Inorganica Chimica Acta, 56 (1981) 89–93

On the Mechanism of Platinum- and Palladium-Albumin Complexes Interaction with (Ca^{2+}–Mg^{2+})-dependent ATP-ase of Sarcoplasmic Reticulum of Skeletal Muscles

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Received February 25, 1981

Complex compounds of platinum and palladium inhibit the activity of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of sarcoplasmic reticulum (SR), interacting with thiol-groups of the enzyme. Thus, K_2PtCl_4 and K_2PdCl_4 form complex I (stable and irreversible) and complex II (unstable and reversible), symbolized as 'platinated' and 'palladinated' albumin, respectively. Both complexes inhibit the (Ca^{2+}–Mg^{2+})-dependent ATP-ase activity by an order more than pure K_2PtCl_4 and K_2PdCl_4 do. By Benesch's method, complexes I and II were found to bond with (Ca^{2+}–Mg^{2+})-dependent ATP-ase through SH-groups of the enzyme, forming a triple complex of albumin–ligand–enzyme. Albumin, concentrated in the region of the enzyme active center, was established to make the interaction of I and II with SH-groups of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR easier, which probably caused the increase of the biological effect of K_2PtCl_4 and K_2PdCl_4 upon the enzyme.

Introduction

Complex compounds of platinum and palladium interact with thiol groups of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of sarcoplasmic reticulum (SR), causing inhibition of enzyme activity. K_2PtCl_4 and K_2PdCl_4 react readily with the SH-groups of albumin to give complexes (I) and (II), symbolized as 'platinated albumin' and 'palladinated albumin', respectively. Complex (I) is irreversible but complex (II) is a reversible one. Both complexes inhibit the activity of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR by an order more than K_2PtCl_4 and K_2PdCl_4 themselves do. Benesch's method of amperometric titration of protein SH-group helped to establish (I) and (II) to be bonded with (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR through thiol groups of enzyme. By means of ESR method, albumin, (I) and (II) were found to be bonded in the region of an active center. Pure albumin being bonded with the active center of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR makes the interaction of (I) and (II) with the SH-groups of enzyme easier. This interaction probably causes the stronger biological action of K_2PtCl_4 K_2PdCl_4 upon the enzyme.

At present great attention is paid to the action of complex compounds of Pt-metals upon the biological systems because of (*i*) their high antitumoral activity and the possibility of their application in cancer chemotherapy [1–4], and (*ii*) the exposure of specific character of allergic deseases among those who work with Pt-metal salts [5].

A rather active effect of a whole number of Pt complex compounds upon the enzyme system has been established [6–10]. It is noteworthy that all mentioned papers deal with direct action of Pt-metals upon the structure and functional state of biopolymers and enzymatic systems. Meanwhile biologically active substances are to be bonded with transport albumin independent of the way they penetrate into the organism and to pass through one or few biological membranes [11, 12] before they reach the receptor and perform their biological effect. However, there are no literary data elucidating the total biological effect of albumin interaction with Pt- and Pd-complex compounds.

The purpose of the present work is to clarify the problem by the example of membrane-bonded enzyme of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR.

Experimental Material and Methods

Purified SR was isolated from the skeletal muscles of the back legs of a rabbit by differential centrifugation as described in [13].

The activity of Ca^{2+} ion transport through the SR vesicules and that of ATP-ase were determined pH-metrically [14].

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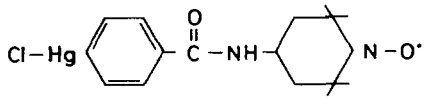
The identification of thiol groups was carried out by amperometric titration [15].

N(1-oxyl-2, 2, 6, 6-tetramethyl-4-piperydinyl-n-chloromercury benzoate)(PCMB-R) spin label interacting specifically with albumin thiol groups



was used in the ESR investigation of membrane-bonded enzyme.

ESR spectra were recorded on the radiospectrometer Varian-9. The experimental conditions are given in Figure and Table captions.

