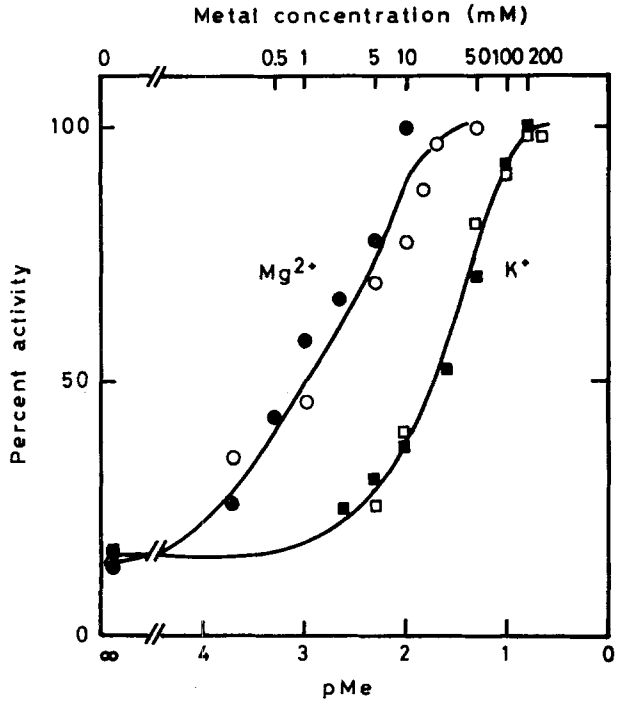

Fig. 2. Efficiency of Mg^{2+} and K^+ in preserving the ATPase activity against heat denaturation. Samples of SR protein were preincubated for 4 min at 45 °C, at a concentration of 5 mg/ml, in a medium containing 5 m*M* *Tris*-Cl pH 7.0, sucrose 0.125 *M* and Mg^{2+} or K^+. Samples were cooled to 20 °C and the ATPase activity was measured with X-537A (100 nmoles/mg SR protein) always present in the assay medium. The data obtained in 2 separate experiments are shown (closed and open symbols).

cording to the method described by INESI et al.[3], or washed with 1 m*M* EDTA at pH 8.5 as described by DUGGAN and MARTONOSI[4].

ATPase activities were measured by monitoring continuously the production of H^+ during the ATP hydrolysis by the method described previously by DEAMER[9] and MADEIRA et al.[10]. The reactions were performed in a thermostated vessel containing 5 ml of reaction medium (5 m*M* *Tris*-Cl pH 6.9, 50 m*M* KCl, 5 m*M* $MgCl_2$, 2 m*M* ATP, 1 m*M* EGTA or 1 m*M* EGTA and 0.9 m*M* $CaCl_2$, and about 0.5 mg of protein per experiment. At the pH of the assay (6.9), 0.7 nmoles of H^+ were produced per nmole of ATP hydrolized. Protein was determined by the biuret method[11]. All the reagents were of analytical grade. X-537A was supplied by Dr. JULIUS BERGER, Hoffmann-La Roche Inc., Nutley, New Jersey, 07110, USA.

Results and discussion. Figure 1 shows the Ca^{2+}-ATPase activities of the 3 types of leaky SR preparations determined after a short period of incubation (4 min) of the membranes at various temperatures (25–55 °C). After the preincubations, the membranes were cooled down to room temperature (20 °C) and the ATPase activity was measured at this temperature. The results show that the Ca^{2+}-stimulated ATPase activity decreases for the 3 preparations incubated at temperatures higher than 45 °C, and is nearly zero if the incubation is performed at 50 °C. However, preincubation at 35 to 45 °C causes a stimulation of the ATPase activity which indicates that the enzyme acquires a more favourable conformation due to the heat treatment. This effect was observed in the 3 types of membranes studied, but was more evident for the ether-treated preparation which, below 35 °C, has a low ATPase activity of about 100 nmoles of ATP hydrolized/min/mg of protein, whereas the controls, treated with X-537A or EDTA, show activities of 350 and 400 nmoles of ATP hydrolized/min/mg of protein, respectively, in the same range of temperature (Figure 1).

It is of interest that the increase in rate of ATP hydrolysis was observed after the enzyme had been preincubated at temperatures just below those which induce pronounced denaturation of the ATPase, as determined from the loss of activity. This suggests that as the enzyme begins to unfold with increase in temperature, it apparently passes through a conformation which is more active than the native form of the enzyme. The stimulation of the enzyme activity due to heat treatment cannot be attributed to an effect of heat increasing the membrane permeability which would cause uncoupling of the ATPase to the Ca^{2+} transport, because the preparations used were in a leaky state as a consequence of treatment with

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X-537A ionophore, with ether or with EDTA. All these agents dissipate the Ca^{2+} gradient across the membrane which loses the capacity to accumulate Ca^{2+} [2–6].

