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YEREVAN PHYSICS INSTITUTE

S.G.GEVORKIAN, S.Sh.TATIKIAN, A.L.SIMONIAN

MECHANICAL STRENGTH OF ENZYME FILMS AS AN INDEX OF ACTIVITY

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Ս.Գ.ԳԵՎՈՐԳՅԱՆ*, Ա.Լ.ՍԻՄՈՆՅԱՆ, Ս.Շ.ԹԱԹԻԿՅԱՆ

ՖԵՐՄԵՆՏԻ ԹԱՂԱՆԹԻ ՄԵԽԱՆԻԿԱԿԱՆ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԸ՝ ԱԿՏԻՎՈՒԹՅԱՆ ՑՈՒՑԱՆԻՇ

Առաջին անգամ չափվել են ուրատօքսիդազայի թաղանթի Յունգի մոդուլը և մարման լոգարիթմական դեկրեմենտը: Ուսումնասիրվել է այդ մեծությունների կախումը լուծիչի կազմից: Չափվել են ուրատօքսիդազայի թաղանթի հիդրատացիայի իզոթերմը՝ կախված լուծիչի կազմից: Չափվել են Է և Ծ կախումը ջերմաստիճանից: Ստացվել է ջրի և բուֆերի մեջ ֆերմենտի ակտիվության կախումը ջերմաստիճանից: Ցույց է տրվել, որ բոր-բորատային բուֆերի մեջ, որտեղ ֆերմենտը ունի մաքսիմալ ակտիվություն, թաղանթները բնութագրվում են Է -ի փոքրագույն արժեքով: Է -ի այդ արբերությունը չի կարելի բացատրել հիդրատացիայի արբերությամբ. ջերմաստիճանի մեծացումը բերում է թաղանթների փափկման և ակտիվության մեծացման:

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С.Г.ГЕВОРКЯН*, А.Л.СИМОНЯН, С.Ш.ТАТИКЯН,

МЕХАНИЧЕСКИЕ СВОЙСТВА ПЛЕНОК ФЕРМЕНТА КАК ПОКАЗАТЕЛЬ АКТИВНОСТИ

Впервые измерены модуль Юнга (Е) и логарифмический декремент затухания (Ѳ) аморфных пленок Уратоксидазы (УО). Исследована зависимость этих параметров от состава растворителя. Измерены изотермы гидратации пленок УО в зависимости от состава растворителя при $t = 25^{0}$ С. Измерены зависимость Е и Ѳ от температуры. Получена зависимость активности от температуры в воде и буфере. Показано, что в борно-боратном буфере (pH =9,25), где фермент имеет максимальную активность, пленки характеризуются наименьшим значением Е . Это различие в Е нельзя объяснить разницей в гидратации. Повышение температуры до 45^{0} С приводит к размягчению пленок и повышению активности.

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Ереван 1991

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S.G.GEVORKIAN*, S.Sh.TATIKIAN, A.L.SIMONIAN

MECHANICAL STRENGTH OF ENZYME FILMS AS AN INDEX OF ACTIVITY

The Young's module E and the logarithmic decrement ϑ of amorphous uratoxidase films are measured for the first time. The dependence of these parameters on the composition of the solution is studied. The isotherms of uratoxidase film hydration are measured as functions of the composition of solution at t=25°C. E and ϑ are measured as functions of temperature. The dependence of activity on temperature in water and buffer is obtained. It is shown that in a borate buffer (pH 9.25), where the enzyme is most active, the films are characterized by lowest E. Variation of E cannot be explained by variation in hydration. Increase in temperature leads to softening of films and an increase in the activity.

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*STC "QUARK" in YEPHI

1. INTRODUCTION

The biological activity of macromolecules is determined by their structure and dynamics. Determination of the equilibrium distribution of conformational states of macromolecules and of the temporary dependence of transitions between them are the main problem for understanding this bond [1-6]. Small-scale fluctuations may serve as a base for local or global conformational transitions, which in their turn may lead to a change in the biological activity [5,7]. The mechanical properties provide an information not only on elastic forces inside a sample, but also on the processes connected with molecular motion at relaxation of the mechanical stress in a solid sample. Investigation of the mechanical properties of some polymers in a wide temperature and frequency range revealed a wide spectrum of relaxational motion off both the main polymer chain and the side groups [8-10]. The low-temperature glassing effect was identified in proteins [11,12].