The following materials for the model system were used: the lyophylic albumin of egg-white ('Serva'), the lyophylic serum albumin of a man's blood ('Reanal'), the lyophylic serum of a bovine's blood ('Reanal') with molecular weights (46000, 69000 and 69000 respectively [16].

It is known that albumin molecules react with the ions of heavy metals to give unsoluble salts. So there was a search undertaken to establish the concentration ratio between albumin and the complex, providing the conservation of homogeneity of the system and it turned out that the 0.05 weight% concentration of albumin and 0.5 weight% content of Pt or Pd (*i.e.* at albumin:Pt = 1:10) the solution remained homogeneous at 4 °C for a month for K_2PtCl_4 and for a fortnight for K_2PdCl_4 [17].

So far the mechanism of enzyme interaction with metal bonded with albumin is of interest, then Pt- and Pd-acidocomplexes were chosen for the investigation (0.05 weight% protein and 0.5 weight% of metal, which makes the molar ratio of albumin to K_2PtCl_4 and K_2PdCl_4 be 1:1.5 and 1:1.3, respectively).

The composition of incubative mixture: NaCl, 100 m*M*; $MgCl_2$, 2 m*M*; $CaCl_2$, 0.1 m*M*; Na oxalat, 5 m*M*; imidazole, 2.5 m*M*; ATP, 1 m*M*; the protein of (Ca^{2+}–Mg^{2+})-dependent ATP-ase specimen, 0.2 mg/ml; pH 6.8; incubation temperature 37 °C. The overall volume of the sample is 5.0 ml. (I) and (II) were prepared immediately before the experiment: 0.05 g of egg albumin was dissolved in a small amount of redistillate, 0.55 mg of K_2PtCl_4 (I) or K_2PdCl_4 (II) in 0.577 mg diluted in redistillate were added and then we brought the volume of the mixture up to 100 ml adding water. Albumin ratios to K_2PtCl_4 and K_2PdCl_4 were 1:1.5 and 1:1.3, respectively (higher concentrations of ligand cause coagulation of protein).

Complex (III) is platinated human albumin;
Complex (IV) is platinated bovine albumin;
Complex (V) is palladinated human albumin;
Complex (VI) is palladinated bovine albumin.

Results and Discussion

The inhibiting action of many complex compounds of Pt and Pd upon the ATP-ase activity and Ca^{2+} ion transport through the membrane of SR have been shown [8, 9, 10]. The mechanism of action of these compounds includes both SH-group enzyme bonding the metal atom with M–S bond formation [6] and the interaction of metal atom with amino-group of protein enzyme with subsequent coordination [10].

Table I presents the data on K_2PtCl_4 and K_2PdCl_4 action on SR in the presence of egg-white albumin. The same Table gives the data on the determination of ATP-ase activity and Ca^{2+} ion transport through the SR membrane in the presence of pure K_2PtCl and K_2PdCl_4 solutions. As it is seen, complex I supresses the enzyme activity by 50–60% already at $K_2PtCl_4 = 10 \times 10^{-6}$ *M*, while pure K_2PtCl_4 does not affect the investigated enzyme in any way at the same concentration.

Complex (II), 'palladinated albumin', exhibits the same effect upon the enzyme at $K_2PdCl_4 = 2.8 \times 10^{-6}$ *M*, while pure K_2PdCl_4 is inactive at the same concentration.

Thus, biological effect of K_2PtCl_2 and K_2PdCl_4 is one order higher in the presence of albumin. (I) and (II), likewise K_2PtCl_4 and K_2PdCl_4, inhibit both ATP-ase activity and Ca^{2+} transport to the same degree.

These data show that the effect of the compounds is caused by their interaction with the enzyme groups participating in ATP-ase reaction, but not by the disconnection of ATP-ase with ionophoric regions nor by the penetration change in the membrane of SR towards the Ca^{2+} ions.

The increase in inhibiting activity of Pt-compounds upon (Ca^{2+}–Mg^{2+})-dependent ATP-ase is also observed in the presence of serum albumin of a man or a bovine (Table I) and upon pure (Ca^{2+}–Mg^{2+})-dependent ATP-ase as well.

