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Aqueous solutions of poly-N-alkyl-substituted acrylamides: Structure and properties related to targeted transport

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Abstract: Temperature dependence of structure and properties of poly-N-alkyl-substituted acrylamides and their aqueous solutions has been studied by IR-Fourier spectroscopy technique. For poly-N-alkyl-substituted acrylamides showing lower critical solution temperature, a specific H-bonded ring structure involving alkyl amide units of a polymer and hydroxyl functions of water was discovered. A new approach to the targeted transport of physiologically active compounds was proposed. The approach involves the immobilization of these compounds on activated poly-N-alkylsubstituted acrylamides and heating the delivery site above critical temperature.

It is known that the solubility of a polymer in a solvent is determined by the balance of polymer-polymer and polymer-solvent interactions (1,2). When a polymer contains groups with very different chemical affinities, for instance polar hydrophilic and non-polar hydrophobic groups, each group contributes independently to solubility. Polymers with such dual affinity can be completely soluble in water, if water is such a good solvent for the hydrophilic part of the polymer unit, that any neighbouring hydrophobic part is also completely hydrated. However, when the temperature of the polymer solution is raised, hydrogen bonds are weakened and hydration is reduced. The previously suppressed hydrophobic groups' interactions grow, solvated hydrophobic groups lose their weakly bound water until they coalesce into a water-insoluble, separate phase and the polymer precipitates. This temperature is called a lower critical solution temperature - LCST (3).

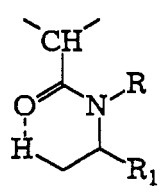
Using polymers showing LCST, for instance, poly-N-alkylsubstitued acrylamides (PSAA), as drug carriers, opens up inherently new possibilities in developing targeted drug delivery systems. The method of the design and application of these systems involves drug immobilization onto PSAA and heating the delivery site above the critical temperature. Taking into account the sensitivity of drugs, especially proteins to the environment it may be proposed that activity of a drug immobilized on such a carrier should be largely controlled by the conformation of the polymer carrier.

Hence, the study of interaction of such polymers with water at various temperatures and the search of the relationships between the biological activity of immobilized molecules and the conformational state of the carrier appears to present a problem of great scientific significance and applied interest.

The specific effect due to different fragments of a polymer molecule and a solvent manifested in the course of the formation of various complexes and aggregates were examined using FT-IR spectroscopy. Solid polymer samples were prepared by evaporation of 2 % solutions of polymers at the temperature below (6°C) and above (70°C) LCST. The resulting samples are referred to as "cold" and "hot" respectively.

Analysis of IR spectra of the "cold" and the "hot" solid samples of PSAA revealed that the amide groups are actively involved in H-bond formation. However, a comparison of the spectra of homologous pairs of these polymers demonstrated that the differences between the "hot" and the "cold" samples depend on whether the polymer exhibit an LCST or not. For instance, for secondary amides in poly-N-methyl acrylamide (no LCST), the hydrogen bond involving NH groups is stronger in the "cold" sample (νNH 3312 and 3096 cm^{-1} in the "cold" sample instead of 3317 and 3109 cm^{-1} in the "hot" sample), whereas in poly-N-isopropylacrylamide (LCST=31-33°C) this bond becomes somewhat weaker (νNH 3319 and 3069 cm^{-1} in the "cold" sample instead of 3315 and 3067 cm^{-1} in the "hot" sample).

However, a significant shift (2970 and 2990 cm^{-1} in the "cold" and "hot" samples, respectively) of the band due to valence vibration of methyl group in polymers exhibiting LCST appears to the feature of principal interest. Taking into account the fact that in the "cold" sample, as compare to the "hot" one, the intensity of this band concomitantly decreases, due to decreased polarization of the bond (4), one finds reason to assume the existence in the "cold" sample of the unusual structure with disguised H-bond, for instance, of the type 1:



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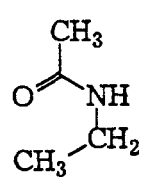

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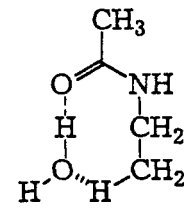

