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THE BINDING OF CHLORAMPHENICOL TO ALBUMIN OF NORMAL AND UREMIC SERA

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Abstract—Albumins isolated from sera of patients with chronic renal insufficiency show the decreased ability to bind chloramphenicol. The bond to single binding center is characterized by constant $k = 5.0 \times 10^3$ l.mole^{-1} while in a case of albumin of healthy individuals by constant $k = 8.2 \times 10^3$ l.mole^{-1}. After charcoal treatment at pH 3 the binding constant of albumin from sera of healthy as well as uremic patients increases to the equal value of 2.1×10^4 l.mole^{-1}. These data are in agreement with the suggestion that the decreased binding is caused by the presence of not identified compounds which bind irreversibly to albumin molecule in uremic sera while changing its binding ability. Using charcoal treatment at pH 3 these compounds can be removed.

In the case of patients suffering from renal disease some changes in the excreted compounds and change of their metabolism have been observed. Frequently, these phenomena involve a decrease of serum albumin concentration. For that reason, it is possible to expect a decreasing tendency to bind pharmaca to serum albumin. The weakness of the binding could then be in connection with exceeding their toxicity limit [1, 2].

Therefore, many recent papers deal with studies on the character of bond extents of various pharmaca to serum proteins in patients with a defective function of kidneys [1–14].

Actually, many compounds show a decrease of the binding ability followed by its reincrease after haemodialysis [1, 2, 5, 7, 9, 12–14]. Only partially the decreased binding capacity of uremic sera was explained to be dependent upon decrease of serum albumin concentration [1, 2, 9, 12]. Some authors [4, 6, 11, 12, 14–16] suppose that the expulsion of pharmaca from binding centres by metabolites or other endogenous compounds concentrated in sera of patients with impaired renal function might be a reasonable explanation for the decreased binding capacity.

However, competitive inhibitions likewise fail to completely explain the decrease of binding ability of uremic sera, considering that their binding extent after dialysis does not reach the value of normal dialyzed sera [12, 14]. There exists an explanation suggesting the possibility of an altered albumin present in sera of patients with chronic renal insufficiency. The work of Shoeman and Azarnoff [6] seems to be a serious argument for such a statement. Using the isoelectric focusing technique they have recorded two separated bands the relative representation of which differs in the normal and uremic serum. In spite of that it is known that in using this method many artefacts have been found. Although Sjöholm *et al.* [14] confirmed that albumin isolated from uremic sera showed a decreased binding affinity, they did not find significant differences in its conformation employing the c.d. specta. Further, they have established that the application of charcoal treatment of albumin at pH 3 restores the binding to normal. The authors therefore suggest the sera of patients with renal insufficiency do not include a qualitatively different albumin, and assume the presence of compounds in the sera capable of forming an irreversible bond to albumin. The inhibition of the binding ability can be then abolished by charcoal treatment at pH 3. Recently, Soltys and Hsia [17] showed that the binding of fatty acids does influence the conformation of albumin molecule under allosteric modification of its binding capacity. They conclude that the fluctuations of fatty acid level in the sera have the modulation influence on the level of free pharmaca and other physiological compounds in sera which bind to albumin. An analogous influence may be suggested for other endo and/or exogenic compounds.

We proved in our paper that in the case of chloramphenicol in patients with chronic renal insufficiency a decreased binding takes place [15]. Using a model of isolated serum albumin we tried to contribute to the explanation of the decreased binding ability of uremic sera.

MATERIALS AND METHODS

Albumins have been isolated from blood sera by precipitation with rivanol [18]. Monomeric fractions were separated by gel chromatography [19] on Sephadex G-150. Starting materials involved sera of healthy blood donors, as well as the sera of patients with polycystic kidneys and chronic glomerulonephritis identical with those in an earlier paper [15]. Some of the albumin samples were further purified according to the procedure of Chen [20] with activated charcoal at pH 3. The

purity of the used samples has been verified by electrophoresis on polyacrylamide gel; for mol. wt the value 65 100 g.mole^{-1} has been used [21].