The purpose of the present work is to investigate, on the example of studying the mechanical properties of amorphous uratoxidase films, the "physical state - biological activity" constraint for enzymes. We have chosen this enzyme, because it is usually used in analytic systems for uric acid concentration determination, and because it is investigated relatively well [13-15].

2. MATERIALS AND METHODS

Amorphous uricase films with specific activity 0.4 U/mg extracted from young pig liver were investigated in a borate buffer (pH 9.1) at t=25°C. The films were prepared as follows. The uricase was solved in a borate buffer (pH 8.6) at room temperature, then the solution was dropped on the polished sur-

face of the teflon support. The support was placed in an air-tight vessel with a relative humidity of 70-75% being supported in it with the help of salts solution. The films with stable mechanical properties were obtained by means of slow drying of the drops at t=10°C. The films were fixed in the vapours of 25% glutaric aldehyde during 48 hours at room temperature. Then they were washed from the remnants of salts and glutaric aldehyde in distilled water and put into a borate buffer, where they were conserved at 6°C. The mechanical properties of uricase films prepared in this manner were stable for 3-4 months. With the aim of measuring the viscoelastic properties, with the help of a special microknife the films were cut in water or a buffer into rectangular plates with length 0.5÷2mm, width 0.1÷0.2mm, and thickness 10÷30μm [15].

We used Morozov's micromethod [15] to measure the Young's dynamical module E and logarithmic decrement ϑ. The method is based on the analysis of the electrically excited transverse resonance vibrations of a plate supported as a cantilever. The method, that allows to measure E and ϑ in a wide temperature range, is developed in [11,12]. The method of measuring the hydration of microsamples weighing up to 0.01mg is described in [16].

The amperometric method of enzyme activity measurement based on measuring the oxygen rate in the uricase reaction, is described in [17].

The relative humidity (A) in the vessel was established by means of water solutions of salts: 97 to 32% by water solution of $CaCl_2$ of different concentrations; A=15 and 10% by saturated solutions of $CaCl_2$ and ZnCl, respectively.

3.RESULTS AND DISCUSSION

Fig.1 shows the Young's module E and the logarithmic decrement ϑ for amorphous uricase films soaked in water and buffer (pH 9.1) as functions of the relative humidity A at 25°C. As the films were conserved in a solution, the experiment was carried out towards decreasing A, starting from A=97%. It is seen that in both cases E varied similarly. The difference between the

modules remained almost a constant in the whole interval of humidities.

The Young's module of the films steeped in a buffer is smaller than E of those steeped in water. As for the logarithmic decrement, at high humidities, for the buffer samples ϑ is larger than for the water-containing ones. This difference vanishes when A=65÷70%. At lower relative humidity the decrements are actually equal. In both cases the difference in the modules may be due to at least two facts: either enzyme hydration is different, depending on whether it the enzyme is steeped in water or a buffer, or the buffer changes the mechanical properties of the enzyme. To check this, we measured the isotherms of hydration of enzymes steeped in water and a buffer (Fig.2). It is seen that there is no significant difference in the isotherms. The slight difference at relative humidities higher than 15% could not lead to such a remarkable difference in E. But there is no difference in hydration when humidity is higher than 75%. So, one may conclude that the difference in E is due to hydration. The other parameter measured, the logarithmic decrement, is proportional to viscosity, i.e. shows what portion of the energy of external mechanical vibrations is expended to overcome viscosity, and what portion is dissipated [18]. Viscosity is due to inter-molecular interaction, intramolecular changes, and water-enzyme interaction. When humidity is lower than 75%, where hydration (Fig.2) and logarithmic decrement (Fig.1) for two different solvents are actually the same, the Young's module of the samples differ by 0.6÷0.8 $GN \cdot m^{-2}$. The parameter measured - the Young's module of amorphous films - is a sum of two components, the module of the enzyme molecules, and elasticity of intramolecular contacts. What can this deference be referred to? If this difference or its any part would arise owing to variation of the contact elasticity, then it would undoubtedly arise also in the viscosity, .i.e. ϑ.