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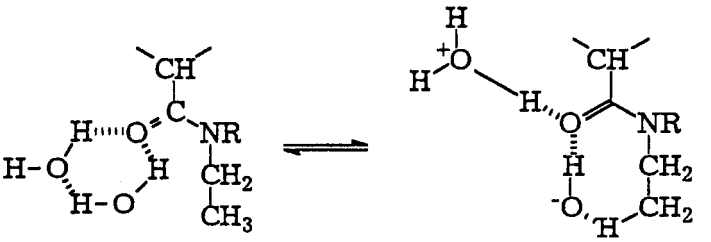
Formation of such six-membered ring structures stabilizes the enolic form of the amide and deters the hydrophobicity of an alkyl radical.

For N-ethyl acetamide, as model structure, we performed a complete optimization of the geometry of all hypothetic conformers using the Hartree-Fock formalism in the AM1 approximation. Structure 2 was computed to possess minimum energy. This structure is characterized by the fact that the CH_3 group, inclined at an angle of 30° with respect to the plane of the ring and, in an equilibrium conformation, is incapable of forming an H-bond. However, in this structure, the bonds are strongly polarized, giving rise to electrostatic interaction between the C=0 and CH_3 groups.

However, if one takes into account that the solid sample contains solvating water, he is likely to arrive at a somewhat different model structure 3. As demonstrated by quantum chemical computation, this is an optimum structure. The CH_3 group is also inclined at an angle 30°, however, this structure involves the formation of two H-bonds: C=0...H-O and H-O...H-C, characterized by the bond indexes of -0.1618 and -0.1103, respectively.

The analysis of the spectra of an aqueous solution of poly-N-diethyl acrylamide demonstrated that it contains a rather large amount of OH^- and H_3O^+ ions, which were absent in pure water, their spectrum being much more intensive than the spectrum of the dissolved polymer (5,6). Moreover, the pH of an aqueous solution of poly-N-diethyl acrylamide was 6.8±0.03, whereas that of pure water was 7.0±0.03.

The latter fact implies that in a system comprising a nonionogenic polymer and water, fragments of cationic nature appear, evidencing polymer-induced dissociation of water and binding of the OH^- anion by polymer. This process can be described by the following scheme:



Two molecules of water solvate the carbonyl roup of a polymer, resulting in their strong polarization. The hydrogen atom of the methyl group of the alkyl radical interacts with the oxygen atom of a molecule of water leading to formation of the H-bonded ring structure. The side groups of the polymer acquire a negative charge. Simultaneously, the proton of one solvating water molecule is transferred to the second molecule of water, forming H_3O^+ cation, which may further interact with the nitrogen atom of the neighbouring unit of the polymer, thus stabilizing the ring structure.

Hence, along the entire chain length, the side groups are involved in hydrogen bonding and electrostatic interactions, causing the polymer chain to extend, whereby, on dissolution, the polymer coil becomes looser, as confirmed by the increased thickness of the "cold" solid sample (Table 1). Samples showing no LCST were found to behave as common solid bodies, that is, they expanded on heating and contracted on cooling. The situation is different with the samples showing LCST ; for these, it is the "cold" samples that are the ones with a loose structure. Such anomalous behaviour (for instance, in water) is well known to be due to formation at low temperatures of stable H-bonded structures deterring a dense packing. The loose structure is most likely associated with the existence of the specific H-bonded structure described above, rather than with the system of amide H-bonds, which are present in all samples, or with the changes in the structure of solvated water, which is actually independent of the existence of LCST.

Thus, the existence of LCST in aqueous solutions of PSAA may be associated with the ability to form disguised H-bonded structures controlling the solubility of a polymer, on the one hand, or with the effect of a hydrophobic fragment in a polymer, resulting in insolubility of a polymer, upon deterioration of the H-bonded structures, on the other hand.

We have used the ability of PSAA to form the precipitate at the temperature above the LCST for the design of universal systems for targeted transport of drugs. Controlling

TABLE 1. Heat-treatment-mediated thickness variation of solid samples of PSAA.

