Formation of a double salt of phosphatidylcholine and zinc chloride

Karoly I. von GLÓS and John C. BOURSNELL*

*Agricultural Research Council Institute of Animal Physiology, Animal Research Station,
307 Huntingdon Road, Cambridge CB3 0JQ, U.K.*

(Received 27 October 1980/Accepted 19 November 1980)

ZnCl_2 forms a 1:1 double salt with phosphatidylcholine. This compound resembles the long-known double salt of CdCl_2 and phosphatidylcholine except that the latter has the composition $(\text{CdCl}_2)_3(\text{phosphatidylcholine})_2$. Treatment of phosphatidylcholine with a mixture of equimolar amounts of ZnCl_2 and CdCl_2 yields the ZnCl_2 double salt. The ZnCl_2 double salt can be obtained as an amorphous flocculent precipitate and as small spherules. These results are discussed in relation to the toxic action of cadmium salts on the mammalian testis and to the protection afforded by zinc salts. It is suggested that membrane phospholipids are essential components of specific sites for reversible binding of Zn^{2+} and Cd^{2+} .

Boursnell *et al.* (1977, 1978) obtained evidence that the zinc in boar seminal plasma was immobilized by added phosphatidylcholine. Thus the transfer of zinc from seminal plasma to spermatozoa, which occurs when the semen is cooled to 4°C, was inhibited by ovophosphatidylcholine. This observation has led to the preparation of a zinc analogue of the well-known double salt of phosphatidylcholine and CdCl_2 . The latter compound, discovered by Strecker (1968), became the basis of procedures for the purification of lecithin from animal sources [See, for example, Pangborn (1951)].

Materials and methods

Anhydrous granular ZnCl_2 , $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ and lecithin ('egg, grade I', as a solution in chloroform/methanol) were obtained from B.D.H. Chemicals, Poole, Dorset, U.K. Ethanol, methanol and peroxide-free diethyl ether were redistilled.

Determinations of all elements except C and H were carried out on solutions of phosphatidylcholine derivatives in 1M-acetic acid. Zinc and cadmium were assayed by atomic-absorption spectrophotometry. Nitrogen was determined by a micro-Kjeldahl method and chloride by means of Diagnostic Kit 830 from Sigma (London) Chemical Co. Total phosphorus was converted into orthophosphate (Umbreit *et al.*, 1957) and this was determined by the procedure of Chen *et al.* (1956). The elemental composition of phosphatidylcholine was calculated on

the assumption that its acyl content consisted of equimolar proportions of esterified stearic, palmitic, oleic and linoleic acids. Melting points were measured on a Koffler stage.

Phosphatidylcholine (250 mg) was freed from solvents in a stream of N_2 at room temperature and dissolved in ethanol to a volume of 10 ml to give a 33 mM solution. Centrifuging was at 1000 g and all operations were at 0–4°C.

Products obtained by adding ZnCl_2 and CdCl_2 to equimolar amounts of lecithin

ZnCl_2 and CdCl_2 were made up as 33 mM solutions in 95% (v/v) methanol.

Experiment 1. To 3 ml of lecithin solution was added 3 ml of ZnCl_2 solution. A bulky amorphous precipitate was centrifuged down 1 h later, washed with 95% methanol and freed from solvents in a stream of N_2 (fraction 1a). The supernatant and washed fluids were stored overnight when small translucent spherules appeared in both. The spherules were firmly attached to the glass walls of the containers and were washed by decantation with 95% methanol, dried and combined (fraction 1s) (m.p. 177–179°C; found: C, 56.6; H, 9.0; N, 1.7; P, 3.6; Zn, 7.9; Cl, 8.3; $\text{C}_{43}\text{H}_{83}\text{O}_8\text{NP} \cdot \text{ZnCl}_2$ requires C, 56.8; H, 9.2; N, 1.5; P, 3.4; Zn, 7.2; Cl, 7.8%). Thus the spherular fraction was an equimolar double salt of phosphatidylcholine and ZnCl_2 . The spherules were not birefringent under a polarizing microscope.