Most likely, the influence of pH manifests itself as a change in the mechanical properties of molecules.

There is another way of changing the mechanical properties of enzyme molecules - increase of temperature. We investigated the dependence of E and ϑ of UO films on temperature. Fig.3 presents the results of such an experiment. The film was first

kept at t=25°C and humidity A=91%. Then if was washed from buffer in distilled water. As if is seen, E decreased, while ν increased in the whole interval of increasing temperature. But the course of these changes is not monotonous. Ignoriry minor changes, which is the subject of separate investigations, the curve may be conditionally divided into three intervals: from 20 to 40°C; 40 to 68°C; 68 to 80°C. By means of minimization, these lengths of the curve for E, are described by straight lines with different slopes. Thus, one can affirm, that the effect of the buffer and temperature oh the mechanical properties of enzyme films is the same - in both cases the Young's module decreases.

To fined the constraint between enzyme activity and mechanical properties of films, we measured the enzyme activity as a function of temperature in water and buffer (Fig.4). It is seen, that activity in water is a lower then in buffer and the dependence on temperature is bell-shaped. The enzyme had the same activity in water at 40°C and in buffer at 25°C. It is seen from Fig.3, that at 40°C the mechanical properties of films begin to change otherwise, the value of E at this temperature in water is equal to that at 25°C, but in a buffer.

Existence of such a constraint between the mechanical properties of enzyme films and activity allows as to conclude, that E can be used as a measure of activity. This opens new possibilities for applied tasks.

