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

P.P. Misra and P.B. Das[1]

Department of Chemistry, R. College, Cuttack-3 (India), 9 March 1979

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/$mole^{-1}$

and in ΔS_t^0 are ± 0.05 J/kg^{-1}/mole^{-1} in all solvent compositions.

The values of ΔG_t^0 are observed to be positive, ΔH_t^0 and ΔS_t^0 are all negative in all solvent compositions and at all temperatures. The positive values of ΔG_t^0 indicate that the salts are in a higher free energy state in dioxane + water mixtures than in water, suggesting that water has more affinity for the salts than dioxane + water mixtures. The entropies in dioxane + water are less than in pure water and hence the net amount of order created by the salts in dioxane + water mixtures is more than in pure water.

The radii of BrO_3^- and IO_3^- have been estimated to be 2.5 Å and 3.2 Å respectively[6]. Utilizing these values, the ΔG_t^0 has been split into 2 parts according to Roy et al.[7]. It consists of an electrostatic part $\Delta G_{t(el)}^0$ corresponding to a change in the dielectric constant of the medium and another non-electrostatic part, chemical contribution, $\Delta G_{t(ch)}^0$ arising from the specific chemical interactions between the ions and the solvent dependent:

Thus $\Delta G_t^0 = \Delta G_{t(el)}^0 + \Delta G_{t(ch)}^0$

The $\Delta G_{t(el)}^0$ has been calculated from the Born equation[8] by utilizing the values of the ionic radii obtained. The $\Delta G_{t(ch)}^0$ was then evaluated. The ΔH_t^0 and ΔS_t^0 have been split up into 2 parts in the manner described by Das et al.[9]. All these values are presented in table 2. It is evident that the $\Delta G_{t(ch)}^0$

Table 1. Free energy, enthalpy and entropy of transfer of $NaBrO_3$ and $NaIO_3$ from water to dioxane + water mixtures at different temperatures

Temperature (°C)	ΔG_t^0/J · mole^{-1} (%)			$-\Delta H_t^0$/J · mole^{-1} (%)			$-\Delta S_t^0$/J · kg^{-1} · mole^{-1} (%)		
	10	20	30	10	20	30	10	20	30
	$NaBrO_3$								
30	541	943	1461	313	915	1123	2.82	6.13	8.53
35	591	1144	1611	714	1125	1524	4.24	7.27	9.28
40	702	1455	2099	692	1684	2876	4.21	10.01	15.86
45	623	1622	2229	912	2423	3215	4.24	12.70	12.09
	$NaIO_3$								
30	807	1178	1868	817	1882	2524	5.36	9.90	14.49
35	808	1228	2114	927	2132	2924	6.66	11.25	17.55
40	954	1577	2417	872	2472	3620	5.83	12.92	19.26
45	1318	2178	3467	1612	2814	4614	9.51	15.99	25.69

Table 2. Electrical and chemical part of the thermodynamic quantities accompanying the transfer of $NaBrO_3$ and $NaIO_3$ from water to dioxane + water mixtures at different temperatures

Temperature (°C)	$\Delta G_{t(el)}^0$/J · mole^{-1} (%)			$\Delta G_{t(ch)}^0$/J · mole^{-1} (%)		
	10	20	30	10	20	30
	$NaBrO_3$					
30	1446	2494	4123	−895	−1551	−2662
35	1113	2322	3972	−502	−1208	−2361
40	1328	2553	4246	−636	−1098	−2165
45	935	2442	4004	−310	− 622	−1775
	$NaIO_3$					
30	1063	1833	3031	−750	− 918	−1103
35	818	1707	2920	−104	− 479	− 806
40	976	1877	3122	− 84	− 193	− 705
45	688	1679	2944	224	744	523
	$-\Delta H_{t(el)}^0$/J · mole^{-1}			$\Delta H_{t(ch)}^0$/J · mole^{-1}		
	$NaBrO_3$					
30	1478	2531	4067	1165	1624	2944
35	1438	2628	4239	724	1503	2705
40	572	2846	4552	−120	1162	1676
45	1561	2860	4661	−649	437	1446
	$NaIO_3$					
30	1091	1866	3190	274	− 16	666
35	1058	1931	3117	131	− 202	192
40	420	2092	3345	−200	− 381	− 275
45	1147	2102	3426	− 51	− 712	− 738
	$-\Delta S_{t(el)}^0$/J · kg^{-1} · mole^{-1}			$\Delta S_{t(ch)}^0$/J · kg^{-1} · mole^{-1}		
	$NaBrO_3$					
30	9.68	16.61	27.03	6.86	10.48	19.50
35	8.28	16.07	26.66	4.04	8.80	17.38
40	6.07	17.25	28.11	1.86	7.24	12.25
45	7.85	16.18	27.25	3.61	3.48	9.45
	$NaIO_3$					
30	7.11	12.21	19.88	1.75	2.31	4.69
35	6.09	11.81	19.60	−6.57	0.56	2.05
40	4.46	12.68	20.66	−1.35	− 0.24	1.40
45	5.77	11.89	20.03	−3.74	− 4.10	− 5.66

