Dab Thr Leu Phe Gly
found: 6.3 2.11 0.95 0.95 3.6
theoretical values: 6.0 2.0 1.0 1.0 –
(for original antibiotic).

**Biological properties of aminocylated derivatives of basic antibiotics**

a) The determination of the antibiotic activity of polymyxin and colistin derivatives was carried out using the plate diffusion method.

Average activity of the derivatives of basic antibiotics, compared with a respective non-substituted standard (in aqueous solutions in the 100—10000 μg/ml concentration range).

<table>
<thead>
<tr>
<th>Derivative</th>
<th><em>Escherichia coli</em> [%]</th>
<th><em>Bacillus subtilis</em> UEM 8/58 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyl-polymyxin</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Glutaminyl-polymyxin</td>
<td>42*</td>
<td>70</td>
</tr>
<tr>
<td>Glycyl-colistin</td>
<td>39 (108 **)</td>
<td>126 **</td>
</tr>
</tbody>
</table>

*When the activity of glycyl-colistin was compared with that of colistin-methanesulfonate as standard.**

At concentrations up to 500 μg/ml the activity is lower than in the case of unsubstituted colistin, while at higher concentrations the activity is also increased. This is probably caused by the change in the diffusion properties of substituted colistin.

b) It was shown that the prepared derivatives distinctly put a stop to the postgerminal development of spores and the vegetative growth of *Bacillus cereus*.

Chemical investigation, as well as biological testing of the prepared derivatives is continued.

It can be assumed that the easy method of preparation described here will also be useful in the case of other basic peptides possessing antibiotic activity.

For the kind performance of microbiological tests our thanks are due to Dr. V. Musilek and Dr. M. Blumaerová of the Institute of Microbiology of the Czechoslovak Academy of Sciences, Prague. We are also grateful to Mr. Z. Zbrožek of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences for the analyses of amino acids.


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**Dielectric Studies of Aggregation Phenomena with Separated α and β Chains of Human Hemoglobin**

**PETER SCHLECHT**

Physik-Department der Technischen Universität München

(Z. Naturforsch. 26 b, 453—457 [1971]; received February 9, 1971)

**Herrn Professor Dr. Nikolaus Riehl zum 70. Geburtstag gewidmet**

The concentration dependence of the dielectric dispersion between 100 kHz and 15 MHz has been investigated for separated α and β chains of human hemoglobin. It is shown that such dielectric measurements can give very detailed information on aggregation phenomena. Specific dielectric increments and dipole moments of monomeric and aggregated chains could be obtained.

In a previous paper we have discussed the mechanism of the dielectric dispersion at 1 MHz for aqueous hemoglobin and myoglobin solutions in some detail and have found that the only possible explanation for this dispersion is an orientational relaxation mechanism. For this mechanism the theory of Onsager gives a connection between the macroscopic dielectric parameters and the microscopic quantities of the individual molecules. A further proof of this mechanism and also an interesting application of this knowledge provides the study of an aggregating system. Such a system was found in the separated α and β chains of human hemoglobin. From the dielectric study of such an
aggregating system one can gain very detailed information, for the three dielectric parameters which are obtained simultaneously give three different aspects of the phenomenon. Such an information is otherwise only available by the combination of several methods. The conclusions one would like to draw from the dielectric measurements of this system could be checked by the results of Bucci et al. which were obtained by sedimentation and electrophoresis experiments.

Method and material

The frequency dependence of the dielectric constant of aqueous protein solutions has been measured in the frequency range between 100 kHz and 15 MHz by means of a Siemens admittance bridge combined with a signal generator and an highly selective receiver. The solution was filled into a capacity cell built as a cylindrical condenser with massive gold electrodes and an electrode distance of 1 mm; cell constant was 5.56 pF, cell volume was 2 ml. The apparatus has been described in more detail elsewhere.

Human hemoglobin has been prepared from full blood by washing red cells repeatedly with 0.9% NaCl solution and hemolysing with distilled water. Ghosts were removed by centrifugation at 16000 rpm. The separated a and b chains of human hemoglobin have been prepared by the method of Bucci and Fronticelli. Part of the chains has been regenerated by cysteine. For the dielectric measurements the material of several columns has been combined; it has been concentrated by vacuum dialysis. For measuring the concentration dependence aliquots of the concentrated stock solution have been taken and properly diluted. Immediately before the dielectric measurement the solution has been completely deionized by a mixed bed ion exchange resin and centrifuged at 16000 rpm. The concentration has been determined optically by measuring the extinction at 540 mX after conversion into cyanmethemoglobin.