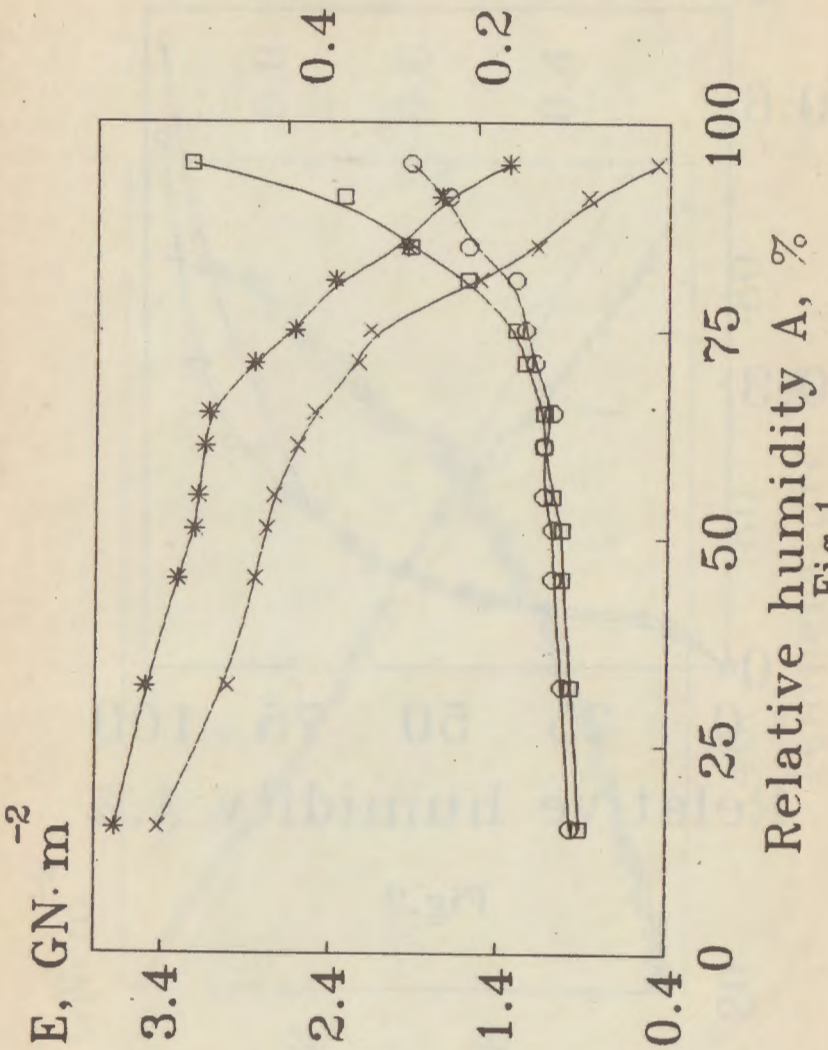

Fig.1

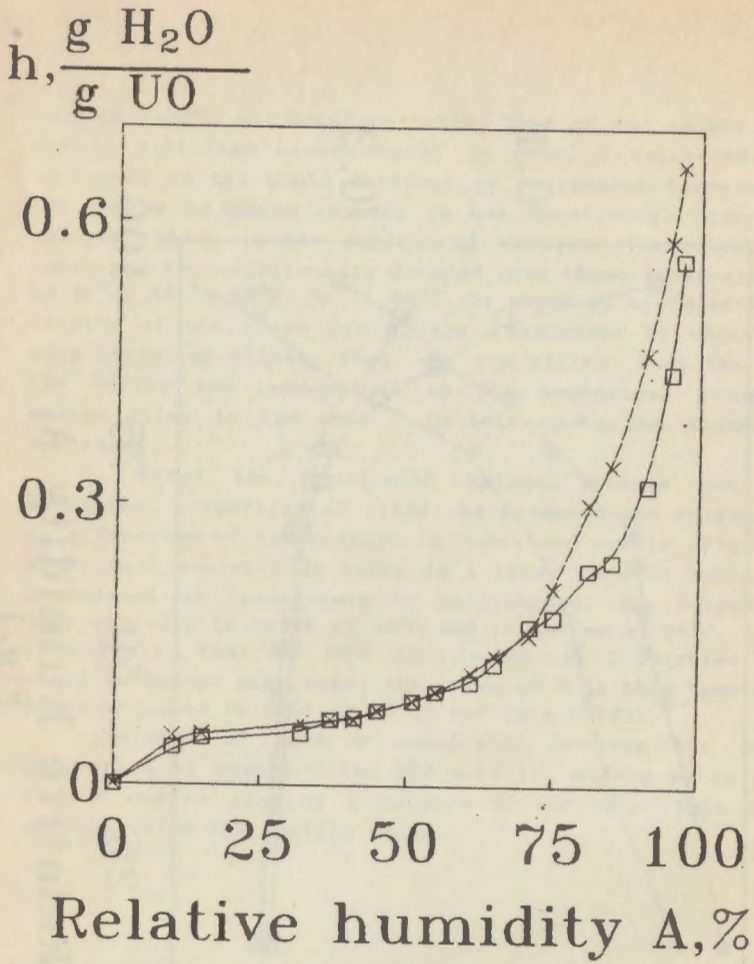
h, g H2O / g UO
0.6
0.3
0
0 25 50 75 100
Relative humidity A,%

