Binding of Cyanide to Methemoglobin

The interaction of methemoglobin (MeHb) with cyanide is of considerable interest in therapy. Indeed, the effectiveness of methemoglobin-producing compounds in counteracting the toxic action of cyanide is based upon cyanmethemoglobin (MeHbCN) formation[1].

In preceeding papers the kinetic of MeHb and MeHbCN formation[2,3] has been studied in vitro, in order to investigate the mechanism of the reactions which represent the basis of the cyanide poisoning treatment.

The present communication reports the data concerning the equilibrium reaction:

$$\text{MeHb} + \text{CN}^- \rightleftharpoons \text{MeHbCN} \qquad \text{(a)}$$

which has been studied in vitro after reacting a fixed amount of MeHb with different amounts of cyanide. Human hemoglobin was obtained from venous blood and transformed into MeHb by means of sodium nitrite, as already described[2]. The MeHbCN formation was quantitated spectrophotometrically according to the procedure thoroughly discussed in our preceding papers[2,3].

Figure 1 shows the fractional saturation ($\overline{Y}$) of MeHb with cyanide, as a function of cyanide concentration (X). The binding parameters were calculated from the straight line obtained by plotting the reciprocals $1/\overline{Y}$ versus $1/X$, according to the molecular interaction theory[4].

Figure 2 shows the SCATCHARD's plot[5], obtained by plotting $\overline{Y}/X$ versus $\overline{Y}$. Both with the reciprocals and with SCATCHARD's method, the same number of binding sites ($n = 1$) and the same association constant value ($K_{ass} = 1.58 \times 10^5$) have been obtained, the linearity of the plots showing that an hyperbolic equation holds for the binding of cyanide to MeHb.

In the present experiment, large amounts of cyanide are necessary for saturating a small quantity of MeHb, since the mass law regulates the equilibrium described by equation (a). The same hyperbolic relation between the concentrations of cyanide and MeHb is valid when a fixed amount of cyanide is reacting with different amounts of MeHb. This makes it understandable why an excess of MeHb is necessary in vivo for counteracting a small lethal dose of cyanide.

We attempted to introduce the data obtained in the present experiment for cyanide binding into HILL's equation[6]:

$$\overline{Y} = \frac{K^n(X)}{1 + K^n(X)} \qquad \text{(b)}$$

which has been proposed for the reaction of reduced hemoglobin with oxygen. In the above equation $\overline{Y}$ is the fractional saturation, K is the binding constant, (X) is the ligand concentration and n is the sigmoid coefficient.

By plotting $ln\ \overline{Y}/(1 - \overline{Y})$ versus $ln(X)$, we were able to calculate an n value of one for the middle portion of the curve so that the HILL's equation yields the general hyperbolic function which is characteristic for the cyanide binding. A thorough discussion on the meaning of n and K is available elsewhere[4] and here we used for MeHb-cyanide equilibrium the theorethical tool which has been developed for studying the hemoglobin-oxigen equilibrium.

An n value of one indicates the presence of one binding site on the protein molecule and the features of SCATCHARD's plot suggest a complete absence of interactions among binding sites. Moreover, a sigmoid coefficient of one indicates, for the hemoglobin system, that no heme-heme interaction takes place during the binding. This means the ligand brings about the modification or disintegration of the quaternary structure of the protein molecule, allowing it to behave as a monomer. Such an effect has been ascertained with some reagents and has been interpreted as heme and protein pushed apart by the ligand[7].

The cyanide ferrihemochrome formation from alkaline ferrihemochrome has been studied carefully[8] with the conclusion that mixed hemochromes are formed in complex equilibria between monomers and dimers.

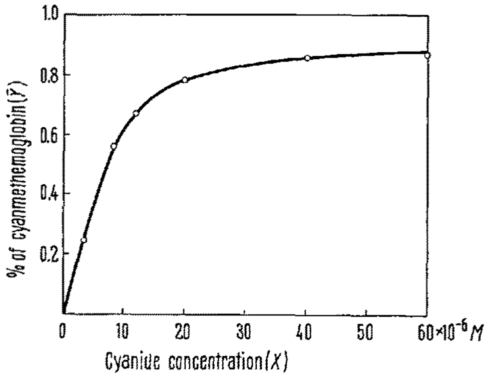

Fig. 1. % of cyanmethemoglobin (Y) formed at 26°C and neutral pH by addition of different amounts of cyanide (X) to 35 nanoMoles of methemoglobin. Final volume 3 ml.

