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POLAROGRAPHIC STUDIES OF THE INFLUENCE OF INCREASING ZINC AND COPPER ION CONCENTRATIONS ON THE CHANGES IN GLOBULAR/ FIBRILLAR RATIO OF SERUM PROTEINS

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Polarographic investigations of the changed globular/fibrillar ratio in serum proteins under the influence of increasing zinc and copper ion concentrations in human blood serum were carried out. Very low concentrations of Zn^{2+} and Cu^{2+} did not exert any effect on the native globular structure of serum proteins at all. Some changes of the size and ratio of both polarographic waves could be detected at certain higher zinc and copper ion levels. This was considered a feature of structural alteration in some serum proteins. The results from the study allowed the estimation of optimal zinc and copper ion concentrations which did not exert any effect on the globular structure of the blood protein. The ions examined at these concentrations possessed a maximally favourable effect on the mechanisms in which they participated.

Key-words: Serum ion concentration, polarography, zinc, copper, protein structure, globular/fibrillar ratio

INTRODUCTION

Roentgenostructural analysis shows that most proteins are globule-like and parts of of polypeptide chains form terminal fibrillar structures (3). Such fibrillar structures can be formed, however, not only at the end of the molecule but also in its middle part. It is possible to consider the protein molecule formed by a spiral and a globular component. Under certain conditions protein molecules display a stability that can be easily upset under the influence of different agents. This results in changes in globular and fibrillar components. Statistical mechanics provides interpretation of possible changes from globular into fibrillar structure, and vice versa (10).

Polarographic analysis can determine the ratio of globular to fibrillar components of a given protein or protein mixture. It can be done by estimating the ratio between the first (h_1) and the second (h_2) polarographic waves (3, 4). This index is very important bearing in mind that both fibrillar and globular parts give the molecule different

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properties. Changing the initial ratio of globular and fibrillar components of the protein molecule induces alterations of its native properties. Proteins demonstrate conformational stability towards weak influences. That is why it is important to be aware of the concentration above which a given agent is capable to cause a change.

The objective of this study was to estimate the effect of increasing concentrations of zinc and copper ions on the changes in the ratio between globular and fibrillar components of blood serum proteins.

MATERIAL AND METHODS

Usage was made of 1 per cent of human blood serum solution. Both $ZnSO_4$ and $CuCl_2$ were added to 1 ml of serum in amounts to obtain zinc and copper ions concentrations given in the Tables presented below. Sulfate and chlorine ions inserted were in very small quantities as compared to those in the serum and they could not influence upon protein conformation. Samples were put into a thermostat for 30 min at 37 °C and after that in a refrigerator for 24 hours at about 4 °C. This experimental design was chosen because it was previously proved that structural changes in serum proteins were significantly delayed under such conditions. After incubation, 2 ml of Brdicka's solution were supplemented to each sample. Polarographic analysis was performed at the presence of air. It started from 0,8 V and was carried out until the terminal automatically registered a double polarographic wave. A LP-60 polarograph with a sensitivity of 1/300 was applied. Brdicka's solution contains 0,001 M $[Co(NH_3)_6]Cl_3$, 0,1 M NH_4Cl, and 0,1 M NH_3 (6). Preparation of $[Co(NH_3)_6]Cl_3$ was done after Brauer's method (9). The hight of both polarographic waves was read in mm.

RESULTS

Table 1 demonstrates the results obtained under the influence of increasing zinc ion concentrations. Each arithmetic mean $\overline{X}$ results from six parallel samples. Total amounts of zinc ions in each sample are indicated. It is evident that zinc ions inserted in concentrations of the series 2 through 4 do not lead to significant changes of the globular/fibrillar ratio of serum proteins. However, insertion of zinc ions in the samples in concentrations of the series 5 through 7 reduces significantly this ratio.

Table 2 presents the changes of the serum influenced by increasing copper ion concentrations. Each arithmetic mean $\overline{X}$ is obtained from six parallel samples. Total quantities of copper ions are shown. It is noteworthy that copper ion insertion in concentrations of the series 2 through 4 do not cause any significant changes of the globular/fibrillar ratio of serum proteins. On the other hand, copper ions inserted in concentrations of the series 5 through 7 diminish statistically significantly this ratio. In contrast to the effect exerted by the zinc ions this decrease in the three series is almost the same.