Experiment 2. This differed from Expt. 1 in that the ZnCl_2 was dissolved in diethyl ether. An amor-

* Deceased 17 December 1977.

Table 1. *Composition of double-salt fractions obtained from phosphatidylcholine and ZnCl₂*

a indicates amorphous products, s indicates spherular products. Atomic ratios are with reference to nitrogen (=1.00).

Fraction number	Yield (%)	Atomic ratios			
		N	P	Zn	Cl ⁻
1a		1.00	0.99	1.10	
1s	17	1.00	1.03	1.00	1.96
2a	89	1.00	0.97	1.01	1.93
4s	37	1.00	0.99	0.98	1.94
5ai	29	1.00	1.02	1.01	1.63
5aii	3	1.00		1.13	
5s	34	1.00	1.01	0.99	1.95
6a		1.00	1.01	1.26	2.03
6s	24	1.00	0.98	1.00	2.05

phous precipitate appeared slowly and was collected the next day (fraction 2a). This was analytically identical with fractions 1a and 1s (Table 1). No spherular fraction was obtained.

Experiment 3. Repeating Expt. 1, but with CdCl₂ replacing ZnCl₂, we repeatedly obtained amorphous and spherular products. However, the molar ratio of CdCl₂/phosphatidylcholine varied in a random manner within the limits 1.0–1.4

Experiment 4. To 3 ml of lecithin solution was added a mixture of 3 ml each of ZnCl₂ and CdCl₂ solutions. No amorphous material appeared but by the next day a spherule fraction (4s) had formed. It proved to be the ZnCl₂ compound. Cadmium was not detected in a 0.6% (w/v) solution of the product. This means that the atomic ratio Cd/Zn was less than 0.001. Tests with standard solutions containing high ratios of ZnCl₂/CdCl₂ gave no evidence that excess of zinc suppressed the spectrophotometer signal given by cadmium.

Reactions between lecithin and excess of ZnCl₂

Experiment 5. Lecithin solution (10 ml) was mixed with 10 ml of dry methanol containing 0.82 mmol of ZnCl₂ (Zn/phosphatidylcholine ratio = 2.5). An amorphous precipitate formed immediately and a spherule fraction (5s) was collected 5 days later. When the amorphous fraction was mixed with 0.1 M-acetic acid most of it (fraction 5ai) dissolved. A small gummy residue (fraction 5aii) was taken up in methanol. Fractions 5ai and 5s both analysed as the 1:1 double salt.

Experiment 6. This was as Expt. 5, except that ZnCl₂ was added as 1 ml of a saturated solution in methanol (Zn/phosphatidylcholine ratio = 7.2). Again the spherule fraction (6s) was the 1:1 double salt.

Results and discussion

Comparison of ZnCl₂ and CdCl₂ double salts of phosphatidylcholine

Thudichum (1844) concluded that CdCl₂ combined with phosphatidylcholine in equimolar ratio, but later authors (e.g. Erlandsen, 1907; Eppler, 1913) found ratios nearer to 1.5 than 1.0. Thudichum also obtained CdCl₂-phosphatidylcholine as spherules, which he assumed to be crystal aggregates. However, lack of birefringence of spherular ZnCl₂-phosphatidylcholine argues against its crystallinity. Phospholipids other than phosphatidylcholine also combine with CdCl₂. Though such double salts are more soluble than the phosphatidylcholine derivative they tend to be adsorbed by precipitates of the latter. Lack of agreement on the composition of CdCl₂-phosphatidylcholine during the 80 years after its discovery (Wittcoff, 1951) is therefore not surprising. The problem was resolved when Baer & Kates (1950) treated three synthetic lecithins with 2.5 molar proportions of CdCl₂ and obtained three double salts with the composition (phosphatidylcholine)₂(CdCl₂)₃. In Expt. 3 the products were presumably co-precipitates of the 2:3 double salt and phosphatidylcholine.