One of the purposes of our investigation was to establish which of the specific molecular groups of egg albumin and SR interact with K_2PtCl_4 and K_2PdCl_4.

Earlier we demonstrated [6] that many of Pt-compounds could interact with thiol groups of SR. Since there are two cystine and five cystein residues in the egg albumin [16] the most active interaction of Pt- and Pd-salts occurs with free thiol groups of protein.

Table II presents the results of (I) and (II) effect, as well as that of K_2PtCl_4 and K_2PdCl_4, upon the thiol groups of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR before and after dialysis. The same Table shows no changes in the content of the thiol groups of SR in the presence of albumin, which is the total quantity of free thiol groups of SR and egg albumin and equals 12, after dialysis as should be expected.

TABLE I. The K_2PtCl_4 and K_2PdCl_4 Influence, as well as that of Their Complexes with Egg Albumin, upon (Ca^{2+}–Mg^{2+})-dependent ATP-ase Activity (in the numerator) and Ca^{2+} Ion Transport through the SR Membrane (in the denominator).

	The inhibiting action of Complexes upon (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR (% to the check samples)				
Complex	Pt- and Pd complexes (M) 7×10^{-7}	1.4×10^{-6}	2.8×10^{-6}	5.6×10^{-6}	1×10^{-5}
Albumin	0	0	0	0	0
K_2PtCl_4	0 / 0	0 / 0	0 / 0	24 ± 1 / 25 ± 1	46 ± 1 / 50 ± 2
Complex (I)	0 ± 3 / 45 ± 2	65 ± 2 / 60 ± 1	100 / 100	–	–
K_2PdCl_4	0 / 0	0 / 0	0 / 0	18 ± 1 / 20 ± 2	38 ± 3 / 40 ± 2
Complex (II)	–	20 ± 3 / 18 ± 2	40 ± 5 / 35 ± 5	71 ± 3 / 80 ± 3	–
Complex (III)	35 ± 1 / 33 ± 1	45 ± 3 / 43 ± 2	53 ± 3 / 50 ± 1	66 ± 4 / 63 ± 4	–
Complex (IV)	20 ± 2 / 20 ± 2	30 ± 4 / 27 ± 3	40 ± 1 / 45 ± 1	–	–
Complex (V)	–	18 ± 2 / 20 ± 1	26 ± 1 / 25 ± 2	45 ± 3 / 44 ± 1	–
Complex (VI)	–	15 ± 3 / 10 ± 1	25 ± 4 / 23 ± 2	43 ± 1 / 41 ± 2	–

TABLE II. The Influence of K_2PtCl_4- and K_2PdCl_4-Albumin Complexes, as well as that of K_2PtCl_4 and K_2PdCl_4, upon the Content of the Thiol Groups in Albumin and Ca^{2+}-dependent ATP-ase of SR before and after Dialysis.[a]

	The content of thiol groups g-eq./10^5 g protein								
	SR	Egg Albumin	SR+ egg albumin	I	II	SR+ K_2PtCl_4	SR+ (I)	SR+ K_2PdCl_4	SR+ (II)
Before Dialysis	10	2	12	1	1	8	8	8	10
After Dialysis	10	2	12	1	2	8	8	10	12

[a]The mean statistic results of 12 experiments are presented with approximation of ± 1 SH-group. $K_2PtCl_4 = 1 \times 10^{-5}$ *M*, $K_2PdCl_4 = 2 \times 10^{-5}$ *M*.

Albumin ratio to K_2PtCl_4 and K_2PdCl_4 was 1:1.5 and 1:1.3, respectively. The compounds were incubated for 45 min at 20 °C. Dialysis was carried out for 24 hrs against the solution containing 0.3 *M* saccharose, 100 m*M* NaCl, 1 m*M* histidine, pH 7.0; after dialysis SR was residued by 1 h centrifugation at 100 000 g, resuspensidized in the minimum quantity of the solution, against which the dialysis was carried out, and was introduced into the incubative mixture.

