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Stability of an anti-stroke peptide: Driving forces and kinetics in chemical degradation

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ABSTRACT

NR2B9c (Lys-Leu-Ser-Ser-Ile-Glu-Ser-Asp-Val) is a 9-amino acid peptide that has been illustrated to be a potential anti-stroke drug. For more effective treatment, suitable drug delivery systems should be developed. However, little is known about the stability of NR2B9c which is essential to its formulation. In this study, a reversed-phase high-performance liquid chromatography (HPLC) was applied to study the forced degradation behavior and stability of NR2B9c. HPLC studies were performed with an C8 column using a mobile phase consisting of acetonitrile (14.5:85.5, v/v) and aqueous solution (0.1% trifluoroacetic acid (TFA) and 0.05 M KH_2PO_4). The flow rate and the wavelength set during HPLC detection were 1.0 mL/min and 205 nm, respectively. The degradation pattern of NR2B9c aqueous solution followed pseudo first-order kinetics. The degradation rate at pH 7.5 was the slowest according to the plotting V-shaped pH-rate profile. The influence of temperature on the rate of reactions was interpreted in terms of Arrhenius equation ($r^2 > 0.98$). Thermodynamic parameters were calculated based on Eyring equation ($r^2 > 0.98$). The concentrations of drug, buffer species, buffer concentrations, oxidation and organic solvents have noticeable effects on the degradation of NR2B9c while ultrasound shows little impact under the experimental conditions. In a word, this study may give a detailed description of stability of NR2B9c.

1. Introduction

Stroke is a common neurological disorder and ranks the third leading cause of death (Feigin et al., 2009). Although the cost of stroke is enormous, clinically available therapeutic drugs provide modest benefit (Iguchi et al., 2011). During the pathogenesis of focal cerebral ischemia, *N*-methyl-D-aspartate glutamate receptors (NMDARs) (Gielen et al., 2009) are over-activated, and excessive NMDARs are coupled with postsynaptic density-95 (PSD-95). The signal is passed to the neuronal nitric oxide synthase (nNOS) and causes the production of NO, leading to neuronal death (Sun et al., 2008). In theory, excitotoxic signaling can be disrupted by blockage of NMDARs. Nevertheless, NMDARs are a major class of excitatory neurotransmitter receptors in the central nervous system. Antagonists of NMDARs can cause physiological dysfunctions, thereby showing no efficacy in clinical trials (Tu et al., 2010). In this case, an alternative strategy should be proposed.

Well-documented experimental evidence from various models of stroke strongly supports that disrupting the interactions of NMDAR NR2B subunits and PSD-95 rendered neurons resistant to focal cerebral ischemia. It is noteworthy that the NR2B-selective antagonist, NR2B9c (Lys-Leu-Ser-Ser-Ile-Glu-Ser-Asp-Val; Fig. 1) (Aarts et al., 2002; Douglas et al., 2012), can specifically bind with PSD-95, perturb NMDAR/PSD-95 interaction and block neuronal excitotoxicity without affecting the normal functions of NMDARs (Mony et al., 2009). The peptide not only remarkably enhanced ischemic tolerance and attenuated ischemic neuronal death, but also avoid the adverse side effects which were usually seen with nonselective NMDAR antagonists (Gogas, 2006). This raised the possibility that NR2B9c had clinical usefulness for ischemic stroke treatment (Soriano et al., 2008).

Currently researches are mainly focused on its mechanism (Cui et al., 2007; Dykstra et al., 2009) while little is known about its

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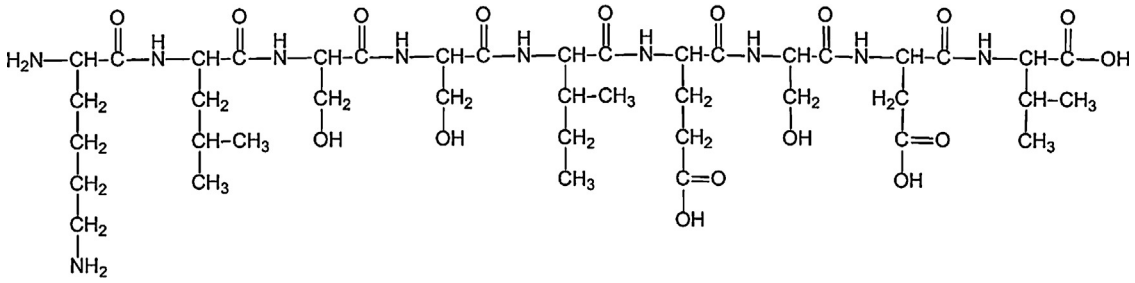

Fig. 1. Structure of NR2B9c.

stability. Chemical stability of pharmaceutical drug is essential to conduct as these studies provide information about the how the quality of a drug substance and drug product changes with time under the influence of various environmental factors such as pH, temperature and specific oxidizing agents, through which we can select favorable formulation and storage style and so on. Studies on chemical stability of a pharmaceutical drug are essential, because they provide information about the how the quality of a drug substance and drug product changes over time under the influence of various environmental factors such as pH, temperature and specific oxidizing agents. Through these studies, we can select favorable formulation and storage style. Forced degradation testing studies are those undertaken at stress conditions to degrade the sample deliberately which can help understand the chemical properties of drug molecules and solve stability-related problems, thus generate more stable formulations. Forced degradation studies, in which the sample is degraded deliberately under stress conditions, can help us understand the chemical properties of drug molecules, solve stability-related problems and produce more stable formulations. Thus, there is a need to evaluate the stability of NR2B9c.

