CHARGE-TRANSFER COMPLEXES OF NICOTINAMIDE ON POLYMERS

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Linear hydrophilic polymers of the methacrylate type with the nicotinamide functional group in side chains and a low-molecular weight model of the structure unit with nicotinamide were prepared. The association constants of the charge-transfer complexes of nicotinamide on polymers and on the low-molecular weight model with iodide and indole as donors were measured by means of the UV spectroscopy. A pronounced increase in the association constant of the complexes of iodide with the polymers compared to the low-molecular weight model and the effect of the polymer structure and of the solvation power of the solvent on the wavelength of the maximum of the absorption band of the CT-complex of iodide with the nicotinamide models are discussed. Properties of the nicotinamide models after the reduction of nicotinamide with sodium dithionite were examined, and it was found that the polymer models of this type (linear soluble polycations) are not suited for studying the redox properties.

Nicotinamide is the functional group of the coenzymes nicotinamidadeninedinucleotide (NAD^+) and nicotinamidadeninedinucleotide phosphate ($NADP^+$), which together with their reduced forms, NADH and NADPH, are the most widespread coenzymes of biological oxidoreduction systems. Although the coenzymes are low-molecular weight compounds, in the oxidoreduction reaction itself in natural systems they do not act as isolated small molecules, but in connection with the macromolecule of the enzyme. The electron transfer between the substrate and the coenzyme is made possible only owing to this connection. The low-molecular models were used for observing the effect of approaching of the reacting bonds which is considered in the enzymatic catalysis. It was found that the approaching of the substrate alone in a suitable position is not sufficient for ensuring the electron transfer from the substrate on the analog of the coenzyme[1,2]. The addition of CN^- on 4th position of NAD^+ and of 1-alkylpyridinium analogs is a well--investigated reaction[3-7] which has been used in several cases for examining the effect of polyelectrolytes[8] and of surface-active and micelle-forming compounds[9] on the reactivity of analogs of the pyridinium coenzymes. Nicotinamide bonded on polystyrene was reduced to 1,4-dihydronicotinamide and was as such capable of reducing some dyes to their leuco derivatives[10].

A characteristic property of the nicotinamide coenzymes is the ability of the pyridinium ring to form the so-called charge-transfer complexes (CT-complexes) with suitable donors; the theory of such complexes in a form which is still valid was proposed by Mulliken[11]. So far there have been no direct proofs of the participation of these complexes in biological oxidoreductions. A widely accepted view, that the exchange of electron and hydrogen on the pyridinium coenzymes occurs via direct contact between the reaction components without the participation of the solvent, supports the theory that CT-interactions are operative in these processes[12]. Up to now, the

existence of CT-complexes among the participants in the electron transfer in biological oxidoreductions has been proven for the case of pyridinium and flavine coenzymes[12–15]. In no way has it been confirmed, however, whether the CT-complex is a necessary condition or only an accompanying effect of the electron transfer reaction between nicotinamide and flavin. The participation of CT-reactions in the attachment of coenzyme on enzyme has been discussed in connection with pyridinium complexes and the indole ring of tryptophan[16].

Our experiments were concentrated on a comparison of the behaviour of nicotinamide units in linear hydrophilic polymers and corresponding low-molecular weight models. Polymer models can be prepared in which nicotinamide is connected with the polymer through a side chain which leaves sufficient freedom of motion for the polymer, as well as the possibility of approaching and mutual influence of the nicotinamide rings. We wanted to find out if nicotinamide in the side chain of the polymer is able to form CT-complexes with the same donors as the low-molecular weight model of the structure unit and to what extent its acceptor properties are affected by the presence or interaction of the adjacent nicotinamide rings. We also tried to demonstrate to what extent the models of this type are suited for studying the properties of the functional group on the macromolecule and whether the facts found by the investigation of the CT-complexes of these models could be related to their oxidoreduction properties.

EXPERIMENTAL

Preparation of Compounds

Monomers. 1-(2-Methacryloyloxyethyl)-3-carbamoylpyridiniumbenzene sulphonate was prepared by a reaction of 2-methacryloyloxyethylbenzene sulphonate and nicotinamide, m.p. 137 to 138°C (ethanol). 3-Methacryloyloxy-2-hydroxypropyltrimethylammonium chloride, a commercial product Sipomer Q 1 (firm Alcolac) was purified by crystallization from 2-propanol, m.p. 180–182°C.