DISCUSSION

It is obvious that native serum protein molecules demonstrate a stability that can be affected by both zinc and copper ions in concentrations higher than a certain level.

Table 1
Polarographic waves influenced by increasing zinc ion concentrations

Series No	Sample	n	h_1 (mm)	h_2 (mm)	h_1/h_2	$\overline{X}$, t, p
I	Control	1	70	90	0,7778	$\overline{X}$=0,7602
		2	69	90	0,7667	
		3	69	90	0,7667	
		4	68	90	0,7556	
		5	68	90	0,7556	
		6	65	88	0,7386	
II	plus 1 ml of	1	69	89	0,7753	$\overline{X}$=0,7579
	$1,53.10^{-7}$M/l	2	69	90	0,7667	t=0,32
	zinc solution	3	68	91	0,7582	p>0,05
		4	68	91	0,7582	
		5	67	90	0,7444	
		6	68	90	0,7556	
III	plus 1 ml of	1	69	91	0,7582	$\overline{X}$=0,7593
	$1,53.10^{-6}$ M/ l	2	68	90	0,7556	t=0,13
	zinc solution	3	69	90	0,7667	p>0,05
		4	68	88	0,7727	
		5	67	90	0,7444	
		6	69	91	0,7582	
IV	plus 1 ml of	1	63	85	0,7412	$\overline{X}$=0,7533
	$1,53.10^{-5}$ M/l	2	68	90	0,7556	t=0,15
	zinc solution	3	64	85	0,7529	p>0,05
		4	64	85	0,7529	
		5	66	87	0,7586	
		6	66	87	0,7586	
V	plus 1 ml of	1	72	105	0,6857	$\overline{X}$=0,6929
	$1,53^{-4}$ M/l	2	72	106	0,6792	t=7,65
	zinc solution	3	74	108	0,6852	p<0,001
		4	75	107	0,7009	
		5	76	105	0,7238	
		6	71	104	0,6827	
VI	plus 1 ml of	1	65	98	0,6633	$\overline{X}$=0,6427
	$1,53.10^{-3}$ M/l	2	62	96	0,6458	t=14,89
	zinc solution	3	61	96	0,6354	p<0,001
		4	60	96	0,6250	
		5	59	93	0,6344	
		6	62	95	0,6526	
VII	plus 1 ml of	1	60	102	0,5882	$\overline{X}$=0,5615
	$1,53.10^{-2}$ M/l	2	57	102	0,5588	t=18,31
	zinc solution	3	56	104	0,5490	p<0,001
		4	60	100	0,6000	
		5	54	101	0,5346	
		6	55	101	0,5445	

Table 2
Polarographic waves influenced by increasing copper ion concentrations

Series No	Sample	n	h_1 (mm)	h_2 (mm)	h_1/h_2	x, t, p
I	control	1	71	85	0,8353	$\overline{X}$=0,7830
		2	68	89	0,7641	
		3	67	89	0,7528	
		4	68	84	0,8095	
		5	71	90	0,7889	
		6	68	91	0,7473	
II	plus 1 ml of	1	68	89	0,7641	$\overline{X}$=0,7575
	$1{,}53.10^{-7}$ M/l	2	68	89	0,7641	t=1,78
	copper solution	3	68	90	0,7556	p>0,05
		4	68	90	0,7556	
		5	68	90	0,7556	
		6	66	88	0,7500	
III	plus 1 ml of	1	67	86	0,7791	$\overline{X}$=0,7702
	$1{,}53.10^{-6}$ M/l	2	68	90	0,7556	t=0,84
	copper solution	3	69	90	0,7667	p>0,05
		4	69	89	0,7753	
		5	71	90	0,7889	
		6	68	90	0,7556	
IV	plus 1 ml of	1	69	83	0,8313	$\overline{X}$=0,7931
	$1{,}53{,}10^{-5}$ M/l	2	69	86	0,8023	t=0,55
	copper solution	3	69	87	0,7931	p>0,05
		4	63	85	0,7412	
		5	69	87	0,7931	
		6	67	84	0,7976	
V	plus 1 ml of	1	90	134	0,6716	$\overline{X}$=0,6741
	$1{,}53.10^{-4}$ M/l	2	86	132	0,6513	t=7,01
	copper solution	3	88	130	0,6769	p<0,001
		4	85	128	0,6641	
		5	89	130	0,6846	
		6	87	125	0,6960	
VI	plus 1 ml of	1	93	136	0,6838	$\overline{X}$=0,6984
	$1{,}53.10^{-3}$ M/l	2	90	125	0,7200	t=5,44
	copper solution	3	90	126	0,7142	p<0,001
		4	87	126	0,6905	
		5	86	123	0,6992	
		6	84	123	0,6829	
VII	plus 1 ml of	1	90	136	0,6618	$\overline{X}$=0,6710
	$1{,}53.10^{-2}$ M/l	2	91	134	0,6791	t=7,62
	copper solution	3	87	132	0,6591	p<0,001
		4	88	129	0,6822	
		5	86	127	0,6772	
		6	84	126	0,6667	

