A Ca^{2+}-sensitive myosin light chain kinase, regulating pig carotid smooth muscle actomyosin ATPase[1]

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Summary. In this paper the correlation between phosphate incorporation into the regulatory light chain of myosin by a Ca^{2+}-dependent myosin light chain kinase, and the Ca^{2+}-sensitive ATPase activity and superprecipitation behaviour of arterial actomyosin, is described.

In all types of muscle an intracellular change in Ca^{2+}-concentration regulates contraction. In smooth muscle, there is no generally accepted model for the way in which the Ca^{2+}-ions actually cause the activation. According to one theory, based on experiments with chicken gizzard[2,3], and nonmuscular actomyosin systems[4], the increase of Ca^{2+}-concentration activates a Ca^{2+}-dependent myosin light chain kinase, which phosphorylates the 20.000 dalton light chain (20 K LC) of the myosin, thus allowing actin - myosin interaction. In recent studies it was shown that in vascular smooth muscle myosin phosphorylation also occurs[5]. In this paper we describe the role of a Ca^{2+}-sensitive light chain kinase in regulating the activity and the degree of phosphorylation of arterial actomyosin.

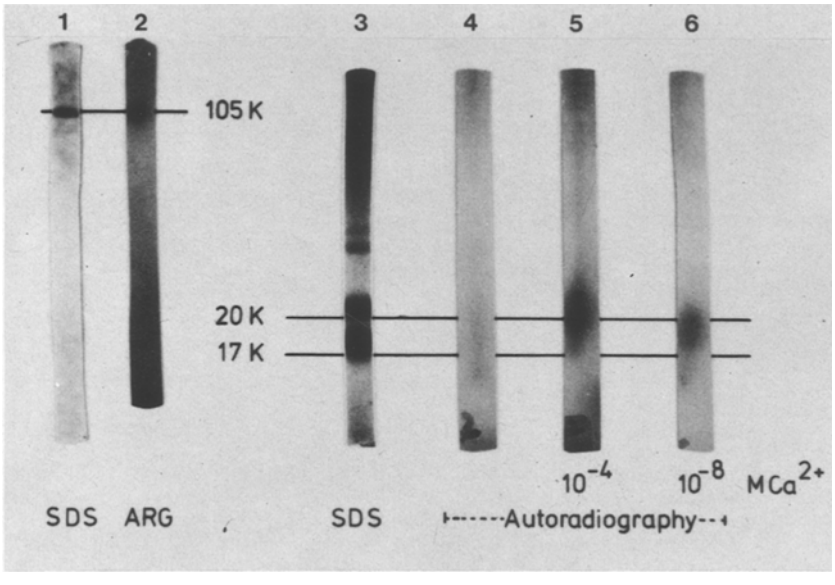
Materials and methods. Crude extracts of arterial actomyosin were obtained as described in[8], desensitization was carried out according to[9]. Light chains were isolated by treatment of actomyosin with 8 M urea[10] and purified by spinning down contaminating actin in the ultracentrifuge. Since control experiments showed that only the 20.000 dalton light chain could be phosphorylated, no steps for further purification of this light chain were taken. Phosphate incorporation into the 20.000 dalton light chain, and ATPase activities of actomyosin, were determined as described in[7]. 6% acrylamid gels containing 1% sodiumdodecylsulfate (SDS) were prepared and run according to Weber and Osborn[11] and autoradiographed on Osray T 4 films. For the superprecipitation experiments, actomyosin was suspended in 30 mM NaCl, 10 mM imidazole / HCl, 10 mM $MgCl_2$, pH 7.0, 22 °C. 2 mM ATP were added to start the reaction[12].

Results and discussion. For the isolation of myosin light chain kinase from arterial muscle the procedure described by Sobieszek and Small[13] was adopted as follows: freshly extracted actomyosin is stirred gently for 3 h at 4 °C in 120 mM NaCl, 40 mM imidazole / HCl in presence of 20 mM $MgCl_2$ at pH 7.7. After centrifugation for 30 min at 45,000 × g the supernatant was dialyzed overnight against 120 mM NaCl and 40 mM imidazole/HCl pH 7. The figure, 1, shows a 6% SDS gel of this extract.