values are negative in both cases (excepting for IO_3^- at 45 °C) which indicates that the transfer of salts from water to dioxane + water is favoured as far as chemical interactions are concerned. The $\Delta G^0_{t(el)}$ is positive in both cases and is of the order $BrO_3^- > IO_3^-$ and hence the ionic solvation is of the reverse order. The $\Delta H^0_{t(el)}$ is negative whereas $\Delta H^0_{t(ch)}$ is positive (excepting in 2 cases) and both increases with the increase in dioxane content. $\Delta S^0_{t(el)}$ is negative in all cases and becomes more and more negative with increase in dioxane content, indicating that the orderedness in the solvent structure $\Delta S^0_{t(ch)}$ is positive in case of $NaBrO_3$ and decreases with increase in temperature, but in case of $NaIO_3$ it is positive at 30 and 35 °C and negative at 40 and 45 °C, which indicates the chemical interaction.

1 To whom reprint requests should be addressed.
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Effect of dietary cholic acid and cholesterol on liver and kidney cystathionase and cysteine sulfinate decarboxylase activities and taurine concentrations in the rat[1]

C. Loriette, Y. Pierre and F. Chatagner

Laboratoire de Biochimie, 96, blvd Raspail, F-75006 Paris (France), 17 May 1979

Summary. Hepatic cystathionase and cysteine sulfinate decarboxylase activities are drastically affected by cholic acid added to the diet without cholesterol. When cholic acid and cholesterol are given together, only cysteine sulfinate decarboxylase activity is changed. Neither kidney enzyme activity nor taurine concentrations in the liver and kidney are noticeably modified, whatever the diet.

It is known that cholesterol is involved in atherosclerosis and there are indications that sulfur amino acids can also be involved in this disease[2]. This suggests that cholesterol and sulfur amino acid metabolisms are not independent and we have been interested in studying the effect of cholesterol on the enzymes of sulfur amino acid metabolism and on taurine concentrations in the liver and kidney. A series of rats was given a cholesterol-rich diet in which cholic acid was added to promote a good absorption of cholesterol. Another series of rats received a control diet containing cholic acid but no cholesterol. 2 enzyme activities, cystathionase (CNase EC 4.4.1.1) and cysteine sulfinate decarboxylase (CSD EC 4.1.1.29), and taurine concentrations were measured. Surprising results obtained even in the control rats made us wonder if they were due to cholic acid and not the other components of the diet. Therefore we also used rats fed with a control diet without cholic acid.

Materials and methods. Several interrelated adult male albino rats from our breeding unit were divided into 2 groups. They were fed (15 g/day) purified diets (formulations are shown in table 1) with free access to water. In experiment 1, 6 rats were given the control A diet and 6 rats were fed a cholesterol-rich diet (chol. group). These 12 rats received cholic acid. In experiment 2, 3 rats were given either the control A or the control B diet (without cholic acid). After 3 or 4 weeks on the diets, rats were killed by decapitation. Immediately upon removal, a fragment of liver and a kidney were frozen for later determination of taurine concentrations according to Anzano et al.[3]. Another sample of liver and the second kidney were homogenized in cold 0.25 M sucrose, 2 mM dithiothreitol (4 ml/g tissue) in a glass homogenizer with a Teflon pestle. The 105000 × g supernatant was used for enzymatic analysis, since these 2 enzymes are present in the cytosol.

These assays were performed as already described[4,5]. The results are expressed as µmoles of H_2S produced per h per g of wet tissue for CNase activity and as µmoles of CO_2

Table 1. Composition of diets (% by wt)

	Control A	High cholesterol diet (chol.)	Control B
Sucrose	64	62	64
Cholic acid	0.4	0.4	
Cholesterol		2	

In addition each diet contained 18% USBC vitamin-free casein, 10% hydrogenated coprah purchased from ITERG (Paris), 2% USBC total vitamin supplement, 4% USBC salt mixture (Wesson modification) and 0.7% sunflower oil for essential linoleic acid supply.

Table 2. Summary of experiment 1

	Control A	Chol.
Initial body wt (g)	423 ± 18	423 ± 12
Final body wt (g)	399 ± 14	392 ± 13
Liver wt (g)	13.8 ± 0.5	18.2 ± 0.9 $p < 0.01$*
Kidneys wt (g)	2.51 ± 0.07	2.53 ± 0.04
Liver enzyme activity		
CNase µmoles H_2S per h per g	103.9 ± 7.8	59.3 ± 3.4 $p < 0.01$*
CSD µmoles CO_2 per h per g	12.26 ± 1.38	11.88 ± 0.82
Kidney enzyme activity		
CNase	18.7 ± 0.2	17.3 ± 0.4
CSD	8.44 ± 0.44	9.10 ± 1.00
Taurine concentration µmole/g		
Liver	1.30 ± 0.07	1.22 ± 0.08
Kidney	9.76 ± 0.45	8.31 ± 0.27 $p = 0.02$*

Dietary treatment for 4 weeks. Values are the mean ± SEM for 6 rats, except CNase activity (4 rats). * Values different from control A.