Polymers. Homopolymerization and copolymerization were carried out in ampoules (inert atmosphere, 60°C, 6·5 h) with 2,2′-azobis(methyl isobutylate) (0·17 mol.% per monomer) as initiator. The initial total concentration of monomers was 0·7 mol/1000 g of aqueous solution (in the reaction of monomeric benzene sulphonate the concentration was 0·3 mol/100 g of solution); the monomer mixture used in the copolymerization contained 10 mol.% or 50 mol.% of benzene sulphonate. On diluting the reaction mixture with methanol, the polymers were precipitated into acetone; conversion was 40–45%.

Low-molecular weight models. 1-(2-pivaloyloxyethyl)-3-carbamoylpyridiniumbenzene sulphonate was prepared by heating equimolar amounts of 2-pivaloyloxyethylbenzene sulphonate and nicotinamide to 90°C for 3,5 h, m.p.: 146–147°C (ethanol); yield 33%.

1-(2-Pivaloyloxyethyl)-1,4-dihydronicotinamide was prepared by a specific reduction of the low-molecular weight model with sodium dithionite[18–21] in 1M-$NaHCO_3$ at 0°C while the mixture was bubbled with a stream of nitrogen for 4 h; after several extractions of the product with dichloromethane the collected organic phase was dried with anhydrous sodium sulphate and evaporated *in vacuo* at 0°C. The light yellow product (hygroscopic, unstable in the presence of moisture) was stored over P_2O_5 at 4°C in a nitrogen atmosphere.

Reduced models in solution. The low-molecular weight model benzene sulphonate or copolymers were reduced with dithionite in a 0·2M glycine-NaOH buffer, pH 9·50, at room temperature for 3 h in a nitrogen atmosphere (conversion 70—80%). On alkalizing with a NaOH, the excess of $Na_2S_2O_4$ was decomposed by bubbling air through the mixture; $BaCl_2$ was added in excess, and the $BaSO_3$ precipitate was removed by filtration. Each time fresh solutions stored in nitrogen were used in the spectrophotometric measurements.

Spectrophotometric Measurements

All absorption spectrophotometric measurements were carried out with a CF 4-Optica spectrophotometer. The cells were thermostated to 25°C by means of a flow thermostat. The emission spectra were recorded with a Hitachi-Perkin-Elmer spectrofluorimeter.

Determination of the molar absorption coefficient of 1,4-*dihydronicotinamide.* The molar absorption coefficient of the reduced form of nicotinamide was determined by the oxidative titration with potassium ferricyanide[21] and by measuring the absorption of a freshly reduced nicotinamide model in solution.

Determination of the equilibrium constant K_c^{AD} *and of the molar absorption coefficient* ε^{AD} of CT-*complexes.* The absorption of a series of solutions of acceptor–donor mixtures was measured, in which the acceptor concentration remained approximately constant while the concentration of the donor component varied. The initial acceptor concentrations $[A]_0$ and the initial donor concentrations $[D]_0$ were calculated from the weighed amounts. The molar concentration of nicotinamide was taken as the acceptor concentration both for the polymers and for the low-molecular weight model benzene sulphonate. The absorption was measured in a 1 cm quartz cell thermostated to 25°C at λ 320 nm for complexes of nicotinamide and iodide and at λ 340 nm for complexes with indole. The correction for the absorption of the more concentrated donor component was read off according to the concentration dependence of absorption determined in advance. The $[A]_0$ and $[D]_0$ values and corrected absorption values, ΔA, were substituted into the Benesi–Hildebrand equation[17]

$$\frac{[A]_0}{\Delta A} = \frac{1}{K_c^{AD} \cdot \varepsilon^{AD}} \cdot \frac{1}{[D]_0} + \frac{1}{\varepsilon^{AD}} . \qquad (1)$$

Eq. (*1*) leads to a linear dependence of $[A]_0/\Delta A$ on $1/[D]_0$ and holds assuming that $[A]_0 \ll [D]_0$ (ref.[17]). The equilibrium constant and the absorption coefficient were calculated from the slope of the straight line and from the intercept on the ordinate. For complexes having a high association constant it appeared more useful to apply Eq. (*1*) rearranged by Scott[18], in which extrapolation to dilute solutions is employed:

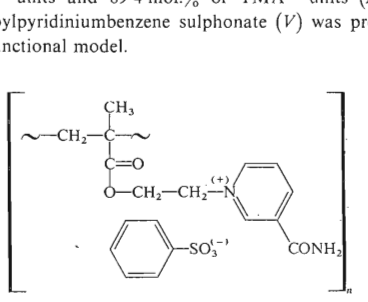
$$\frac{[A]_0 \cdot [D]_0}{\Delta A} = \frac{1}{K_c^{AD} \cdot \varepsilon^{AD}} + \frac{1}{\varepsilon^{AD}} \cdot [D]_0 . \qquad (2)$$

Experimental data were calculated by means of a programmable Wang 600 computer; linear extrapolation was carried out by using the least squares method.