There is a prominent band corresponding to a component with an apparent mol. wt of 105.000 dalton. In presence of [γ^{32}P] ATP it shows [^{32}P] phosphate incorporation on the autoradiograph of the SDS gel (figure, 2), suggesting that the kinase can be phosphorylated either by selfphosphorylation or by a cyclic AMP dependent kinase[14]. This crude preparation does not have any ATPase activity, in spite of the fact that there is a slight contamination with actomyosin visible on the SDS gels. Furthermore, no phosphate incorporation into the contaminating light chains occurs after incubation with [γ^{32}P] ATP at 10^{-5} M Ca^{2+}, indicating that the contaminating light chains are probably denatured and cannot serve as a substrate for the kinase.

By further purification on Sepharose 4B enrichment of the 105.000 dalton band could be achieved, but kinase activity was rapidly lost during this purification steps. The ability of the myosin light chain kinase to transfer phosphate to the 20 K light chain was tested with isolated light chains (figure, 3). Incubation of the light chains with myosin light chain kinase at 10^{-4} M Ca^{2+} with [γ^{32}P] ATP resulted in phosphate incorporation only into the 20 K light chain (figure, 5). At 10^{-8} M Ca^{2+} only very little phosphate incorporation can be detected (figure, 6). When the light chain fraction is incubated with [γ^{32}P] ATP in the absence of light chain kinase, no phosphate incorporation occurs at all (figure, 4).

Since our major interest was to clarify the role of this enzyme in Ca^{2+}-regulation of carotid muscle its influence on the Ca^{2+}-sensitivity of the superprecipitation behaviour and ATPase activity of actomyosin was investigated. The



1 SDS gel of myosin light chain kinase. *2* Autoradiograph of SDS gel of myosin light chain kinase (protein incubated with [γ^{32}P] ATP prior to electrophoretic separation). *3* SDS gels of isolated light chains. *4* Autoradiograph of SDS gel of isolated light chains incubated with [γ^{32}P] ATP at 10^{-5} M Ca^{2+} prior to electrophoretic separation. *5* Autoradiograph of SDS gel of isolated light chains mixed with light chain kinase, incubated with [γ^{32}P] ATP at 10^{-5} M Ca^{2+} prior to electrophoresis. *6* Same as 5 but Ca^{2+}-concentration reduced to 10^{-8} M.

Correlation of ATPase activity and superprecipitation of actomyosin with phosphate incorporation into the 20.000 dalton regulatory light chain of myosin

[Ca^{2+}]	ATPase activity nm P_i mg^{-1} min^{-1} 10^{-5}	10^{-8}	Phosphate incorporation nm P_i mg AM^{-1} 10^{-5}	10^{-8}	Super-precipitation 10^{-5}	10^{-8}
Ca sensitive actomyosin	9.2	1.8	0.9	0.2	+ +	–
Desensitized actomyosin	3.2	2.8	0.21	0.19	–	–
Desensitized actomyosin plus light chain kinase	7.4	2.2	0.6	0.17	+	–

ATPase activities, superprecipitation and phosphate incorporation were measured in 50 mM K Cl, 10 mM imidazole/HCl, 10 mM $MgCl_2$, pH 7.2; 22 °C, 2 mM Ca EGTA/EGTA buffer as required, 2 mM ATP. Turbity changes during superprecipitation were monitored at 550 nm. [^{32}P]-phosphate incorporation into the regulatory light chain was determined after 5-min incubation of actomyosin with [$\gamma^{32}P$] ATP at 10^{-5} M Ca^{2+}, the reaction was terminated by precipitating the protein with trichloracetic acid on glass-fibre filters.

results are summarized in the table. Freshly extracted actomyosin exhibits a Ca^{2+}-sensitive ATPase activity, it superprecipitates at 10^{-5} M Ca^{2+} and relaxes at 10^{-8} M Ca^{2+}. Phosphate is incorporated (up to 0.9 nm P_i/mg AM) into the 20 K light chain only at 10^{-5} M Ca^{2+}, but not at 10^{-8} M Ca^{2+}.

By repeated precipitation of the actomyosin at 200 mM KCl (desensitization[9]), we obtained an actomyosin lacking kinase activity. This protein was dephosphorylated, irrespective of the Ca^{2+}-concentration, probably because of the presence of a phosphatase, and it did not superprecipitate after addition of 10^{-5} M Ca^{2+}. Re-addition of the isolated kinase results in Ca^{2+}-sensitive phosphate incorporation into the 20 K light chain, which is accompanied by a partial restoration of the Ca^{2+}-dependent activation of ATPase activity and superprecipitation of actomyosin.