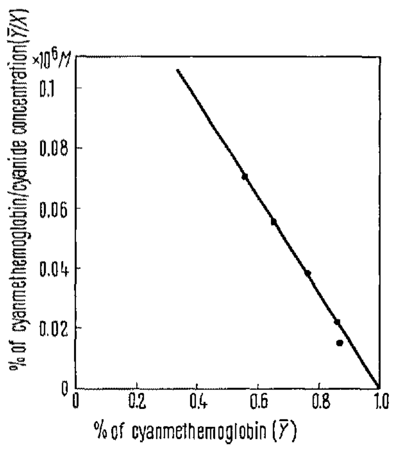

Fig. 2. The experimental values obtained as given in Figure 1 are plotted according to SCATCHARD[5].

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In our case only 1 mole of cyanide seems to be bound per mole of protein and this may account for the easy transformation of MeHbCN back to hemoglobin, which is carried out in vivo by means of thiosulfate and represents the final step of cyanide psoioning treatment.

Riassunto. È stata studiata l'interazione tra metemoglobina e cianuro. Il cianuro sembra legarsi ad un solo eme e l'equilibrio non sembra influenzato da interazioni eme-eme.

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Microbiological Transformation of 6,14-*endo*-Ethenotetrahydrothebaine Alkaloids

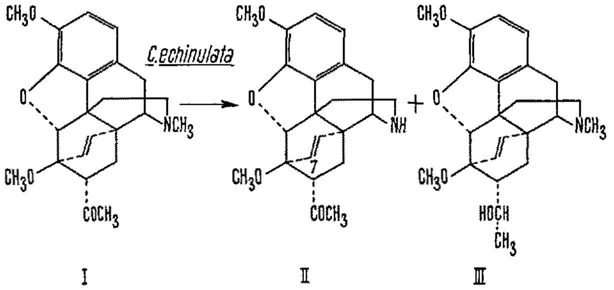
The 6,14-*endo*-ethenotetrahydrothebaine alkaloids have attracted considerable scientific attention because they often exhibit extremely high analgesic potency[1]. The results of extensive chemical investigations have been reported recently[2–8]. We have attempted to prepare new members of this class by microbiological means.

In a typical example, a cell suspension of *Cunninghamella echinulata* (NRRL-A-11498) was prepared by shaking the organism for 24 h in a medium composed of 1% glucose, 0.1% yeast extract, 0.1% beef extract, 0.2% bactopeptone and 0.3% corn steep liquor. The substrate (I) was added (100 γ/ml final concentration) and the products were isolated after 6 days fermentation by chloroform extraction. The products were separated and obtained crystalline in 3–10% overall yield after partition chromatography using methanol-saturated hexane on acid washed diatomaceous earth. The products were identified readily from their characteristic micro NMR-spectra[8] which were obtained with a Varian Associates A-60 instrument using a computer of average transients attachment. The formation of secondary alcohol III[3] is the result of a common biological reaction but it is interesting to note that only 1 of the 2 possible stereoisomers was found[3]. The second product (II) clearly lacked the *N*-methyl function, for the characteristic three-proton singlet at 2.33 ± 0.03 ppm was missing[8]. No other major change in the spectrum was apparent and the spectrum was in fact identical to that of authentic II[4]. This identification was supported by alkylation studies with methyl iodide[2]. A third, minor, product turned out to be the C_7 epimer of II and may have been an artifact of the isolation procedure[1].

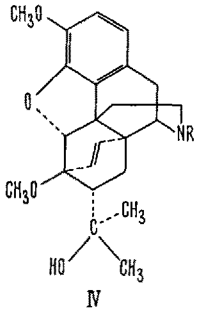
Dealkylation through chemical means in this series sometimes leads to difficulties via rearrangements so the microbiological process may be of some utility in difficult cases[4]. Interestingly, dealkylations of this type have not been reported previously through microbiological reactions on alkaloids. These reactions also find an interesting parallel in the proposal that morphine analgesics are dealkylated at the in vivo receptor site whereupon they exert their characteristic biological effects[9,10]. For these various reasons the reaction was studied in greater detail. From the results set out in the Table, it can be seen that the reactions described are fairly general. Furthermore, the nature of the alkyl group on nitrogen is not critical

Microbiological transformation of 6,14-*endo*-ethenotetrahydrothebaine alkaloids

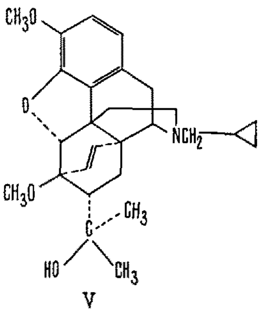
Organism	Substrate	Products
Cunninghamella bertholletiae	I	II, III
C. echinulata	I	II, III
C. bainieri	I	II, III
Xylaria sp.	I	II III
Xylaria sp.	IV (R = CH_3)	IV (R = H)
Xylaria sp.	V	IV (R = H)



I II III



IV



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