Fig.2

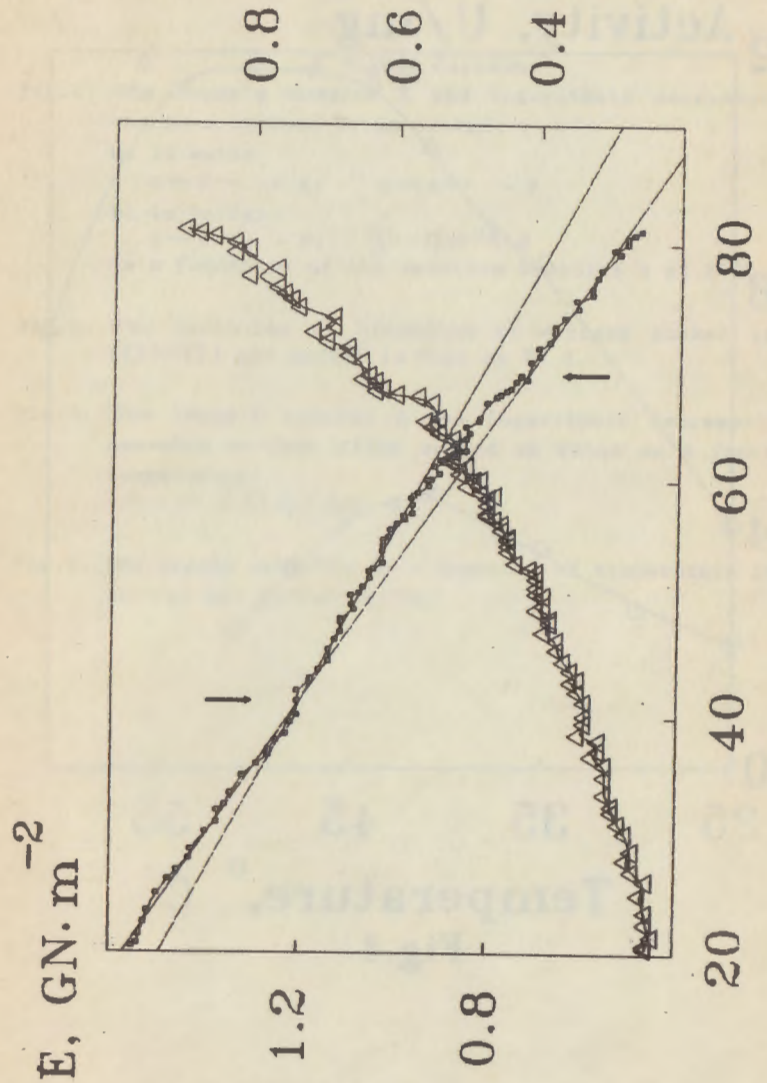
E, GN·m⁻²
1.2
0.8
0.4
0.6
0.8
20 40 60 80
Temperature, °C