This study represents the first report that deals with the development of a simple, sensitive and rapid HPLC method for determining the content of NR2B9c. Furthermore, the influence of pH, ionic strength, buffer species, buffer concentrations or organic solvent conditions, oxidation agent, ultrasound, light and stirring on stability of NR2B9c in solutions under accelerated conditions was investigated to provide the basis for preparing effective drug delivery system in the treatment of stroke.

Table 1
Composition of the tested buffer systems and pHs at different temperatures.

Reaction solution	Buffer concentration (M)[a]		pH			
			25 °C	65 °C	75 °C	85 °C
Formate	HCOOH	HCOONa				
	0.0427	0.0073	2.88 ± 0.12	2.84 ± 0.09	2.94 ± 0.12	3.03 ± 0.06
	0.0408	0.0592	2.88 ± 0.16	2.94 ± 0.1	2.95 ± 0.07	3.06 ± 0.04
	0.0816	0.1184	2.98 ± 0.13	3.04 ± 0.07	2.87 ± 0.08	3.02 ± 0.12
	0.018	0.032	3.93 ± 0.07	3.92 ± 0.12	3.94 ± 0.12	3.99 ± 0.07
	0.036	0.064	3.92 ± 0.14	4.02 ± 0.1	3.98 ± 0.09	4.01 ± 0.10
	0.072	0.128	4.05 ± 0.04	4.02 ± 0.09	4.02 ± 0.08	4.00 ± 0.08
Acetate	CH_3COOH	CH_3COONa				
	0.041	0.009	3.91 ± 0.07	3.95 ± 0.09	3.94 ± 0.08	4.00 ± 0.09
	0.082	0.018	3.95 ± 0.11	3.97 ± 0.12	3.97 ± 0.13	4.07 ± 0.10
	0.164	0.036	3.91 ± 0.07	3.98 ± 0.09	4.03 ± 0.06	4.04 ± 0.04
	0.018	0.032	4.87 ± 0.10	5.01 ± 0.03	5.07 ± 0.04	4.93 ± 0.10
	0.036	0.064	4.96 ± 0.06	4.93 ± 0.12	4.93 ± 0.14	5.09 ± 0.06
	0.072	0.128	4.91 ± 0.08	4.87 ± 0.10	4.95 ± 0.09	5.05 ± 0.06
Phosphate	KH_2PO_4	K_2HPO_4				
	0.0444	0.0056	5.93 ± 0.08	5.95 ± 0.11	5.91 ± 0.12	5.88 ± 0.08
	0.0888	0.0112	6.02 ± 0.09	6.02 ± 0.06	5.94 ± 0.08	5.91 ± 0.02
	0.1776	0.0224	5.95 ± 0.06	5.96 ± 0.08	6.02 ± 0.08	6.03 ± 0.10
	0.0209	0.0291	6.86 ± 0.08	6.96 ± 0.07	7.00 ± 0.09	7.01 ± 0.09
	0.0418	0.0582	7.02 ± 0.10	6.95 ± 0.06	7.04 ± 0.10	6.94 ± 0.10
	0.0836	0.164	6.96 ± 0.08	6.98 ± 0.06	6.94 ± 0.09	6.99 ± 0.11
Tris	Tris	$Tris\text{-}H^+$				
	0.016	0.034	7.44 ± 0.06	7.42 ± 0.04	7.45 ± 0.06	7.44 ± 0.06
	0.032	0.068	7.43 ± 0.10	7.50 ± 0.04	7.46 ± 0.07	7.45 ± 0.08
	0.064	0.136	7.45 ± 0.07	7.47 ± 0.09	7.49 ± 0.02	7.46 ± 0.02
	0.0208	0.0292	8.00 ± 0.08	7.98 ± 0.08	7.99 ± 0.08	7.93 ± 0.08
	0.0416	0.0584	7.90 ± 0.05	8.06 ± 0.04	7.99 ± 0.10	7.85 ± 0.07
	0.0832	0.1168	7.89 ± 0.06	7.84 ± 0.13	7.83 ± 0.03	7.90 ± 0.04
Borate	H_3BO_3	$H_2BO_3^-$				
	0.0296	0.0204	8.86 ± 0.07	9.01 ± 0.06	8.94 ± 0.08	8.95 ± 0.07
	0.0592	0.0408	9.02 ± 0.05	8.93 ± 0.10	8.97 ± 0.08	8.94 ± 0.10
	0.1184	0.0816	8.95 ± 0.03	9.02 ± 0.04	8.83 ± 0.09	8.95 ± 0.08
	0.0180	0.0320	9.47 ± 0.05	9.44 ± 0.06	9.44 ± 0.08	9.49 ± 0.04
	0.0360	0.0640	9.52 ± 0.04	9.48 ± 0.04	9.49 ± 0.02	9.41 ± 0.06
	0.0720	0.128	9.47 ± 0.04	9.43 ± 0.06	9.47 ± 0.04	9.49 ± 0.05

[a] Buffers were prepared at room temperature. All reactions were adjusted to ionic strength of 0.5 M with NaCl and the concentration of NR2B9c is 200 μg/ml.