The dielectric parameters and their interpretation

In the frequency range of our experiments we observe a dielectric dispersion, that means the dielectric constant of the solution decreases from a low frequency value \( \varepsilon_0 \) to a high frequency value \( \varepsilon_\infty \). Such a dispersion can be described by the Debye-Cole-Cole equation:

\[
\varepsilon = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (i \omega \tau)^{1 - \alpha}}
\]

According to this equation the dispersion is characterized by the three dielectric parameters dielectric increment \( \Delta \varepsilon = \varepsilon_0 - \varepsilon_\infty \), the relaxation time \( \tau \) or its reciprocal, the relaxation frequency \( f_r = 1/2 \pi \tau \), and the Cole-Cole parameter \( \alpha \) with values between 0 and 1. As \( \Delta \varepsilon \) is strictly proportional to concentration for non associating substances, the specific dielectric increment \( \Delta \varepsilon/c \) is characteristic for the special protein (c = concentration in g/l). It is related to the dipole moment of the molecule by a formula given by Onsager according to Onsager's theory. The relaxation time \( \tau \) is related to molecular quantities by the formula:

\[
\tau = \frac{4 \pi a b^2}{kT} \eta \psi
\]

Here \( (4 \pi/3) a b^2 \) is the volume of an ellipsoid of revolution with the axes \( a \) and \( b \), \( \eta \) is the viscosity of the solution, \( \psi \) is the Perrin factor considering the deviations from a sphere of equal volume. \( \psi \) depends on the ratio \( b/a \) and the rotation axis. Therefore, \( \tau \) is directly proportional to the volume of the molecule. Values of the Cole-Cole parameter \( \alpha \) greater than zero indicate, that we do not have a single sharp relaxation time but a more or less broad distribution of relaxation times.

In mixtures of several different proteins each species causes a dispersion. They are additive and give the formula:

\[
\varepsilon = \varepsilon_\infty + \sum \frac{\Delta \varepsilon_r}{1 + (i \omega \tau_r)^{1 - \alpha}}
\]

But from the experimental dispersion curve they can only be separated if the relaxation times are different by more than a factor of 10. Otherwise we should analyze the dispersion again in terms of equation (1) with a mean relaxation time and a Cole-Cole parameter somewhat greater than for a single protein. This description is mathematically not exact because \( \alpha \) always corresponds to a distri-

2. L. Onsager, J. Amer. chem. Soc. 58, 1486 [1936].
4. P. Schlecht, H. Vogel, and A. Mayer, Biopolymers 6, 1717 [1968].
bution of relaxation times and not to several discrete values. The difference between both formulas is, however, within experimental error.

In this paper we report measurements with separated $\alpha$ and $\beta$ chains of hemoglobin. Here one expects aggregation from monomers to dimers and tetramers. We should therefore consider this case here in more detail. On aggregation the volume of the particles changes by a factor of two or four. Therefore, the relaxation time must change by the same factor. The shape of the monomers, dimers and tetramers is not so much different from a sphere that the Perrin factor $\psi$ would be markedly affected. These considerations are valid for hemoglobin and myoglobin, as has already been shown.

In the case of these aggregations it is not possible to separate the different dispersions. So we should try to express the mean dielectric parameters of the mixture by the dielectric parameters of the pure components and consider the expected changes of the mean parameters during aggregation. The mean dielectric increment is simply the sum of the contributions of the components. On aggregation we find a transition from the increment of the monomer to another increment of the aggregated species. In the middle of the transition the concentrations of both components are equal ($c_1 = c_2$). For the relaxation frequency we again expect a transition between the relaxation frequencies of the pure components. But here the components contribute according to their specific dielectric increment. So the middle of the transition (when $f_r$ is plotted on a logarithmic scale) is not at $c_1 = c_2$, but at $(\Delta \varepsilon/c)_1 c_1 = (\Delta \varepsilon/c)_2 c_2$. For the relaxation frequency the component with the higher specific dielectric increment is more important. The Cole-Cole parameter of the mixture increases with increasing inhomogeneity and shows a maximum in the middle of the relaxation frequency transition. The maximum value of $\alpha$ depends on the difference between the relaxation frequencies of the components. When we assume for the pure components $\alpha = 0.1$ (what we usually found for hemoglobin or myoglobin), then in a mixture of monomers and dimers $\alpha$ should not exceed 0.15, but in a mixture of monomers and tetramers $\alpha$ can go up until 0.3.