RESULTS AND DISCUSSION

Nicotinamide Models

New hydrophilic polymers of the methacrylate type substituted in the side chain with the nicotinamide group (NA^+) or with the trimethyl amine group (TMA^+), which served as a control group without function but preserving the positive charge of the quaternary nitrogen atom were prepared. The homopolymers used were poly 1-(2-methacryloyloxyethyl)-3-carbamoylisopyridiniumbenzene sulphonate (*I*), poly 3-methacryloyloxy-2-hydroxypropyltrimethyl ammonium chloride (*II*); the copolymers used contained 46 mol.% of NA^+ units and 54 mol.% of TMA^+ units (*III*), or 10·6 mol.% of NA^+ units and 89·4 mol.% of TMA^+ units (*IV*). 1-(2-Pivaloyloxyethyl)-3-carbamoylpyridiniumbenzene sulphonate (*V*) was prepared as the low-molecular weight functional model.



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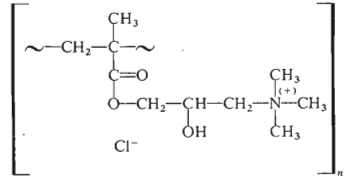

II

Both the low-molecular weight model *V* and polymers *I*, *II*, *III* and *IV* are compounds readily soluble in water and soluble in methanol; their absorption properties were measured in these solvents. The absorption spectrum of *I*, *III*, *IV* and *V* in the UV region has a band with a maximum at 265 nm in an aqueous solution and at 265·4 nm in methanolic solution. It appears as a result of the joined bands of nicotinamide

and of the benzene ring of the benzene sulphonate anion ($\varepsilon_{263.6} = 330$ l mol^{-1} cm^{-1}). The common molar absorption coefficient, the same for all models, is $\varepsilon_{265} = 4130$ l mol^{-1} cm^{-1}; it was used for precise determinations of the molar concentrations of nicotinamide units in polymer solutions in all measurements. No hypochromism of polymer models with respect to the low-molecular model *V* was proven. Homopolymer *II* does not absorb in the range from 250 to 450 nm.

CT-Complexes – Equilibrium Constant

UV spectroscopy was used in the investigations of the CT-complexes of our models (acceptors) with iodide, indole, tryptohphan, tyrosine, phenylalanine, histidine, methionine, adenosine, and adenosine monophosphate (donors). The complexes with iodide and indole were investigated in greater detail. The other complexes are either not pronounced and difficult to measure, or they possess porperties similar to those with indole.

Since polymer models are polycations, we regarded it as suitable to examine the CT-complexes with iodide as donor carrying a negative charge and indole as the electroneutral donor. We observed a very pronounced increase in the association equilibrium constant for complexes of iodide with polymers compared to that with the low-molecular model *V*. The equilibrium constant values were obtained from linear relationships according to Hildebrand and according to Scott by an interpolation of experimental data by the least squares method. The correlation coefficient from 9 and more points was higher than 0·998 in all cases. The association constants are summarized in Table I.

The greatest increase in the association constant is that for homopolymer *I*, which forms a complex with iodide whose equilibrium constant is higher by three orders of

TABLE I

Association Constants of CT-Complexes of Nicotinamide Models with Iodide and Indole

Acceptor	K_c^{AD}(1 . mol^{-1})	
	iodide (KJ, 25°C, H_2O)	indole (25°C, methanol)
Homopolymer *I*	$1{\cdot}91 \cdot 10^3$	0·51
Copolymer *III*	$1{\cdot}67 \cdot 10^3$	0·47
Copolymer *IV*	$0{\cdot}87 \cdot 10^3$	0·42
Low-molecular weight model *V*	2·25	0·59

magnitude than that of the low-molecular weight model *V*. The association constant of copolymers decreases with decreasing participation of the nicotinamide units in the copolymer, but even for copolymer *IV* it is 400 times higher than that for the low-molecular weight model *V*. Homopolymer *II* carrying the trimethylammonium cation does not form a CT-complex with the iodide.