D-Chloramphenicol (mol. wt. 323 g.mole^{-1}) was a product of Léčiva, Prague. The extent of binding of chloramphenicol to serum albumin was determined using equilibrium dialysis. Two compartments of dialysis cell [22] having volume of 4 cm^3 were separated by membrane Kalle (Wiesbaden–Biebrich, BRD). Before use the membrane was stored for 24 hr in corresponding buffer. One of the cells has been filled with a solution of albumin of a concentration of 2 g/100 cm^3 in 0.05 M phosphate buffer at pH 7.2 while the other cell contained solutions of chloramphenicol in the same medium. The experiments have been carried out in the range of 1.5×10^{-4} to 9×10^{-4} mole. l^{-1} of chloramphenicol concentrations obtained by dilution of stock solution of 9.285×10^{-4} M, i.e. 300 μg/l cm^3 with the buffer employed. To prepare this solution chloramphenicol had been first dissolved in ethanol and the solution was then diluted to the final content of 1 g ethanol/100 cm^3. The dialysis of chloramphenicol solution against pure buffer showed that the period of 48 hr is quite sufficient to attain equilibria when mechanical stirrer was applied. In addition, it has been proved that under these conditions the antibiotic does not bind to dialysis membrane.

The concentrations of serum albumin solutions have been determined from weight of total solids obtained by drying at 105°C and checked by spectrophotometry using Unicam SP 700 when absorption at 280 nm of 1 mg/1 cm^3 solution was taken as 0.55 [23]. The concentrations of antibiotic solutions have been determined by measuring the absorption at 280 nm; the molar absorption coefficient has been determined as 9.65×10^3 cm^2 m-mole^{-1} according to a series of independent experiments. The dependence of absorption upon concentration has been found to be linear. Using an identical method the free antibiotic equilibrium concentrations in the albumin-free dialysate compartment have been determined.

The mean extent of binding ($\bar{r}$) has been calculated as

$$\bar{r} = \frac{C_A - 2(A)}{C_p}$$

where C_A and C_p represent initial molar antibiotic and albumin concentrations and A corresponds to free antibiotic concentrations as assessed after equilibrium in the protein-free dialysate compartment.

The c.d. spectra of studied albumins have been obtained from JASCO ORD/UV—5 with c.d. equipment in quartz cell of 1 mm optical path. The samples have been measured in concentrations of 0.1 g of protein in 100 cm^3 0.05 M phosphate buffer at pH 7.2.

RESULTS AND DISCUSSION

In our earlier paper [15] we have studied the binding of chloramphenicol to sera using samples diluted with the equal volume of buffer. To be close to these conditions we have used in model experiments the solutions containing 2 g of albumin in 100 cm^3. For this protein concentration the binding isotherm, i.e. the dependence of mean extent of binding ($\bar{r}$) upon equilibrium concentration of free antibiotic (A) for albumin from sera of health individuals, is given in Fig. 1. It has been constructed using ten different samples of albumin involving three purified monomers. The results obtained with monomers did not differ from those obtained with unpurified albumin. Because of the similarity in the results obtained with various preparations the presented data represent the combined results of all the experiments.

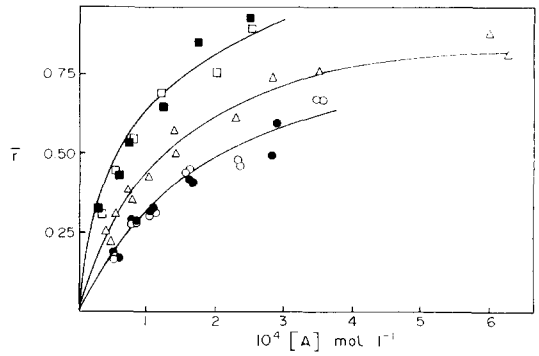

Fig. 1. The binding of chloramphenicol to albumin from serum of healthy individual (△) and from sera of patient O.T. with polycystic kidneys (○) and of K.M. with chronic glomerulonephritis (●). The upper curve illustrates the binding to albumin after charcoal treatment at pH 3 isolated from sera of healthy individuals (□) and from uremic patients (■). The dependence of mean moles number of antibiotic bound by one mole of protein, $\bar{r}$, on equilibrium molar concentration of free antibiotic (A). Fully contoured curves correspond to calculated theoretical dependences.

As follows from the good agreement of experimental data with theoretical curve the depence of $\bar{r}$ on A could be described as

$$r = \frac{nk(A)}{1 + k(A)}.$$

This is a mathematical expression of binding curve by a simple Schatchard model [24] of non interacting centres, where n gives the number of binding centres on protein molecule and k is their microscopic binding constant. These values were determined using curve n fitting method by calculating the S.D. of differences between experimental points and the theoretical dependence calculated for arbitrarily chosen values n and k. These were then gradually changed using digital computer till reaching minimum S.D. The program was constructed in FORTRAN IV language and it was applied in digital part of computer EAI 640. By this way the values $n = 1.05$ and $k = 8.2 \times 10^3$ l.mole^{-1} for albumin from blood serum of healthy individuals have been determined as well as the theoretical binding isotherm, the representation of which is given in Fig. 1 by a fully contoured line in correlation with experimental data.

