THE INFLUENCE OF CATIONS ON THE DISSOCIATION OF HEMOCYANIN OF THE SCORPION *ANDROCTONUS AUSTRALIS GARZONII* PROVOKED BY 1 M UREA

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1. Introduction

It is well known that Ca^{2+} and Mg^{2+}-ions protect hemocyanins of molluscs [1,2] and of certain species of arthropods [3,4] against a pH-induced dissociation.

In the scorpion *Androctonus australis*, we have shown that after extensive dialysis, low concentrations of urea (1 M) dissociate the native hemocyanin into six fragments in 24–48 h. The dissociation is subtotal (> 90%) and the same products are obtained by exposing the native form to alkaline pH-values.

This mild dissociation by urea is the basis of a technique for subunit isolation [5]. Five subunits of different electrophoretic mobilities have sedimentation constants near 4.8 S. The sixth fragment, having a sedimentation constant of 7 S, is dissociable by SDS into protomers whose molecular weight is near that of the five subunits [6,7].

The original purpose of this paper was to define the action of divalent cations on 1 M urea-induced dissociation, but the control study with monovalent alkaline cations have shown that the latter have the same effect although at higher concentrations.

The effect of mono- and divalent cations on the 1 M urea-induced dissociation of hemocyanin in *A. australis* is reported here.

2. Experimental

The hemolymph, obtained by cardiac puncture, is centrifuged for 10 min at 800 × *g* to eliminate hemocytes. The hemocyanin is then precipitated three times by 55% saturated ammonium sulfate.

We studied the following concentrations for each cation: 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, $5 \cdot 10^{-1}$, 1 and 2 M, in a pH 7.5, 0.05 M Tris-buffer.

Dissociation is obtained by adding 7 M urea to the sample in such a way that the final urea concentration is 1 M. After two days of contact, the solutions are analyzed by thin-layer gel-filtration (TLG).

This analytical technique has three advantages:

(i) It needs little biological material (5 μl, 15 mg/ml solution)

(ii) It gives a better resolution than column gel-filtration

(iii) It is possible to treat ten different samples comparatively on the same plate.

An 8 mm thick Sephadex G-150 SF, eluted with 0.05 M Tris-HCl buffer, pH 7.5, was used. The development lasts 5 h at 20°C with a slope of 15°. The spots are colored with Coomassie Brilliant Blue R on a replica [8].

3. Results

Figure 1 is a replica of TLG obtained for two cations, NH_4^+ and Ca^{2+}. The upper spot corresponds to those subunits with sedimentation constants of 4.8 S, the intermediate spot corresponds to the fragment whose sedimentation constant is 7 S, and the lower spot corresponds to excluded proteins, essentially non-dissociated hemocyanin (34 S). The control, performed in the absence of cations is the 0-point.

It can be clearly seen that, as the concentration of divalent ions increases, the proportion of dissociation products (upper spots) decreases, reaches a minimum, and then increases again.

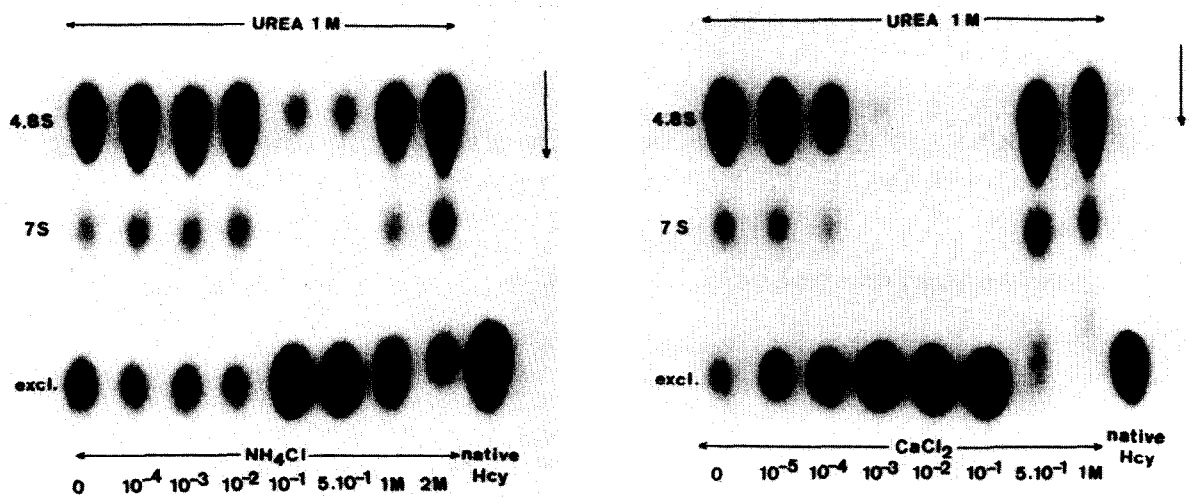

Fig.1. Action of NH_4Cl and $CaCl_2$ on 1 M urea-provoked hemocyanin dissociation in *A. australis* (replica of TLG). The arrow indicates the direction of migration.