In conclusion, addition of a myosin light chain kinase resulted in the conversion of a Ca^{2+}-insensitive, inhibited actomyosin into a Ca^{2+}-activated actomyosin, in which ATPase activity and superprecipitation are regulated by Ca^{2+}-dependent phosphate incorporation into the 20.000 dalton regulatory light chain.

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2 A. Gorecka, M.D. Aksoy and D.J. Hartshorne, Biochem. biophys. Res. Commun. *71*, 325 (1976).
3 A. Sobieszek and V.S. Small, J. molec. Biol. *102*, 75 (1976).
4 R.S. Adelstein and M.A. Conti, Nature *256*, 597 (1975).
5 U. Mrwa and J.C. Rüegg, in: Excitation contraction coupling in smooth muscle, p.353. Ed. R. Casteels. Elsevier/North Holland Biomedical Press, Amsterdam 1977.
6 D.M. Cohen and R.A. Murphy, J. gen. Physiol. *72*, 369 (1978).
7 U. Mrwa, M. Troschka, C. Gross and L. Katzinski, Eur. J. Biochem. *103*, 415 (1980).
8 W.E. Russel, Eur. J. Biochem. *33*, 495 (1973).
9 M. Sparrow and F.M. v. Bocksmeer, J. Biochem. *72*, 1075 (1972).
10 W.T. Perrie and S.V. Perry, Biochem. J. *119*, 31 (1970).
11 A. Weber and M. Osborn, J. biol. Chem. *244*, 4406 (1969).
12 S. Ebashi, T. Mikawa, M. Hirata, T. Toyo-oka and Y. Nonomura, in: Excitation contraction coupling in smooth muscle, p.325. Ed. R. Casteels. Elsevier/North Holland Biomedical Press, Amsterdam 1977.
13 A. Sobieszek and V.S. Small, J. molec. Biol. *112*, 1386 (1977).
14 R.S. Adelstein, M.A. Conti and D.R. Hathaway, J. biol. Chem. *253*, 8347 (1978).

Thermodynamics of bromate and iodate of sodium in dioxane-water mixtures at different temperatures

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Summary. The free energy transfer (ΔG_t^0), enthalpy of transfer (ΔH_t^0) and entropy of transfer (ΔS_t^0) of $NaBrO_3$ and $NaIO_3$ from water to 10, 20 and 30% dioxane-water mixtures have been studied using conductance measurements. The chemical and electrical parts of these transfers of thermodynamic parameters have been estimated.

Studies of electrolytic conductance in dioxane-water media of varying dioxane content were initiated by Das and Das[2] at 30, 35, 40 and 45 °C±0.01. In the present communication attempts have been made to evaluate the thermodynamic functions ΔG_t^0, ΔH_t^0 and ΔS_t^0 for the transfer of $NaBrO_3$ and $NaIO_3$ from water to the respective dioxane-water media, which would give some information regarding ionic solvation.

Materials and methods. The salts and dioxane used were from E. Merck ('extra pure' varieties). Purification of dioxane, preparation of solvents solutions and measurement of conductance have been reported earlier[2]. The conductance measurement was of an accuracy of ±2 in 1000. The concentration range was from 0.01 to 0.001 moles/l^{-1}.

Results and discussion. The plot of λ of $C^{1/2}$ was found to be linear, and λ^0 has been obtained from the extrapolated values. Since the dielectric constant of the medium is low, the dissociation constant 'K' has been calculated by the methods of Fuoss and Krauss[3] and Shedlovsky[4]. The values obtained by both the methods are in good agreement. The standard thermodynamic parameters ΔG^0, ΔH^0 and ΔS^0 have been calculated. The plots of these thermodynamic parameters vs solvent compositions were found to be linear and the extrapolated values gave the thermodynamic parameters for water. The transfer of these thermodynamic parameters could then be calculated by Feakins[5] method and the values are tabulated in table 1. The probable uncertainties in ΔG_t^0 are ±15 J/$mole^{-1}$, in ΔH_t^0 are ±18 J/$mole^{-1}$