For solution corresponding approximately to albumin content in normal serum (dilution in ratio 1:1, i.e. in range of antibiotic concentrations maximum 1×10^{-3} mole l^{-1}) only one binding center is occu-

pied having binding constant of order 10^4 l.mole^{-1}. It represents a medium strong binding center. According to chloramphenicol structure the main source of binding forces involves hydrophobic interactions similar to those of other ligands having a non polar structure [25].

The theoretical curve of dependence of binding extent upon the concentration of albumin (which has been calculated from binding constants), has shown that in a range of concentrations simulating the concentrations of albumin in sera of patients (dilution 1:1) the binding extent is significantly changed. This is to be considered when the binding abilities of normal and uremic sera should be correlated.

In our earlier paper [15] the group of sixteen patients suffering from chronic renal insufficiency was examined after repeated hemodialyses. In the course of 2 yr generally five series of measurements have been carried out. Plotting the mean values of binding extent (related to 1 g of serum albumin) which have been calculated for each particular series of measurement, vs mean values of albumin concentrations in sera, we obtain the curves of analogous character as in the case of theoretical curves for pure albumin isolated from sera of normal individuals. In Fig. 2 the dependence of sera of patients before and after haemodialysis and the dependence for normal sera are compared. It is obvious that albumin of sera in patients with chronic renal insufficiency shows a decreased ability to bind chloramphenicol and that this ability is re-increased after dialysis. In accordance with this fact the albumins isolated from blood sera of two patients showed the decreased binding of antibiotic as given in Fig. 1. From the experimental data identical curves for both samples have been obtained, corresponding to $n = 1.02$ and $k = 5 \times 10^3$ l.mole^{-1}.

Using calculated n and k values and starting concentration of chloramphenicol 20 μg/l cm^3 which corresponds to the medium level of effect (it has been also used in the preceding paper [15]) and further considering the mean albumin content in serum diluted 1:1 it is possible to get the binding value of 0.103 mole of chloramphenicol/mole of albumin for a healthy individual and 0.085 mole/mole for uremic patient. At the mean content of proteins 6.86 g/100 cm^3 in normal sera and 6.65 g/100 cm^3 in patients [15], it corresponds to the binding of 0.336 mg and 0.239 of chloramphenicol as related to 1 g of serum proteins. However, after addition of chloramphenicol into sera of healthy individuals the mean value (0.188 ± 0.004) mg/g and in case of patients in chronic renal insufficiency the mean value of (0.165 ± 0.003) mg/g has been found [15]. These decreased values are in agreement with the fact that the ability of blood sera to bind chloramphenicol is not equal to the binding ability of isolated pure protein. Nevertheless, the determined values from both sera and isolated serum albumins are decreased in patients with chronic renal insufficiency.

The difference of binding ability to isolated serum albumins and to whole serum has been observed also by other authors using different pharmaca [12–14]. Thus, Sjöholm *et al.* [14] explain this phenomenon

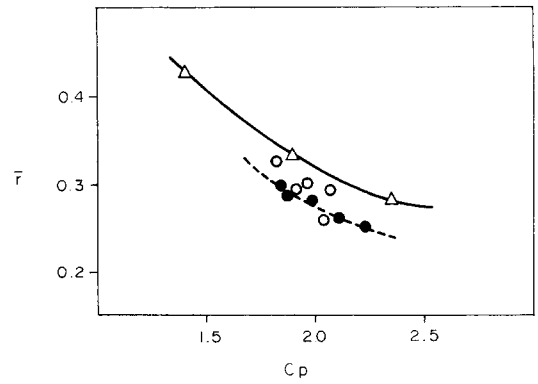

Fig. 2. The binding of chloramphenicol in normal sera (△) and uremic sera before (●) and after (○) dialysis. The dependence of $\bar{r}$, given in mg of antibiotic bound by 1 g of serum albumin, on concentration of this protein in serum (diluted 1:1). The total starting concentration of chloramphenicol was 2 mg/100 cm^3, C_p is given in g/100 cm^3.

by presence of binding inhibitors in normal sera forming an irreversible bond to albumin.

In accordance with papers of Sjöholm *et al.* [14] and of Soltys and Hsia [17] we proved that in the case of chloramphenicol the treatment of albumin with charcoal leads to the increased binding. The protein isolated from sera of healthy individuals as well as of uremic patients show then the equal binding ability; their identical binding curves (Fig. 1) correspond to parameters $n = 1.01$ and $k = 2.1 \times 10^4$ l. mole^{-1}.