To summarize these considerations, we can gain information on the aggregation phenomena first from the concentration dependence of the relaxation frequency and of the dielectric increment, then from the maximum value of the Cole-Cole parameter, and further from the fact that the transitions of the specific dielectric increment and of the relaxation frequency depend in a different way on the composition of the solution. Additional information can be obtained from the steepness of the transition. According to mass law, a monomer dimer transition needs a broader concentration range than a monomer tetramer transition, which is almost completed within one order of magnitude in concentration.

Results

Dielectric measurements have been done with all the species available from the separation procedure, first with $\alpha_{PMB}$ and $\beta_{PMB}$, where the sulphydryl groups are blocked with PMB, than with $\alpha_{SH}$ and $\beta_{SH}$, the regenerated native chains. Also equimolar mixtures of both chains have been investigated. The temperature of experiments was 2°C. The solutions were deionized, their conductivity was less than

![Graph](image-url)
5 μmho/cm. The pH of the solutions was near the isoionic point of the species (α_{PBM} 7.3, β_{PBM} 5.8, α_{SH} 7.6, β_{SH} 6.3). The ligand on the heme was always oxygen.

The measurements gave both capacity and conductivity of the cell; but only the capacity which is proportional to the dielectric constant was used for the analysis. The frequency dependence of the capacity gave good relaxation curves, from which the dielectric parameters were obtained. The way of analysis has already been described. All the results are shown in Fig. 1. Here the concentration dependence of the specific dielectric increment, of the logarithm of the relaxation frequency, and of the Cole-Cole parameter α are shown. The concentration is plotted on a logarithmic scale. The experimental error depends very much on the concentration. It is indicated in the Figure.

**Interpretation of the results**

These results shall now be discussed in terms of an aggregation process by the criteria developed above. The conclusions can be compared with the results of Bucci et al. who studied the same system by sedimentation and electrophoresis.

a) The α chains clearly are monomeric and show no aggregation. We find no concentration dependence of the parameters. The relaxation frequency is the same as expected for monomers.

b) β_{PBM} on the other hand shows a marked concentration dependence. The relaxation frequency shows a transition from a value characteristic for monomers to a value expected for dimers. The transition is too steep for a pure monomer-dimer transition. So we should expect that at higher concentrations the aggregation would proceed to tetramers. The Cole-Cole parameter which only slightly exceeds 0.15 supports this view. Bucci et al. found here an aggregation from monomers to dimers which does not depend on pH and ionic strength.

c) A similar interpretation holds for the results of the mixture α_{PBM} + β_{PBM}. We must assume an aggregation from monomers to dimers and tetramers, with much emphasis on the dimers as is shown by the Cole-Cole parameter not exceeding 0.15. The dimers must be α - β dimers. This can be concluded from the fact, that the specific dielectric increment deviates from a medium value just between α_{PMB} and β_{PMB}, and that f, shows more aggregation than β_{PMB} alone. This picture agrees very well with the findings of Bucci et al.

d) Again a marked concentration dependence is shown by β_{SH}. The steepness of the transition of the relaxation frequency shows that it aggregates directly from monomers to tetramers. The high value of α (=0.3) agrees with this picture. The dielectric increment, however, shows only the tail of a transition. Above 3 g/l it is constant. That must be due to the fact that the monomers have a much higher specific dielectric increment; it should be as high as 0.6 or 0.8. Therefore, in our whole concentration range β_{SH} exists as a tetramer, and it is only a small fraction of monomers which has so much influence on the mean relaxation frequency. Bucci et al. found β_{SH} as a tetramer equivalent to hemoglobin H.

e) The same explanation holds also for the results of the mixture α_{SH} + β_{SH} which should be the same as the original hemoglobin. It is a tetramer. The concentration dependence of f, and α is due to a small fraction of unassociated material. Small mixing errors or incomplete regeneration may be the reason. The dielectric measurement is very sensitive to such impurities. The specific dielectric increment of the mixture is higher than for native hemoglobin. This can be due to the fact that only one of the two sulphydryl groups of the β chain can be regenerated by the method of Bucci and Fronticelli.