In the case of calcium, the dissociating effect of 1 M urea disappears for concentrations between 10^{-3} M and 10^{-1} M inclusive. For ammonium, the effect attenuates without entirely disappearing between 0.1 M and 0.5 M.

The results obtained with other cations are shown in table 1. It can be noted that all divalent cations at a concentration of 10^{-2} M protect the hemocyanin completely from 1 M urea-provoked dissociation. The monovalent cations show the same characteristic, but the protection is generally incomplete, the maximum occurring between 0.1 M and 1 M, that is, for concentrations nearly 100-times greater than those of divalent cations.

Likewise, monovalent cation protection appears at a concentration 100-times greater (10^{-2} M as compared to 10^{-4} M for divalent cations).

Finally, it can be seen on this table that, in most cases, the protection disappears at high ionic strength.

4. Discussion

Mild dissociation through low concentrations of urea is not widely used and its exact mechanism is not yet clear. In the absence of cations, scorpion hemocyanin dissociates easily, but this is not true for all arthropods. In fact, the preliminary results have shown that the hemolymph of the spider *Dugesiella californica* [9] does not dissociate at all in the presence of 1 M urea, at pH 7.5.

With *A. australis*, the native molecule (34 S) always breaks down to five 4.8 S fragments and one 7 S fragment, and never gives a 16 S compound regardless of the dissociation process; this is the opposite of what was observed in *Cancer magister* [10] and *Limulus polyphemus* [11].

The protection phenomenon is seen in divalent as well as monovalent cations, but the monovalent ones act as concentrations 100-times greater. Although all our experiments have been carried out in $5 \cdot 10^{-2}$ M chloride (pH 7.5, Tris-HCl buffer), the addition of a chloride or a sulfate of the same cation does not modify the effect of a given cation at a given concentration. The ionic strength cannot alone explain the protective effect. With the exception of $MgSO_4$ and KCl, protection disappears or diminishes in most cases at high salt concentrations. In this last case, according to the salt used, the pH can vary by 0.5 unit, but this cannot be responsible for the observed phenomena. On the other hand, a salting out effect prevents very often the study of high concentrations.

A precise chemical analysis of the salts used has shown that in no case a divalent impurity of monovalent salts can be responsible for the effect observed.

These phenomena can possibly be explained in part

Table 1
Effects of different cations on 1 M urea induced dissociation of hemocyanin in *A. australis*

Cations		Moles per liter							
		10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	5.10^{-1}	1	2
Divalent	Mg^{2+} (Cl^-)	–	++	+++	[++++]	[++++]	a	–	–
	Mg^{2+} (SO_4^{2-})	–	++	+++	[++++]	[++++]	[++++]	[++++]	[++++]
	Ca^{2+} (Cl^-)	–	++	+++	[++++]	[++++]	+	–	a
	Mn^{2+} (Cl^-)	+	+++	+++	[++++]	[++++]	+	+	a
	Mn^{2+} (SO_4^{2-})	–	++	[++++]	[++++]	[++++]	a	a	a
Monovalent	Na^+ (Cl^-)	–	–	–	+	++	++	+	+
	Na^+ (SO_4^{2-})	–	–	–	–	+	[++++]	[++++]	a
	K^+ (Cl^-)	–	–	–	–	++	a	++	++
	Li^+ (Cl^-)	–	–	–	+	+++	+++	++	+
	Li^+ (SO_4^{2-})	–	–	–	+	+++	a	[++++]	a
	NH_4^+ (Cl^-)	–	–	–	–	+++	+++	++	+
	NH_4^+ (SO_4^{2-})	–	–	–	–	+++	a	[++++]	a

[++++] = complete protection (dissociation = 0)
+++ = 3/4 protection
++ = 1/2 protection
\+ = beginning of protection
– = no protective effect
a = untested concentration (protein precipitation in most cases)

by the hypothesis according to which the stabilizing action of calcium and magnesium on the quaternary structure diminishes at high ionic strength [12].

The reversible denaturaing action of urea thus becomes perceptible, even at very weak concentrations, on a hemocyanin molecule in unstable equilibrium.

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