The situation observed for complexes with indole is quite different. The equilibrium data of these complexes were measured in methanol at λ 340 nm. An extrapolation according to Hildebrand leads to values which decrease in the acceptor series $V > I > III > IV$ (Table I). On the other hand, the association constant of the complex of indole with the low-molecular model *V* is higher than for complexes of indole with polymers, but the difference is not large and all constants are of the same order of magnitude.

We measured the dependence of the equilibrium association constant of the complex of homopolymer *I* with iodide on the amount of added homopolymer *II*. The equilibrium constant decreases in the mixture of homopolymers with increasing amount of homopolymer *II* added or with increasing $TMA^+ : NA^+$ ratio. At the *II* and *I* ratio 9 : 1, when the TMA^+/NA^+ ratio in the mixture is the same as in copolymer *IV*, the association constant drops from $1{\cdot}9 \cdot 10^3$ l mol^{-1} to $0{\cdot}6 \cdot 10^3$ l mol^{-1}. No drop of the association constant was found in measurements of the same dependence for the complex with indole. The equilibrium association constant was independent of the $TMA^+ : NA^+$ ratio in the mixture of both homopolymers, *I* and *II*.

The different behaviour of the iodide and indole complexes is explained by the different mechanism by which the polymer properties affect the equilibrium of these complexes. Electrostatic forces between the iodide anion and polymer cation cause the local concentration of iodide in the surroundings of each nicotinamide unit, increasing in this way the measured equilibrium association constant. The estimated positive charge density along the polymer chain led to a conclusion that the local concentration of iodide ions in the polymer coil might be higher by two to three orders of magnitude than in a solution of a low-molecular weight electrolyte having the same average concentration. In those copolymers in which the partial concentration of nicotinamide units between the positive charges of the polycation is lower the measured association constant is also lower, depending on this concentration. If indole is used as donor, the electrostatic effects have a different influence. The positive charges of the polymer acceptor attract electrostatically predominantly counterions present in the mixture; the comparatively large molecule of indole which cannot compete with them in coulomb interactions has more difficulties in getting into a suitable position for a complex with the pyridinium ring of nicotinamide on polymer than with a freely accessible low-molecular weight model. One may also expect that the probability of complex formation will be the lower, the lower is the ratio of the acceptor units to the other positive charges, *i.e.* NA^+/TMA^+ in copolymers.

CT-Complexes – Energy of Absorption

A change in the affinity towards electrons can be reflected in the energy of the CT transition. We measured the wavelength of the maximum of the CT-absorption band of complexes of the nicotinamide models with iodide. The absorption band of these complexes is sufficiently separated from the absorption of the components and has a maximum at 290 nm. Fig. 1 shows the difference spectra of aqueous solutions of the nicotinamide models with iodide without any further electrolyte added. In the case of polymers the concentration of nicotinamide was the same, and the iodide concentration did not change either. Besides a different absorption value due to different association constant, one can also see a shift in the spectra of polymers towards longer wavelength compared with the spectrum of the complex of the low-molecular model *V*. This shift is most pronounced in the spectrum of the complex of homopolymer *I* and lowest for copolymer *IV*.

By adding an electrolyte (*e.g.*, homopolymer *II* or a low-molecular weight electrolyte which does not form a CT-complex, *e.g.* $NaHCO_3$) to a solution of the complex of homopolymer *I* with iodide, the maximum of the absorption band of the complex can be shifted towards shorter wavelength, until in the limiting value at the TMA^+/NA^+ ratio approx. 20 λ reaches 289 nm. The maxima of absorption of both copolymers, *III* and *IV*, as complexes with iodide also lie at this wavelength, if the same TMA^+/NA^+ ratio is obtained for them by adding homopolymer *II*. A further increase in the ionic strength, an increase in the content of homopolymer *II* in the polymer mixture or the addition of a low-molecular electrolyte ($NaHCO_3$) do not cause any further shift towards shorter wavelengths. The maximum of absorption of the complex of the low-molecular weight model *V* with iodide in a mixture with homopolymer *II* is also shifted in the same direction; for the TMA^+/NA^+ ratio equal to 20, the maximum of absorption of the complex lies at λ 282 nm. The difference between the wavelength of the maxima of the absorption bands of complexes of the low-molecular weight and polymer model, ($^{\Delta}\lambda$) 7 nm, remains unchanged.

The energy of the CT-transition is, among others, inversely proportional to the acceptor affinity. In spite of this, however, the observed shift towards lower transition energies for complexes of polymer acceptors can hardly be attributed to a change in the electron affinity of the acceptor unit alone. The dielectric constant and the solvation power of the medium inside the polymer coil can greatly differ from the solvation power of the solvent outside the coil.