Dialysis of (I) as well as of SR in the presence of K_2PtCl_4 or (I), does not affect the quantity of free thiol groups of proteins, which gives evidence of the stable bond of the Pt-complex with protein.

Another picture is observed at the interaction of albumin with (II). Dialysis of Pd-ated egg albumin leads to the release of two thiol groups of protein. Dialysis of SR in the complex with K_2PdCl_4 reduces

ten SH-groups, and SR+(II) releases all the twelve free thiol groups of enzyme and albumin. These data enable us to draw the conclusion that the Pd-complex with proteins is characterized by considerably less stable chemical bonds.

The results obtained lead to the conclusion that apparently K_2PtCl_4 forms a complex of protein-ligand-protein with albumin and SR as a result of the stable bondage with thiol groups of proteins. The interaction of Pd-compounds with thiol groups of SR and albumin, forming an analogous complex, is unstable, which probably explains the much weaker inhibiting action of (II) upon (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR. To elucidate the character of (I) and (II) interaction with an active center of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR the experiments with potassium ferricyanide titration of iminoxyl radical (PCMB-R) bond with SR were carried out. The procedure caused broadening of ESR spectrum (Fig. 1) which is explained by the comparatively free access of PCMB-R for this compound.

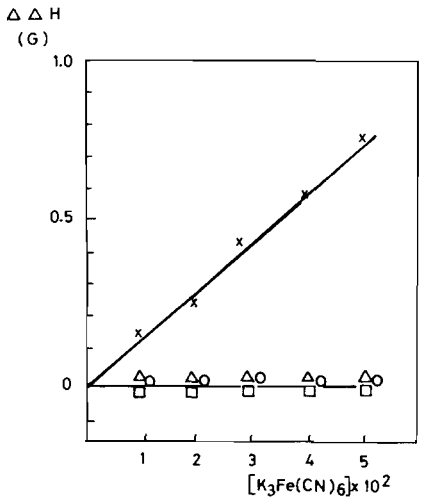


Fig. 1. The broadening of ESR spectrum of (SR + PCMB-R) complexes at the addition of potassium ferricyanide: (×) the broadening of ESR spectrum of the (SR + PMCB-R) complex, (○) the broadening of ESR spectrum of the (SR + PCMB-R) + egg albumin complex, (□) the broadening of ESR spectrum of the (SR + PCMB-R) + platinated albumin (I) complex, (△) the broadening of ESR spectrum of the (SR + PCMB-R) + palladinated albumin complex.

SR = 1.6×10^{-4} *M*, albumin = 1.7×10^{-7} *M*, platinated albumin with K_2PtCl_4 = 2.6×10^{-4} *M*, palladinated albumin with K_2PdCl_4 = 2.2×10^{-4} *M*, PCMB-R = 1.6×10^{-4} *M*.

Quite a different picture is observed in the case when SR, labelled with PCMB-R is in contact with egg albumin and (I) and (II). As is seen from Fig. 1 in these cases the potassium ferricyanide titration of the samples does not lead to the noticeable broadening of the ESR spectra which points to the nitroxyl radical screening by (I), (II) and albumin. The same behavior is observed in the case of individual (Ca^{2+}–Mg^{2+})-dependent ATP-ase.

In the other series of experiments the titration was carried out in the presence of saturating concentrations of ATP (2 m*M*).