Fig.3

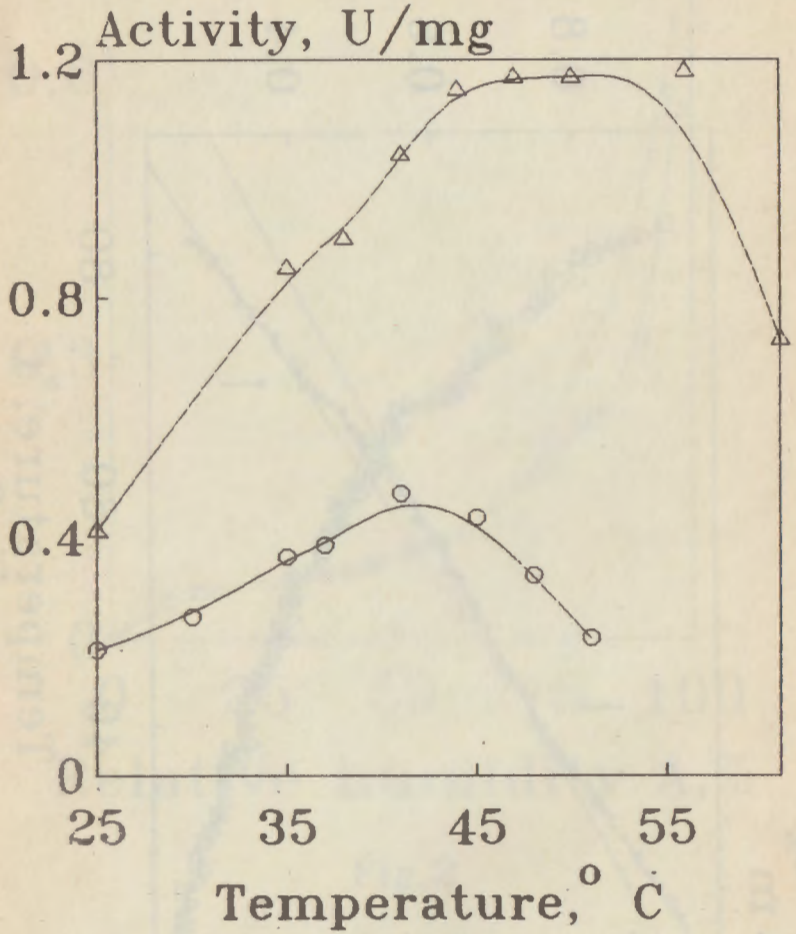

Fig.4

Figure Captions

Fig.1. The Young's modulus E and logarithmic decrement ϑ for amorphus uricase films soaked:
a) in water
—— - E; o—o— - ϑ
b) in buffer
x—x— - E; □—□— - ϑ
as a functions of the relative humidity A at 25°C.

Fig.2. The isotherms of hidration of uricase soaked in water (□—□) and buffer (x—x) at 25 C.

Fig.3. The Young's modulus E and logarithmic decrement ϑ for amorphus uricase films soaked in water as a function of temperature:
•—•— - E; Δ—Δ— ϑ

Fig.4. The enzyme activity as a function of temperature in water (o—o) and buffer (Δ—Δ).

REFERENCES

1. Karplus M., Mc Common J.A. The internal dynamics of globular proteins. CRC Crit. Rev. iochem., 1981, 9, pp. 292-349.
2. Cooper A. Protein fluctuations and the thermodynamics uncertainty principle. Prag. Biophys. Mol. Biol., 1984, 44, pp.181-214.
3. Austin R.H., Beeson K.W., Eisenstein L., Frauenfelder H., Gunsulbus I.C. Dynamics of ligand binding to myoglobin. Biochemistry, 1985, 14, pp. 5355-5373.
4. Elber R., Karplus M. Multiple conformational states of proteins: a molecular dynamics analysis of myoglobin. Science, 1987, 235, pp.318-421.
5. Beecem J., Haas E. Simultaneous determination of intramolecular distance distributions and conformational dynamics by globule analysis of energy transfer measurements. Biophys. J, 1989, 55, pp.1225-1236.
6. Axelsen P.H., Prendergast F.G. Molecular dinamics of tryptophan in ribonuclease-T1. II Correlation with fluorescence. Biophys. J., 1989, 56, pp. 43-66.
7. Абатуров Л.В. Тепловые движения белка: мелкомасштабные флуктуации и конформационные подсостояния. Молекуляр. Биология, 1983, 17, № 4, с. 683-704.
8. Ferry T.D. Viscoelastic properties of polymers. Wiley. Interscience, N.Y, 1961.
9. Hopkins J.L., Kurcjian C.R. In physical Acoustics, Principles and Methods. vol.2, Part B, Mason W.P. Ed., Academie N.Y., 1965, pp. 171-196.
10. Перепечко И.И. Свойства полимеров при низких температурах. М., Химия, 1977, с. 171-196.
11. Gevorkian S.G., Morozov V.N. Acoustic properties of protein solids in a wide temperature range. In Symposium on acoustical properties of biological objects, 1984, Pushchino, pp. 21-22.
12. Morozov V.N., Gevorkian S.G. Low-temperature glass transition in proteins. Biopolymers, 1985, 24, pp. 1785-1799.
13. Conley Th.G., Priest D.G. Thermodynamics and stoicheiometry of binding of substrate analogues to uricase. Biochem. J., 1980, 187, pp. 727-732.

14. Bongarts G.P.A., Uitzetter J., Brouns R., Vogels G.D. Uricase of *Bac. fastidiosus*. Properties and regulation of synthesis. Biochem. Biophys. Acta, 1978. 527, pp. 348-358.
15. Morozov V. N., Morozova T. Ia. Viscoelastic properties of protein crystals. Biochemistry, 1981, 20, № 3, pp. 451-467.
16. Геворкян С.Г., Морозов В.Н. Зависимость изотерм гидратации белков от способа упаковки в полистироле, Биофизика, 1983, 28, с.944-948..
17. Симонян А.Л., Татикян С.Ш., Кулис Ю.Ю. Кинетические свойства и термоинактивация бактериальной уриказы. Биохимия, 1985, 50, вып.5, с.782-785.

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