From equimolar amounts of ZnCl₂ and phosphatidylcholine we invariably obtained a double salt containing the two components in a 1:1 ratio (Table 1). When ZnCl₂ was in excess spherular fractions consisted of the same double salt, but some amorphous fractions contained excess of zinc over phosphatidylcholine though no excess of chloride. This can be explained by the very high affinity of ZnCl₂ for water and its resulting hydrolysis. Hot methanolic solutions of either form of the ZnCl₂ derivative deposit spherules when slowly cooled. The appearance of insoluble ZnCl₂-phosphatidylcholine in Expt. 4 suggests that phosphatidylcholine has a higher affinity for ZnCl₂ than for CdCl₂. Thus in absence of the ZnCl₂ the CdCl₂ double salt would have been deposited. All results in Table 1 were obtained with freshly prepared phosphatidylcholine solutions. Stored solutions tended to give gummy non-stoichiometric products.

It is interesting that CdCl₂ and glycerophosphocholine yield a 3:2 double salt that dissociates to a 1:1 salt on recrystallization from aqueous ethanol (Baer & Kates, 1948).

Competition between Zn²⁺ and Cd²⁺ in vivo: phospholipids as possible mediators

Pařízek (1957, 1960) showed that subcutaneous injection of rats with 0.04 mmol of CdCl₂/kg body wt. caused regressive changes in the spermatogenic epithelium within a few hours. At 24–48 h all testicular tissue was severely necrosed. Simultaneous injection of an excess of zinc acetate gave complete

protection against this cadmium necrosis. During a histochemical study of rat testis Timm & Schulz (1966) found high concentrations of membrane-bound zinc in the germ cells and interstitial cells. But when rats were injected with CdCl_2 , the bound zinc of the Leydig cells and Sertoli cells was rapidly replaced by bound cadmium.

In an attempt to correlate the information in the present paper we put forward the hypothesis that some membrane phospholipids can form double salts incorporating Zn^{2+} . Such phospholipids would be essential components of membrane binding sites (and possibly of translocation systems) for this metal. The same sites would also have affinity for cadmium and perhaps for other heavy-metal ions.

K. I. v. G. thanks Dr. E. F. Hartree for a critical reading of the manuscript.

References

- Baer, E. & Kates, M. (1948) *J. Am. Chem. Soc.* **70**, 1394–1399
- Baer, E. & Kates, M. (1950) *J. Am. Chem. Soc.* **72**, 942–949
- Bournsnel, J. C., Partridge, D. R. & von Glós, K. I. (1977) *J. Reprod. Fertil.* **49**, 391–393
- Bournsnel, J. C., Niemczuk, P. & von Glós, K. I. (1978) *J. Reprod. Fertil.* **52**, 327–332
- Chen, P. S., Toribara, T. Y. & Warner, H. (1956) *Anal. Chem.* **28**, 1156–1158
- Eppler, J. (1913) *Hoppe-Seyler's Z. Physiol. Chem.* **87**, 233–254
- Erlandsen, A. (1907) *Hoppe-Seyler's Z. Physiol. Chem.* **51**, 71–155
- Pangborn, M. C. (1951) *J. Biol. Chem.* **188**, 471–476
- Pařízek, J. (1957) *J. Endocrinol.* **15**, 56–63
- Pařízek, J. (1960) *J. Reprod. Fertil.* **1**, 294–309
- Strecker, A. (1868) *Justus Liebigs Ann. Chem.* **148**, 77–90
- Thudichum, J. L. W. (1884) *A Treatise on the Chemical Constitution of the Brain*, pp. 42–48, Baillière, Tindall and Cox, London
- Timm, F. & Schulz, G. (1966) *Histochemie* **7**, 15–21
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957) *Manometric Techniques*, 3rd edn., p. 273, Burgess Publishing Co., Minneapolis
- Wittcoff, H. (1951) *The Phosphatides*, p. 95, Reinhold Publishing Corp., New York