R_1	R_2	LCST	d"cold"/d"hot"	
			"hot" - "cooled"	"cold" - "heated"
H	H	no	0.56	0.64
H	CH_3	no	0.37	0.58
CH_3	CH_3	no	0.91	0.87
C_2H_5	C_2H_5	exists	1.59	1.67
H	$CH(CH_3)_2$	exists	1.21	1.20

"Cooled sample - "hot" sample after keeping at 6°C for 16 hr;
heated sample - "cold" sample after keeping at 70°C for 6 hr.

complex chemical and biochemical processes presents a number of sophisticated problems, of which the selective concentration of only one of the dissolved components of a multicomponent solution appears to be very important. The dissolved compound may be a catalyst, one of the reagents or one of the reaction products, whose elimination may alter the reaction rate or even its direction. A particular case of vital significance concerns the selective accumulation of a drug in a target organ of a living body. It is not merely economic aspects that give rise to this problem [it is noteworthy, however, that during the conventional administration of drugs, up to 90 % of its amount does not reach the damage organ, and is in fact wasted (7)]. The main reason for the vitality of this task is the fact that many highly active drugs, for instance, enzymes, cytostatic agents, etc., are toxic to healthy tissues.

Most of the approaches to targeted drug delivery systems design involve coimmobilization of a drug and a vector molecule, displaying biospecific interaction with a diseased organ, on a soluble polymeric carrier (8). Within the framework of this approach, for each distinct drug and target organ, corresponding vector molecules to be found, isolated, and appropriately immobilized, each of these steps requiring laborious study.

A drug can also be concentrated by using an external physical effect, for example, a magnetic field (9) or heating. Using thermal activation as a driving force for drug transport appears to be an interesting trend, because very frequently inflammatory areas and tumors show a local temperature increase (10). In addition, the target organ can almost always be locally providing the means for drug transport to the organ.

In this study, water-soluble copolymers of N-isopropyl acrylamide (IPAA) were used as polymeric carriers, trypsin and horseradish peroxidase being used as a model physiologically active substances. A reactive copolymer was synthesized by copolymerization of NIPAA with N-acryloylphthalimide (API) providing a functional group which is able to react with the amino group of the proteins.

To obtain a polymer system with LCST in the physiological temperature range, acrylamide (AA) was introduced into the copolymer of IPAA with API. The LCST of copolymer systems increased with an increase in AA content in the copolymer. Studies of thermally activated transport were carried out with the copolymer exhibiting LCST slightly exceeding body temperature. The copolymer composition was AA:IPAA:API=7:92:1 (LCST = 37.0±0.2°C).

The activity of trypsin conjugates as a function of temperature is presented on the Fig. 1. The activity of the native trypsin continuously increases with an increase in temperature from 10 to 40°C, whereas the activity of immobilized enzyme becomes slightly lower and independent of temperature above LCST. The decrease in activity of the immobilized trypsin is reversible: on cooling the solution the immobilized trypsin completely recovers its activity.

The reduced activity of the immobilized trypsin at temperature above LCST may be related to the conformational changes in the enzyme molecules. These changes could be the result of either the enzyme conjugation with the copolymer or the enzyme interaction with hydrophobic fragments of the macromolecule. We have shown that the circular dichroism spectrum of the native trypsin at temperature below LCST is similar to that of immobilized trypsin; the helix content calculated from the spectra is almost the same for native and immobilized enzyme (10.2±0.9% and 9.6±0.8%). The enzymatic activity of trypsin immobilized on the model AA-API copolymer (no LCST in aqueous solution) was equal to the activity of the native enzyme within the temperature range examined.

Thus, a change in the catalytically active conformation of trypsin immobilized on AA-IPAA-API copolymer at temperature above LCST may result from hydrophobization of the

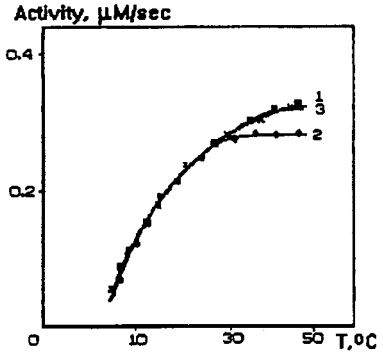


Fig.1 Trypsin activity as a function of temperature: 1-native trypsin, 2-trypsin immobilized on IPAA-API copolymer, 3-trypsin immobilized on AA-API co-polymer.