This index is very important as above this level considerable alterations appear in the structure and properties of the protein molecules. This is the toxic effect of the microelements.

Lower concentrations of zinc and copper ions in which protein molecules can not be altered are of great importance as it is well-known that these microelements are essential for the normal functioning of most enzymes and some specialized proteins (1, 2, 5, 7, 8). That is why these results can help the determination of optimal concentrations of zinc and copper ions in the serum of a healthy organism. On the other hand, these levels established in the present investigation above which the ratio between globular and fibrillar components of serum proteins changes are of significance when studying the properties of serum proteins under the influence of zinc and copper ions.

CONCLUSION

Our data suggest that further studies should be directed towards the revealing the type of serum proteins which are labile in their globular structure under the conditions of enhancing zinc and copper ion concentrations.

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Polarographische Untersuchungen über den Einfluß wachsender Zn^{2+} und Cu^{2+}-Konzentrationen auf die Veränderungen des Verhältnisses der globulären zu den fibrillären Abschnitten in den Serumproteinen

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Zusammenfassung: Es wurden polarographische Untersuchungen über die Veränderungen des Verhältnisses der globulären zu den fibrillären Abschnitten in

den Proteinen unter der Einwirkung wachsender Zn^{2+} und Cu^{2+}-Konzentrationen im menschlichen Blutserum durchgeführt. Sehr niedrige Konzentrationen von Zn^{2+} und Cu^{2+} bewirkten die native globuläre Struktur des Serumproteins überhaupt nicht. Es wurden Veränderungen in der Größe und im Verhältnis der beiden polarographischen Wellen bei bestimmten höheren Konzentrationen von Zn^{2+} und Cu^{2+} festgestellt. Das wurde als ein Zeichen der Strukturänderung in einigen Serumproteinen angenommen. Die Untersuchungsergebnisse erlaubten die optimale Zink- und Kupferionenkonzentration, die keinen Einfluß auf die globuläre Struktur des Eiweißes im Blut hatte, zu ermitteln. Die in diesen Konzentrationen studierten Ionen besitzten einen maximal günstigen Effekt auf die Mechanismen, an denen sie beteiligt waren.

Études polarographiques sur l'influence des concentrations croissantes des ions de zinc et de cuivre sur le changement de la relation des tronçons globulaires et fibrillaires dans les protéines du sérum sanguin

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Résumé: Des études polarographiques sur le changement de la relation des tronçons globulaires et fibrillaires dans les molecules des protéines d'un sérum sanguin humain sous l'influence des concentrations croissantes des ions de zinc et de cuivre ont été réalisées. Les petites concentrations des ions de zinc et de cuivre ne changent pas la structure globulaire native des protéines du sérum. A des valeurs déterminées des ions de zinc et de cuivre introduites dans les prélèvements du sérum sanguin s'effectuent des changements dans le grandeurs et la relation des ondes polarographiques, ce qui est un indice du changement de la structure globulaire de certains protéines du sérum. La constatation de ces changements donne la possibilité d'établir des concentrations optimales de zinc et de cuivre dans un organisme sain lesquels n'exercent pas d'influence sur les structures globulaires des protéines et ont un effet favorable maximal sur les mécanismes exigeants une participation de ces ions.