Properties of the Reduced Form of Nicotinamide Models

The nicotinamide models were reduced with sodium dithionite. This reaction allows as to reduce specifically N-substituted nicotinamide to the respective dihydronicotinamides. The degree of reduction and the final product were investigated by UV spectro-

scopy. In all cases a product could be obtained having the characteristic absorption of 1,4-dihydronicotinamide[20]. The spectrum of models with dihydronicotinamide is characterized by a decrease in the absorption of the oxidized form with the quaternary nitrogen atom at 265 nm and by the formation of a new absorption band with a maximum at 353 nm (Fig. 2). The form. of the absorption curve is the same for both the low-molecular weight and the polymer model. The molar absorption coefficient of the reduced form was measured in a freshly prepared sample, $\varepsilon_{353} = 6300$ l. . cm^{-1} mol^{-1}

The emission spectra of models after reduction excited by light of a wavelength of 353 nm were also recorded. The fluorescence maximum at λ 456 nm corresponds to the literary data for derivatives of 1,4-dihydronicotinamide[21].

The low-molecular weight model *V* could be reduced in solution and thus transformed into 1-(2-pivaloyloxyethyl)-1,4-dihydronicotinamide. The crystalline dihydro derivative could not be prepared, as it is unstable in the presence of moisture and changes into a product which is probably formed by the addition of water to the double bond between 5th and 6th carbon atom of 1,4-dihydronicotinamide[22].

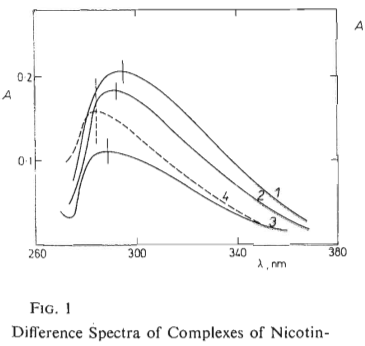

FIG. 1

Difference Spectra of Complexes of Nicotinamide Models with Iodide

Obtained by subtracting the spectra of nicotinamide and iodide from the total spectrum of the mixture; *1*, *2* and *3* 0·025 mM nicotinamide in 2 mM-KJ; *4* 2 mM nicotinamide +0·1M-KJ.

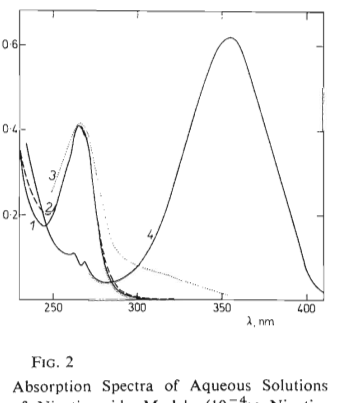

FIG. 2

Absorption Spectra of Aqueous Solutions of Nicotinamide Models (10^{-4}M Nicotinamide, 1 cm, 25°C)

1 Low-molecular model *V*; *2* homopolymer *I*; *3* homopolymer *I* + 8 . 10^{-4}M-KJ; *4* 10^{-4}M 1,2-pivaloyloxyethyl-1,4-dihydronicotinamide in 1·0M-$NaHCO_3$.

The presence of this product is reflected in the spectrum in a decrease in absorption at 353 nm and in the formation of a new absorption band at 292 nm. The same instability after reduction was also observed for polymer models.

It follows from the equilibrium constants and wavelengths of the absorption maximum of the CT-complexes determined in this work that the properties investigated are predominantly affected by the polyelectrolyte character of the polymer as a whole. It cannot be inferred that the polymer structure in this case influences considerably the acceptor properties (and consequently also the oxidoreduction properties of the functional group itself).

The polymer models of this type, that is, linear soluble cations, are not suited for the investigation of the redox properties. The properties of the models are predominantly determined by their polycationic character. All reactions with the anions (J^-, OH^-, HSO_3^-, $S_2O_4^{2-}$, CN^-) proceed on the polymer at a much higher rate (by two to three orders of magnitude) owing to the electrostatistic effects of the polycation. Reactions leading to the loss of charge of nicotinamide (*e.g.*, addition of CN^-, OH^-, reduction) then considerably alter the physical properties of the polymer molecule (depending on the ratio of charged and uncharged groups *i.e.* on the ratio of reacted and unreacted groups), and the quantitative evaluation of the properties of functional groups themselves becomes very difficult.

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