Further we tried to correlate the conformation of albumins of normal sera and uremic sera using c.d. spectra in the far u.v. region, the dichroic bands of which reflect the secondary structure. Concerning this level of molecular structure we did not find any differences in conformity with Sjöholm *et al.* [14] who compared c.d. spectra of normal and uremic sera. But it is not possible to recognise small conformation differences taking place e.g. in the vicinity of binding centres by use of chiroptic methods.

The results of our model experiments are fully in agreement with the explanation published by Sjöholm *et al.* [14]. These authors suppose that the decreased binding of various pharmaca in uremic sera is caused by presence of not yet identified compounds bound to albumin under change of its binding affinity. However, at pH 3, these compounds can be removed using charcoal treatment. Such type of compounds could be involved in pathologic peptides or other compounds of medium mol. wt described for example by Adam, Dzúrik *et al.* [26, 27].

REFERENCES

1. M. M. Reidenberg and M. Affrime, *Am. med. J. Acad. Sci.* **226**, 115 (1973).
2. M. M. Reidenberg, *Med. Clins. N. Am.* **58**, 1103 (1974).
3. M. M. Reidenberg, I. Odar-Cederlöf, C. van Bahr, O. Borga and F. Sjöqvist, *N. Engl. J. Med.* **285**, 264 (1971).
4. A. H. Anton and W. T. Corey, *Acta pharmac. tox.* **29**, 134 (1971).

5. P. C. Farrell, N. L. Grib, L. Fry, R. P. Popovich, J. W. Broviac and A. L. Babb, *Trans. Am. Soc. Artif. Int. Organs* **18,** 268 (1972).
6. D. W. Shoeman and D. L. Azarnoff, *Pharmacology* **7,** 169 (1972).
7. D. S. Campion, *Toxic. Pharmac.* **25,** 391 (1973).
8. M. M. Ghoneim, S. E. Kramer, R. Bannow, H. Pandya and J. I. Routh, *Anesthesiology* **39,** 410 (1973).
9. F. Andreasen, *Acta pharmac. tox.* **32,** 417 (1973).
10. W. A. Craig, P. G. Welling, J. P. Wagnild and C. M. Kunin, in *Abstr. 8th Int. Congr. of Chemoth., Athens* (1973).
11. F. Andreasen, *Acta pharmac. tox.* **34,** 284 (1974).
12. I. Odar-Cederlöf and O. Borgä, *Clin. Pharmac. Ther.* **20,** 36 (1976).
13. O. Borgä, I. Odar-Cederlöf, V. Ringberger and A. Norlin, *Clin. Pharmac. Ther.* **20,** 464 (1976).
14. I. Sjöholm, A. Kober, I. Odar-Cederlöf and O. Borgä, *Biochem. Pharmac.* **25,** 1205 (1976).
15. J. Grafnetterová, Z. Vodrážka, D. Jandová, O. Schück, R. Tomášek and J. Lachmanová, *Clin. Nephrol.* **6,** 448 (1976).
16. R. H. McMenamy, C. C. Lund, J. Van Marche and J. L. Oncley, *Archs Biochem. Biophys.* **93,** 135 (1961).
17. B. J. Soltys and J. C. Hsia, *J. biol. Chem.* **252,** 4043 (1977).
18. K. Heide and H. Haupt, *Behringwerke-Mitteilungen* **43,** 161 (1964).
19. K. O. Pedersen, *Archs Biochem. Biophys., suppl.* **1,** 157 (1962).
20. R. F. Chen, *J. biol. Chem.* **242,** 173 (1967).
21. R. H. McMenamy, H. M. Dintzis and F. Watson, *J. biol. Chem.* **246,** 4744 (1971).
22. H. Holeyšovská, *Coll. Czech. Chem. Commun.* **31,** 130 (1966).
23. G. H. Beaven, S. H. Chen, A. D'Albis and W. B. Gratzer, *Eur. J. Biochem.* **41,** 539 (1974).
24. G. Scatchard, I. H. Schenberg and S. H. Armstrong, *J. Am. chem. Soc.* **72,** 535 (1950).
25. L. O. Anderson, A. Rehnström and D. L. Eaker, *Eur. J. Biochem.* **20,** 371 (1971).
26. J. Adam, R. Dzúrik and E. Valovičová, *Clinca Chim. Acta* **36,** 241 (1972).
27. P. Božek, H. Daněková, R. Dzúrik and J. Řezníček, *Biochem. Clin. Bohemoslov.* **4,** 17 (1975).