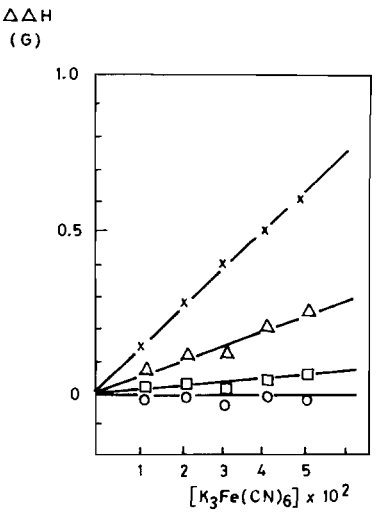


Fig. 2. The broadening of ESR spectra of (Ca^{2+}–Mg^{2+} ATP-ase + PCMB-R) + egg albumin (I, II) in the presence of the saturating concentrations of enzyme substrate and without it: (○) the broadening of ESR spectrum of the (ATP-ase + PCMB-R) + egg albumin complex, (×) the broadening of ESR spectrum of the (ATP-ase + PCMB-R) + ATP + egg albumin complex, (□) (ATP-ase + PCMB-R) + ATP + I, (△) (ATP-ase + PCMB-R) + ATP + II.

Ca^{2+}-ATP-ase = 3×10^{-5} *M*, ATP = 3×10^{-3} *M*, PCMB-R = 3×10^{-5} *M*, albumin = 6×10^{-5} *M*, palladinated albumin = 5×10^{-5} *M*, platinated albumin = 5×10^{-5} *M*.

As is seen from Fig. 2, in this case egg albumin, (I) and (II) do not completely prevent the PCMB-R interaction with potassium ferricyanide, though the access of nitroxyl radical to the action of potassium ferricyanide is considerably lower ($k = 1.5 \times 10^{-8}$), than in SR without albumin, (I) and (II) ($k = 2.1 \times 10^{-8}$), which points to the same place of seating of enzyme substrate, albumin, (I) and (II), *i.e.* to the catalytically active center of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR.

Figure 3 shows that in the presence of 1 *M* NaCl egg albumin, and (I), (II) to a less degree, do not prevent potassium ferricyanide interaction with PCMB-R, which evidences to an ion character of egg albumin interaction with SR and to an additional bond of (I) and (II) with the other (thiol, first of all) groups of protein. The aliquative action of NaCl upon SR at the investigated concentrations is hardly probable as the NaCl addition to the protein solution does not cause any noticeable changes in the solution.

It might be supposed that the increase of the inhibiting action of Pt- and Pd-compounds in the presence of egg albumin is conditioned with the

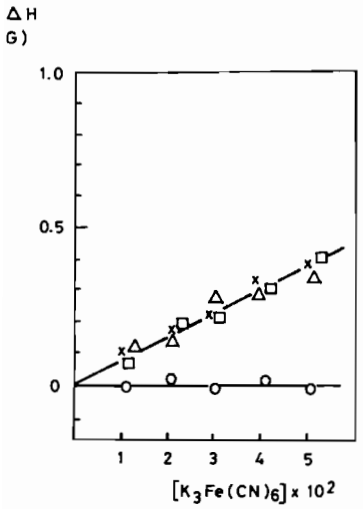


Fig. 3. The broadening of ESR spectra of (ATP-ase + PCMB-R) complex at the addition of potassium ferricyanide in the high NaCl concentration: (○) the broadening of ESR spectrum of (Ca^{2+}-ATP-ase + PCMB-R) + albumin, (×) the broadening of the spectrum of (Ca^{2+}-ATP-ase + PCMB-R) + NaCl + albumin, (□) (Ca^{2+}-ATP-ase + PCMB-R) + NaCl + (I), (△) (Ca^{2+}-ATP-ase + PCMB-R) + NaCl + (II).

Ca^{2+}-ATP-ase = 3×10^{-5} *M*, NaCl = 10^{-2} *M*, albumin = 6×10^{-5} *M*, PCMB-R = 3×10^{-5} *M*, (I) = 6×10^{-5} *M*, (II) = 6.10^{-5} *M*.

albumin ability to interact with the hydrofobous parts of the protein, which are present in the active center of (Ca^{2+}–Mg^{2+})-dependent ATP-ase of SR. This, in its turn, makes the interaction of Pt and Pd with thiol groups of enzyme active center easier. The similar situation can be observed in the case of other transport proteins, human or bovine serous albumin, for example.

Thus when determining the biological activity of the Pt- and Pd-compounds in the organism, one should take into account the specific increase of their activity at the interaction with protein, which has been described in the present work.

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