So we have seen that from the concentration dependence of the dielectric parameters one can gain reliable and detailed information on an aggregating system. In addition to the mean relaxation time, which is equivalent to the result of a sedimentation experiment, the method indicates by the Cole-Cole parameter α the homogeneity of the solution as far as particle size is concerned. By the difference between the transition regions of the specific dielectric increment and the relaxation frequency the method is quite sensitive to small amounts of a species with high specific dielectric increment. The applicability of such dielectric measurements is, however, limited to favourable cases because the errors become increasingly large at lower concentrations, and because the measurements must be performed in saltfree solutions.
Dipole moments

In addition to the information concerning the aggregation phenomena these measurements gave results for the specific dielectric increments of the investigated species. One interesting finding is that the specific dielectric increment seems usually to decrease on aggregation. This is not necessary, and an increase would be equally possible. The specific dielectric increments of the pure species can be obtained by extrapolation and used for the calculation of molecular dipole moments. These results are summarized in Table I. For the molecular interpretation of these dipole moments similar considerations as for myoglobin should apply.

| \( \alpha_{\text{PMB}} \) | 0.23 | 170 ± 10 |
| \( \alpha_{\text{SH}} \) | 0.23 | 170 ± 10 |
| \( \beta_{\text{PMB}} \) | 0.54 | 260 ± 15 |
| \( \beta_{\text{SH}} \) | 0.35 | 300 ± 15 |
| \( \beta_{\text{SH}} \) | 0.6 | 275 |
| \( (\beta_{\text{SH}})_4 \) | 0.23 | 340 ± 15 |
| \( (\alpha \beta)_{\text{PMB}} \) | 0.35 | 300 ± 15 |

Table I. Specific dielectric increment and dipole moment of separated chains.

The author is very much indebted to Professor Dr. N. RIEHL and Dr. A. MAYER for stimulating discussions and to Dr. H. FORMANEK for valuable help in the preparation of the chains.

8 P. SCHLECHT, Biopolymers 8, 757 [1969].

Über die Ionen-Abhängigkeit des Rezeptorpotentials der Retina von Astacus leptodactylus

The Ion-dependence of the Receptor Potential of the Visual Cell of the Crayfish

H. STIEVE und CHR. WIRTH

Institut für Zoologie der Technischen Hochschule Aachen

(Z. Naturforsch. 26 b, 457—470 [1971]; eingegangen am 11. Februar 1971)

The mass response of the photoreceptor cells of the isolated crayfish-retina (Astacus leptodactylus) (receptor potential ReP) resulting from short and long light stimuli, has been measured with external electrodes.

The ionic composition of the saline by which the preparation was perfused was changed and the influence of those changes on the ReP was measured under constant stimulus conditions.

When all sodium ions are substituted by potassium ions, the height of the ReP is reversibly both decreased and shortened (Figs. 3 and 4).

When all sodium ions are almost substituted by choline ions, (the remaining sodium concentration at the photoreceptor cells being less than 1 mMol/Z) the sensitivity to light adaptation is drastically and reversibly increased. Mainly due to light adaptation the ReP becomes smaller and shorter whereas the latency period increases (Figs. 5—8).

If the concentration of extracellular calcium is reduced to less than 0.1 mMol/Z, the decrease of the REP is considerably and reversibly slowed down while the increase of the REP remains almost unchanged (Fig. 9). If magnesium ions are also lacking the effect is even more pronounced (Figs. 10 and 11).

All these changes of the ReP are probably due to actions of the ions on the membrane of the photoreceptor cells or its immediate vicinity.

The results do not allow an unequivocal decision whether the ReP is due to an increased conductance (CIM) of the photoreceptor cell membrane or due to a change in the action of an electrogenic ion pump (EPM). But considering the results as a whole, and especially those from the low Ca- and low Mg-experiments, it seems unlikely that the ReP is caused by an EPM.

In einer früheren Veröffentlichung (STIEVE 1) wurde die Abhängigkeit des extrazellulär gemessenen Rezeptorpotentials der Retina des Einsiedlerkrebses Eupagurus von dem extrazellulären Ionenmilieu beschrieben.

Aus drei Gründen wurden diese Untersuchungen nun auch an der Retina des Sumpfkrebses Astacus leptodactylus E s c h s c h o l z fortgesetzt.

1. Astacus leptodactylus ist im Gegensatz zu dem marinen Eupagurus ein Süßwasserkrebs, bei dem...