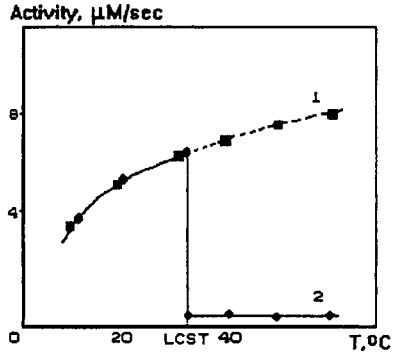


Fig.2. Horseradish peroxidase activity as a function of temperature: 1-native peroxidase, 2-peroxidase immobilized on IPAA-API copolymer.

polymeric carrier. If this is really the case, the difference between the activities of the native and immobilized enzymes should increase with an increase in temperature due to enhanced hydrophobic interaction (11). This behavioral pattern was confirmed by experimental studies (Fig. 1).

If these speculations with respect to the basic influence of hydrophobic interactions on trypsin activity are correct, then the use of horseradish peroxidase, which is more sensitive to hydrophobic interactions (12,13) should lead to greater loss of activity at temperature above LCST. Based on Fig. 2, this is indeed the case. The immobilized horseradish peroxidase activity falls abruptly to a very low value (5-10 % compared to the native enzyme) when the temperature reaches LCST. Thus, the less sensitive an enzyme is to hydrophobic interactions, the higher its activity at higher temperatures when immobilized on the AA-API-IPAA copolymer.

The proposed approach to the targeted transport involves the following. The drug immobilized on the thermo-sensitive polymer is introduced to the blood (the LCST of the polymer being slightly higher than physiological temperature). The accumulation of the drug occurs directly in the damaged area due to the higher temperature in this region which precipitates the polymer (the targeted organ or tissue can also be locally heated using some physiotherapeutic treatment). The model for the thermally activated transport study is presented in Fig. 3. The apparatus includes two interconnected vessels that are thermostatted independently. A solution is continuously pumped by the peristaltic pump (P) through both vessels at a rate 1.5 ml per minute. Fibrin clots 0.5 ml in size were placed into each vessel and the system was filled with 10 ml of native or immobilized trypsin solution. The concentration of enzyme was 0.03 mM.

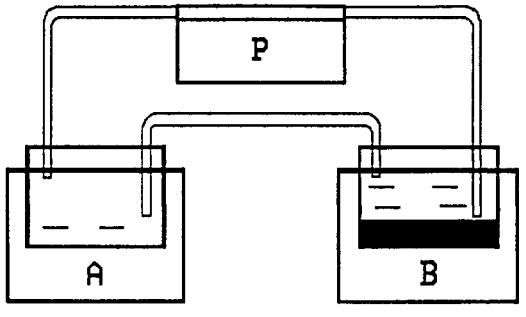


Fig.3.
Model for the study of immobilized trypsin targeted transport.

When the temperature of both vessels below LCST (36°C) the time for the dissolution of the fibrin clot in each of the two vessels was equal to 15 hr for the native trypsin (sample 1) as well as for its two immobilized forms: trypsin immobilized on AA-API copolymer (sample 2), and trypsin immobilized on AA-IPAA-API copolymer (sample 3). Warming vessel A to 38°C produced no effect on the dissolution rate when samples 1 and 2 were used: in both vessels clot dissolution time was still the same, approximately 15 hr. However, when sample 3, having LCST 37°C, was used, it took only 1 hour for the clot to be dissolved in vessel A at the temperature 38°C, whereas in vessel B at 36°C the clot not dissolved even after 24 hr.

Obviously, accumulation of the entire amount of immobilized trypsin in vessel A, occuring due to phase separation of the copolymer with the bound enzyme, appears to be the only cause to account for the observed effect. When vessel A was cooled down to 36°C (after dissolution of clot) and vessel B was warmed to 38°C, immobilized trypsin accumulated in vessel B and the clot was dissolved within an hour.

Thus, water-soluble polymeric carriers having LCST could be used for targeted delivery systems by accumulation the drug in the affected region due to the higher temperature in the region, which precipitates the drug bound copolymer.

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