The preincubation of the SR membranes whose ATPase activities are shown in Figure 1 was performed in ionic media (5 m*M* $MgCl_2$, 50 m*M* KCl) which we previously found to preserve optimally the ATPase activity during preincubation at temperatures which in ion free media cause complete denaturation of the enzyme. Figure 2 shows the relative efficiency of Mg^{2+} and K^+ in stabilizing the enzyme activity of SR during preincubation at 45 °C. It is evident from these results that whereas about 5 to 10 m*M* Mg^{2+} already gives maximal stabilization of the enzyme, about 100 m*M* K^+ are necessary for obtaining the same effect. The concentrations for half maximal effect of Mg^{2+} and K^+ were estimated to be 1.0 and 25 m*M*, respectively.

The S-shape curves shown in Figure 2 indicate that the direct plots of ion concentration against ATPase activity are classical saturation curves. The protective effect of these ions is probably due to the binding of Mg^{2+} or K^+ to the membranes which also exhibits saturation[12]. The fact that Mg^{2+} is much more effective than K^+ in protecting SR ATPase from heat inactivation is compatible with the binding results of these cations to the membranes[13], which show that the affinity of the binding sites in the membranes for Mg^{2+} ($pK_M = 4.2$) is much higher that the affinity for K^+ ($pK_M = 1.3$).

The heat activation of the ATPase of SR membranes which occurs between 40 and 45 °C, as well as the effect of divalent cations on the stability of the enzyme, should be taken into consideration when Arrhenius plots of the activity of the enzyme are constructed to obtain information about the various thermodynamic parameters of the enzyme.

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Fibrillation of Tropoelastin Induced by Proteoglycan

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Summary. Electrostatic interaction between tropoelastin, the native precursor of elastin, and proteoglycan results in tropoelastin fibrillation. The finding suggests a possible involvement of proteoglycans in elastogenesis.

It has been shown that simple coacervation of both α-elastin, a degradation product of insoluble elastin, and tropoelastin, the native precursor of elastin, results in elastin fibril formation[2,3]. In a recent work[4], we have described a different mechanism of α-elastin fibrillation, that is ionic interaction with proteoglycan. In the complex coacervate, which is formed as a result of this interaction, fibrillar structures were clearly observable. The present paper gives evidence that this mechanism of fibrillation applies also for the native precursor of elastin, which suggests a possible biological significance of the tropoelastin-proteoglycan interaction.

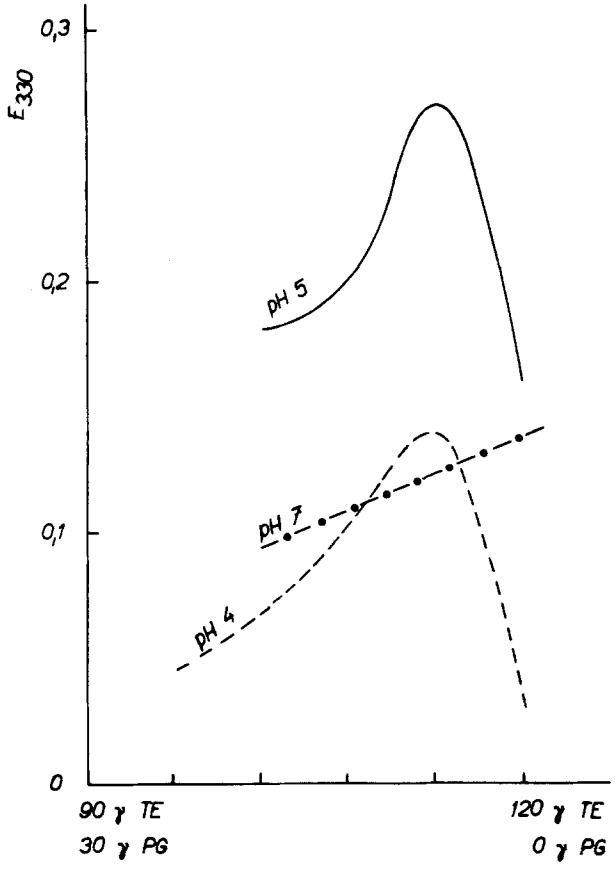

Fig. 1. The plot of optical density at 330 nm vs. tropoelastin: proteoglycan ratio. The amounts given are in μg/ml.

Materials and methods. Tropoelastin was a generous gift from Dr. L. B. Sandberg (University of Utah, Salt Lake City, USA). It was prepared from Cu-deficient pig aorta and its amino acid composition was in agreement with the criteria of purity. Proteoglycan prepared from bovine nasal cartilage by the dissociative method[5] was the same preparation as in the previous report[4]. It did not contain any contaminating substances.

Optical density measurement at 330 or 440 nm was used for the detection of the interaction between the tropoelastin and proteoglycan. The measurement was carried out at various pH values and at varying weight ratios of both components while the total concentration remained constant and was 120 μg/1 ml.

The interaction products (complex coacervates) for electron microscopic observation were prepared either by mixing the compounds in solution directly at pH 4.0 and 20 °C (see the results) or by dialysis at 20 °C of mixed solutions prepared at pH 7.0–8.0 (no interaction occurs at these pH values) against several changes of water ad-

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